Seeing inside cells

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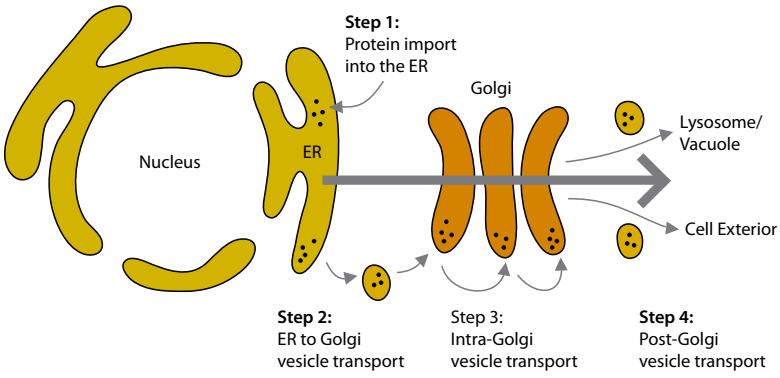
As you run across a grassy field, it would be difficult to imagine, without biological knowledge, the billions of intricately organized cellular processes which are occurring under your feet. Each individual blade of grass consists of various tissues, each made of cells in their thousands. In these cells an as yet unknown number of reactions and interactions are constantly taking place. The cells contain different parts known as organelles, which can be thought of as the cellular equivalent of the organs in animals and plants. Plant and animal cells differ in many ways (see the table below).

The secretory pathway

If you zoom into the cell cytoplasm you will see intricate organization of the different organelles which have processes occurring inside them.

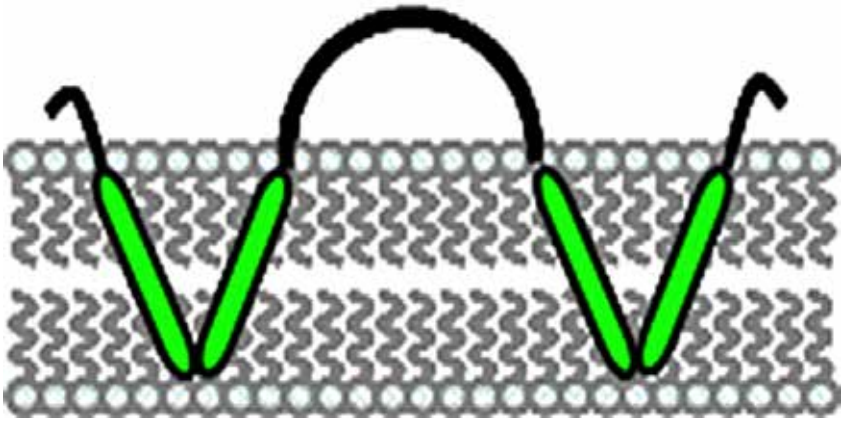
The genetic information that codes for all of the proteins of the cell is in the nucleus. Once a protein has been assembled in the cytoplasm, it enters the part of the endomembrane system known as the endoplasmic reticulum, or ER for short. The basic protein molecules, just a chain of amino acids, travel through this membrane system along a route we call the secretory pathway. Like an initially unmodified product sent down the production line of a factory, the proteins are folded and modified with additional components to produce the final product. Any faulty proteins are sent to be destroyed, like quality control on factory products.

Following this the proteins are moved to the next station in the secretory pathway, the Golgi apparatus. This is similar to the sorting centre of the factory where the proteins are packaged and sent for distribution based on the associated sorting signal, like an address. This occurs in little 'bubbles', or vesicles, made of lipids and proteins, which can merge with membranes, expelling their contents. This can be to the outer membrane so that the proteins are released into the surrounding area, to other organelles inside of the cell for a specific function, or to be destroyed.



The steps in the secretory pathway in a typical cell

Oxford Brookes University houses a whole group of plant cell biologists in the Department of Biological and Medical Sciences and we are very interested in finding out more about the structure and function of these organelles. I am researching a family of proteins called reticulons, known to shape the lipid membranes of the ER into tubules. To find out about the details of these structures which are invisible to the naked eye we use a range of different microscopes and techniques.



A generalized reticulon protein and how it is thought its topology may be in a lipid bilayer a membrane. The wedge like shape is what is thought to force the membrane to curve.

How can we find out more?

An optical (light) microscope is made up of multiple lenses, like magnifying glasses. Light passes through the specimen, is collected by the lenses, focussed and magnified into an image we can see.

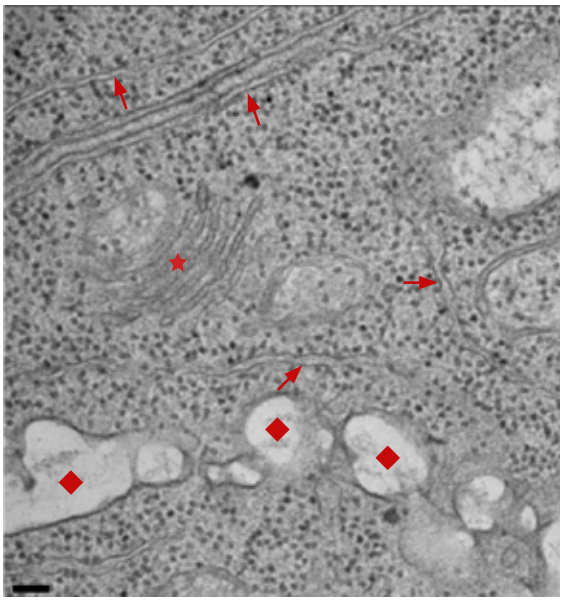
Since the beginning of microscopy it has moved far past the limitations of light microscopy and allowed us to see with great clarity the structural organization inside cells and even inside organelles. Powerful electron microscopy (EM) combined with other modern scientific techniques such as immunocytochemistry (the use of 'probes' to label specific molecular targets) allows us to see the precise place where specific proteins are located in a sample.

There are two types of electron microscope, transmission which can have a magnification of up to one million times, and scanning, which can magnify up to two hundred thousand times (TEM and SEM). Scanning electron microscopes look at larger 3D samples and scan an electron beam across the surface. The scattered electrons are used to create images of the surface detail of the sample.

I use transmission electron microscopy to look at the structure of the ER in my research. Firstly the samples need to be prepared by fixing the cells using chemicals. This keeps the structures preserved as they are at the time when they were 'fixed' in place, maintaining the shape of the organelles so they do not degrade. The cells are then embedded in resin that is hardened, so that thin sections can then be cut. The sections I usually cut of the cells are one one-thousandth of a millimetre thick and to do this I use a small diamond knife.

The sections are then placed on tiny grids to support them and placed inside the microscope for viewing and taking images to study. This entire process takes about a week to complete. The final information collected by the microscope depends on the density of the different parts of the sample and its interaction with the electrons. Therefore the images produced are black and white and not coloured.

A transmission electron microscope



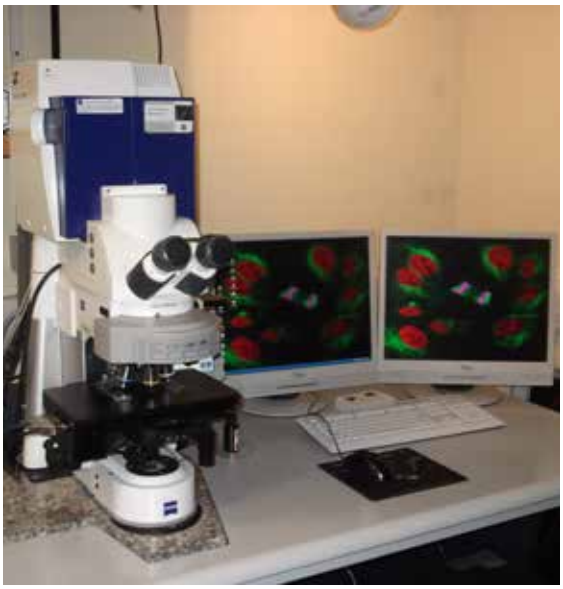
An electron micrograph of Nicotiana tabacum (tobacco) suspension culture cell showing a Golgi apparatus (star), ER tubules (arrows) and material forming the new cell wall in a dividing cell (diamonds). Scale bar = 100 nm

Electron microscopes, for all their power and the detail they reveal, have one major limitation due to the fact that they use electrons inside of a vacuum. This means that only dead cells can be studied and we cannot see how proteins move or interact over a period of time. I am interested in what happens to the proteins I am researching when the cells divide and how their distribution changes. I especially want to see what happens during the stage when a new cell wall forms between the two daughter cells.

Confocal microscopy

Fortunately we have another type of microscope with which we can also view living cells at a high resolution, although not to the extent that we can with electron microscopes. Fluorescence microscopes allow us to see fluorescent molecules such as different stains which bind to different parts of the cell, enabling us to see them clearly. A more advance system is known as a confocal laser scanning fluorescence microscope and is in essence a much further developed light microscope.

These microscopes enable us to look at different types of proteins in live cells at a magnification of up to 5000 times. We can not only see where in the cells the proteins are located but also how they move and interact with organelles in the cell. Using lasers we can produce light of different wavelengths and excite specific molecules in the specimen to produce fluorescence. The fluorescent molecules are excited by the wavelengths of light specific to them and then emit another wavelength of light which we can detect. The results are imaged on a computer which is linked to a detector in the microscope.

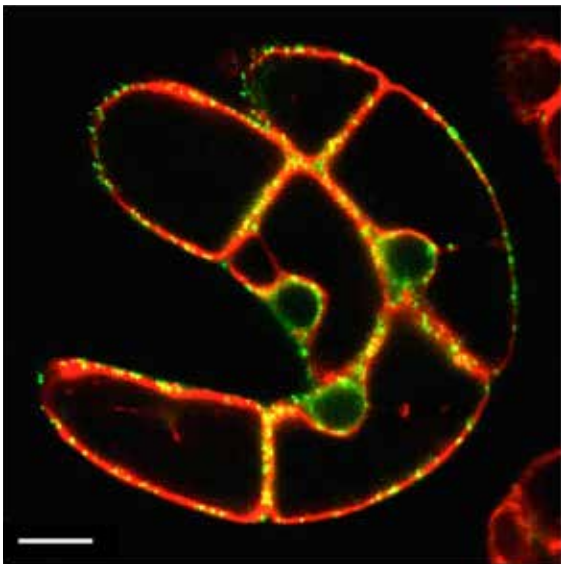


A confocal microscope in use to look at plant cells

I use confocal microscopy in my work to image cells which contain a piece of modified DNA with a portion containing the code for the protein I am interested in attached to the code for a fluorescent protein. Then, when I look at the cells under the microscope, I select a wavelength that excites the proteins produced from this DNA code and wherever the fluorescent proteins are they are excited and fluoresce.

As the fluorescent proteins are attached to the proteins I am studying, I know that the fluorescence indicates where those proteins are. Also, I know that the brighter the fluorescence in a certain area of the cell, the higher the number of my proteins in that location. From this I can look at how the distribution of protein changes as the cells undergo division and try to figure out how this is important to the functioning of the cell.

Plant Cells	Animal Cells
Have cell wall so stronger but less flexible than animal cells	No cell wall
Have vacuoles, membrane bound sacs which store various substances. They also contain water in variable amounts which can alter the rigidity of the cell	No permanent large vacuole
Have chloroplasts which trap light energy which is used to make food such as sugars. The chloroplasts also give off all the oxygen which is in the atmosphere.	No chloroplasts



A confocal micrograph of Nicotiana tabacum (tobacco) suspension culture cells expressing the Golgi marker ST-GFP (a green fluorescent protein fused to sialyltransferase). The plasma and vacuolar membranes are stained red with the dye FM4-64. Scale bar = 20 µm

3D and video

Prior to photographic technology and computer imaging, cells could only be viewed under the microscope by eye and diagrams drawn to record the data. With modern technology we can not only record individual photographs but also reconstruct 3D images and record live video of the movements of the fluorescently-labelled proteins inside the cells. We can also use stains to label larger structures such as entire organelles or their components, instead of individual proteins.

So next time you wander across a field try, to imagine the complexity of the billions of cells making up all of the living things that you see around you.

Petra Kiviniemi is a cell biologist currently working towards a PhD as a postgraduate researcher at Oxford Brookes University. She is investigating a family of proteins called reticulons in plant cells using a variety of cell imaging and molecular techniques.

Look here!

Some fascinating images made using a variety of microscopes: *http://www.nikonsmallworld.com/*