At Home in the Cell

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The cell is home to me. It is the level of biological organization with which I feel most comfortable. I discovered this home in the mid-1960s as a college freshman. My general biology professor had put a book of electron micrographs of cells on library reserve, and I fell in love with those pictures. That there was so much complexity, so much form and organization, in something so small, was exciting to me, and I have never lost my attraction to such images. So when Rockefeller University sponsored a symposium on "Imaging in the Cell" in May 1998, I *had* to go, and I learned a lot from the experience including, to quote the cell researcher Dennis Bray (1997), "Cells were much simpler when I was a student."

Of course, it isn't that cells have gotten more complicated, but that biologists' understanding of the cell has become much richer in the past 30 years. Progress has been made in a number of areas. Genetic engineering has increased knowledge of genes and gene products, the components of cells. More and more sophisticated computer technology now makes it possible to work out the structures of proteins and other molecules much more quickly and with greater clarity. As the symposium at Rockefeller made clear, techniques for imaging the cell have come a long way from the electron microscopy of the 1950s and 1960s. In a recent book on the history of the electron microscope, Nicholas Rasmussen (1997) writes that this instrument has become passé, that newer technologies have supplanted it in the field of cell imaging. I'm not sure that things have moved quite that far—many research articles on the cell still include electron microscope images—but it is true that there are now many other imaging techniques that biologists can draw upon to investigate not only what is in the cell, but how it functions.

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Imaging the Cell

In his introduction to the symposium, Sanford Simon of Rockefeller University noted that many of the techniques used today are designed to get around two limitations of electron microscopy: that the specimen must be dead because it is placed in a vacuum chamber and that, to make the specimen opaque to electrons, it must be coated with heavy metals that change cellular forms. The physicist Andreas Engel of the University of Basel, Switzerland spoke on atomic force microscopy (AFM), a technique in which the surface of a specimen is scanned with a fine needle that picks up information about atoms at the surface and then a computer turns this information into an image. Engel uses AFM to study membrane proteins, in particular aquaporins that regulate the flow of water through cell membranes.

Fluorescence microscopy (FM) is one example of a technique that allows imaging of living material; cell constituents are labeled with fluorescent dyes and then their motion can be tracked. Daniel Axelrod of the University of Michigan uses a form of FM—total internal reflection FM—to follow the motion of proteins in the cell membrane, while John Sedat of the University of California, San Francisco probes the organization of chromosomes within the nucleus with four-dimensional FM. Like most of those presenting at the symposium, these two researchers are biophysicists. With many of these techniques, knowledge of the physics of the instrumentation is as important as is knowledge of cell biology.

My favorite presentation at the symposium was given by Joachim Frank of the State University of New York at Albany. In his work on analyzing the structure of ribosomes, Frank uses a technique called three-dimensional cryo-electron microscopy (cryo-EM) that gets around some of the problems of traditional EM. A sample, in this case of ribosomes, is quickly frozen in liquid ethane at a temperature of nearly −196° Celsius; the ribosomes are thus covered with a thin layer of ice which holds them in place in random orientation on a grid that is then exposed to radiation levels much lower than those used in traditional EM. Because the ribosomes are encased in ice, they don't shrink as they would with ordinary EM, and though the lack of a heavy-metal coat means that the image formed is less sharp, it is possible to reconstruct from the images of individual ribosomes in random orientations a three-dimensional image of the intact ribosome. This requires the processing of a large number of images—Frank has use 30,000—but the result is a much clearer picture of a ribosome that can be produced with other technologies. Frank (1998) has achieved a resolution of 15 angstroms, which is not at the level of individual atoms, but which does reveal a great deal about the ribosome's internal and external form. For example, Frank has identified the areas of the ribosome where the mRNA and the tRNAs bind and how this binding changes as the

protein chain lengthens. While it has long been known that the ribosome is the site of protein synthesis and that these RNA species must bond to it, it is still exciting to see precisely where and in what orientation this bonding occurs. I can remember when the two subunits of the ribosome were pictured as flattened spheres, the smaller one on top of the larger one, forming something that always reminded me of the body of a snow person. It is very satisfying to "see" that there is so much more to the ribosome than that.

Much of what has been learned about the cell in the past 30 years involves just such increasing clarity, not only in terms of images, but also in terms of understanding what is going on at the molecular level within the cell. What was once described in general terms can now be described in much more detail. Sometimes this detail can be daunting, and this is particularly true of cell-signaling mechanisms. For example, there are the G proteins, or more formally, guanine-nucleotide-binding proteins. These are attached to the inner surface of the cell membrane and are linked to membrane receptors for many neurotransmitters and hormones. The G proteins act as on-off switches for cellular signaling (Lefkowitz 1995). Each G protein is composed of the three subunits—α,B,γ—and this is where things start to get complicated. In the resting state or "off" position, the three subunits are bound to each other and the nucleotide guanosine diphosphate (GDP) is bound to the α subunit. When the membrane receptor is activated, it interacts with the G protein. This causes the GDP to dissociate from the α subunit, which then rapidly bonds a guanosine triphosphate (GTP) molecule, activating the G protein and causing the α subunit to dissociate from the βγ segment; both α and βγ can then go on to activate other effectors. But the α subunit is also a GTPase, so it breaks GTP down into GDP; this inactivates the α subunit which then rejoins Bγ, and the G protein is back to its resting state again. Yet this is really only the beginning of the story. It gets more complicated because there are many different G proteins, each associated with a different type of membrane receptor and each capable of activating different pathways within the cell. It also turns out that there are proteins that control the activities of the subunits, with, for example, a protein called RGS regulating the activity of the α subunit (Iyengar 1997).

The Golgi Apparatus

I just picked the G protein system to describe because it is particularly important and relatively well understood, but this kind of multistep process with multiple levels of control is found wherever you look in the cell. Researchers are now able to detect and explain more and more of such complexity. While in the past, processes would be described in terms of activities involving organelles, they are now described in terms of molecules. For example, it has been known for years that proteins to be exported from cells were synthesized on ribosomes on the endoplasmic reticulum; then these proteins were transported to the Golgi apparatus where they were packaged into vesicles that fused with the cell membrane and released the proteins from the cell. Now, if I were to accurately describe this process in detail, it would take a lot longer because I would have to include the

proteins involved in processing the newly synthesized proteins within the endoplasmic reticulum as well as those responsible for transporting the new proteins into the Golgi apparatus (Riezman 1997), and then there are the molecules in the vesicles that shuttle the proteins around.

A very good article on these vesicles was written by James Rothman and Lelio Orci (1997) who have collaborated since the mid-1980s. At the beginning of the article, they write that "nonscientists often think of scientific discovery as an impersonal process in which pure intellect leads by way of inexorable logic to a clear solution to a problem. This view underestimates the role played by false steps, occasional good luck and dogged persistence" (p. 70). While in many such articles authors pay lip service to these ideas, here Rothman and Orci follow through and describe not only the results of their research, but how they achieved those results—and they do it very humorously. This is an entertaining and insightful article.

Rothman and Orci describe everything from the language difficulties they encountered during their first telephone conversation to the experiences of two researchers in Rothman's lab. They discovered that one was throwing out the unwanted fraction in a protein purification process—the very proteins that another researcher in the same lab was trying to find. This is the kind of difficulty that usually doesn't make its way into published work, and mention of it is one of the things that makes this article so refreshing and interesting. Rothman, who does the biochemistry, and Orci, who does the electron microscopy, describe how they discovered COP-coated vesicles that bud from the Golgi apparatus. COP stands for coat proteins and is a complex of seven proteins that attach to the Golgi membrane as a single unit, with another single protein called ARF binding separately. These proteins are essential to the formation of the vesicle, but are quickly removed and recycled when the vesicle has formed.

Before leaving the Golgi apparatus, I should note that 1998 was the 100th anniversary of its discovery (Mazzarello & Bentivoglio 1998). Camillo Golgi first found the structure in nerve cells, but his students soon showed that it was also present in other type of cells. Golgi himself hypothesized that it was involved in secretion, but the very existence of this organelle was debated for decades, even after it was officially named for Golgi in 1913. One reason for this was the variability of the results with the metallic stains which Golgi developed and which continued to be used long after him. It wasn't until the Golgi apparatus was seen with the electron microscope that its existence was widely accepted and later work confirmed its role in secretion. This is a good reminder of the difficulties of identifying structures in microscopic work and figuring out what is a real cell structure and what is an artifact, to say nothing of the problem of linking structure with function. Nicholas Rasmussen (1993) describes the case of the mesosome which some argues was a cell organelle but which was eventually found to be an artifact of the staining methods used.

The Nucleus

Joseph Gall, who himself is a noted cell biologist, has for a number of years been selecting important images from the history of cell biology for the cover of the journal *Molecular Biology of the Cell*. He has now collected 60 of these images into a wonderful book called *Views of the Cell* (1996). Naturally, Robert Hooke's drawing of cells in a piece of cork and Antony van Leeuwenhoek's microscope are here, but in many of the 19th and early 20th century images, the focus is on the nucleus and on cell division. The nucleus is probably the cell organelle that has the longest history because in many cells it is by far the most prominent structure, and it can often be easily seen with a light microscope. Yet despite the long history of work on the nucleus, it is still the object of much research and it is still yielding interesting findings. Like every other part of the cell, it has been subjected to new imaging and biochemical techniques and has been found to have more and more structure and complexity. In many older images of the cell, the nucleus appears to be little more than a large rounded sack filled with diffuse material, with perhaps a darkly stained nucleolus as the only obvious landmark. But there is a great deal of evidence that the nucleus is just as complex as the other organelles, and perhaps even more so, which is not surprising considering its central role in cellular control processes.

The nuclear membrane has become a particularly interesting place. As the cell biologist Stephen Adam of Northwestern University noted recently, "there are a larger number of nuclear transport pathways than we thought" (Pennisi 1998). There are many different, though structurally related, proteins that escort proteins and mRNAs through the nuclear membrane. Some move mRNA out of the nucleus, while others work only to get proteins in. Researchers have found that these transport systems are relatively independent of each other: shutting down one system does not have an affect on others.

The nuclear membrane is dotted with structures called nuclear port complexes that are composed of about 100 proteins organized to form a tunnel. Proteins need to be attached to a carrier protein such as one called an importin in order to move through this tunnel into the nucleus, and proteins destined for the nucleus have a stretch of amino acids that designates them as such and to which the importin binds. Also involved in nuclear transport are proteins called Rans which are small guanosine triphosphatases or GTPases (Goldfarb 1997). They bond to the importin-protein complex once it has entered the nucleus, and this binding causes the protein to dissociate from the carrier which can then be exported from the nucleus so it can be used again to transport another protein molecule. There is evidence that Rans are involved in exporting molecules from the nucleus as well. But the Rans story is very much a work in progress and there are indications that a number of other transport factors may also be essential components of this process.

Also a work in progress is the deciphering of the structure within the nucleus, the nuclear matrix, which is composed of proteins and RNA. The matrix appears to organize the transcription of genes and is involved in regulating gene expression (Baskin 1995). There are transcript domains within the nucleus where synthesis of RNA and splicing take place, but the existence of such areas and their pre-

cise structure and function are still open to question (Pennisi 1997). There is evidence that RNA polymerase II, which copies DNA into RNA, also plays an important role in insuring that the splicing machinery is assembled properly. I really like this idea that the enzyme responsible for one function is also involved in coordinating the next function. It is a beautiful example of the kind of organization that makes the complex processes in the cell function so smoothly.

There is definitely an elaborate spatial organization within the interphase nucleus (Lamond & Earnshaw 1998). Individual chromosomes occupy discrete patches called chromosome territories. Within individual chromosome territories, the chromatin fiber is contorted, lopping back and forth between the nuclear interior and the periphery, it is at the periphery where transcript domains seem to occur, so the chromatin in this area is probably composed of the active genes of the chromosome. There is also evidence that chromosomes or parts of chromosomes change location within the nucleus. Some of these movements may be linked to DNA replication because it appears that replication only occurs at a fixed number of sites in the nucleus. These sites, called replication factories, contain large multienzyme complexes that do not move, so the chromosome must come to the factory for replication.

An example of beautiful coordination of the nucleus with the cytoplasm is the discovery that mechanical forces transmitted through the protein filaments of the cell cytoskeleton alter nuclear structures which may in turn lead to changes in gene expression. What this means is that the binding of effector molecules to receptors on the cell surface, which can be anchored to cytoskeleton on the cytoplasmic side of the membrane, can very rapidly cause changes in gene expression in the nucleus and can also increase protein synthesis in the cytoplasm (Vogel 1998). This is a great example of the linking of structure and function and of the tremendous dynamism of the cell. It is easy to forget what a busy place the cell is when your experience of the cell is, as it is from many of our students, based primarily on the two-dimensional frozen images found in textbooks. These cells can most readily be compared to morgues while an appropriate metaphor for living cell would be a very busy factory or, more to my liking, a busy kitchen where some cooks are cutting food up while others are taking the resulting pieces and putting them together into elaborate meals.

Conclusion

My problem now is that I have come near the end of this column, and yet I have only scratched the surface of what's new in the cell and of the newly discovered layers of complexity to be found there. I haven't had a chance to tell about the integrins, proteins in the cell membrane that link the extracellular matrix to the intracellular actin cytoskeleton (Ruoslahti 1997). The integrins activate many signaling molecules associated with growth factor signaling, so they are important in development; there is also evidence that they may be involved in the con-

trol of apoptosis or programmed cell death. Apoptosis has itself become a big topic over the past few years because it has become more and more obvious that this process is crucial in normal development where the selective death of cells is as important as is the survival of other cells. The prevention of normal apoptosis is involved in the development of a number of cancers (Barinaga 1996).

I also haven't mentioned the latest on telomeres, the chromosome ends that have gotten a lot of attention because they are linked to aging: the more times a cell divides, the shorter the telomeres become (de Lange 1998a). Whether reversing this process could lead to immortality is definitely an open, if not dubious, question, especially in light of the fact that in most cancer cells, the enzyme telomerase that lengthens telomeres is active. I have also neglected the latest on how homologous chromosomes find each other during meiosis (de Lange 1998b), even though I find this fascinating. Meiosis is something that is drilled into every biologist, usually in several different courses, yet there is still so much about this intricate process that is a mystery. And I've failed to mention the most recent work on cell evolution: a new hypothesis that eukaryotes arose from an ancient symbiosis between a member of the Archaea and a bacterium that excreted H2 and CO2, the two molecules needed by modern methane-producing archaeans (Doolittle 1998). This would help to explain the rather close association between the gene sequences in archaeans and those of eukaryotes, but only further research will indicate the viability of this hypothesis.

In an older book on the cell that is still worth reading, L.L. Larison Cudmore (1997) begins with the sentence: "All cell biologists are condemned to suffer from an incurable secret sorrow: the size of the objects of their passion" (p. 5). She goes on to write that "this universe we love is a distant one and a hard to share one. . . .Cells have everything. But visibility" (p. 6). Cudmore's ideas rang very true to me. The cell is a place I love, and it bothers me that so many of my students find it less than thrilling. While the new work on the cell may make it seem an even more daunting and complex place to try to get to know, it also makes it much more dynamic and exciting. I can't expect students in introductory biology to thrill to the cascade of proteins involved in cell signaling or become as engrossed as I am by the controversies on the structure of the nucleus. But I do want them to have some feel for my home turf.

I was recently rereading Lewis Thomas' (1974) *The Lives of a Cell*, and in the title essay and another on "Organelles as Organisms" I rediscovered Thomas' very intimate attitude toward cells. Mitochondria and nuclei and membranes were very real to him, and he felt both a connection to them and an affection for them. He writes that "my mitochondria comprise a very large proportion of me" (p. 72), and "I like to think that they [organelles in his cells] work in my interest, that each breath they draw for me" (p. 4). Thomas' personification of organelles might turn off some biologists as being very unprofessional, but I think his words have been subtly working on my subconscious during the more than 20 years since I first read them; they have helped me to feel very intimate with the cell, helped me to think of the cell as home. Though the research done since Thomas wrote those words has made this home much more upscale than it was, it is still a place where I would like my students to spend time. Yes it is complex and even chaotic, but it is also exiting and endlessly fascinating.

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