Catalytic mechanisms of topoisomerases
Interchromosomal interactions

Lecture 12: 2
10/25/2006

Congratulations to Drs. Richard Axel and Linda Buck on winning the Nobel Prize in Physiology or Medicine 2004.

Our research is primarily concerned with olfaction, or the sense of smell. For most animals, smell is the primal sense, one they rely on to identify food, predators and mates. Humans can distinguish approximately 10,000 scents, yet we know relatively little about how the nose and the brain cooperate to perform this amazing task.

Altered sexual and social behaviors in trp2 mutant mice.

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We have used gene targeting to generate mice with a homozygous deficiency in trp2, a cation channel expressed in the vomeronasal organ (VNO). Trp2 mutant animals reveal a striking reduction in the electrophysiological response to pheromones in the VNO, suggesting that trp2 plays a central role in mediating the pheromone response. These mutants therefore afford the opportunity to examine the role of the VNO in the generation of innate sexual and social behaviors in mice. Trp2 mutant males and nursing females are docile and fail to initiate aggressive attacks on intruder males. Male-female sexual behavior appears normal, but trp2 mutant males also vigorously mount other males. These results suggest that the cation channel trp2 is required in the VNO to detect male-specific pheromones that elicit aggressive behaviors and dictate the choice of sexual partners.
The expression of a single odorant receptor (OR) gene from a large gene family in individual sensory neurons is an essential feature of the organization and function of the olfactory system. We have used chromosome conformation capture to demonstrate the specific association of an enhancer element, H, on chromosome 14 with multiple OR gene promoters on different chromosomes. DNA and RNA fluorescence in situ hybridization (FISH) experiments allow us to visualize the colocalization of the H enhancer with the single OR allele that is transcribed in a sensory neuron. In transgenic mice bearing additional H elements, sensory neurons that express OR pseudogenes also express a second functional receptor. These data suggest a model of receptor choice in which a single trans-acting enhancer element may allow the stochastic activation of only one OR allele in an olfactory sensory neuron.
"Confocal" is defined as "having the same focus." What this means in the microscope is that the final image has the same focus as or the focus corresponds to the point of focus in the object. The object and its image are "confocal." The microscope is able to filter out the out-of-focus light from above and below the point of focus in the object. Normally when an object is imaged in the fluorescence microscope, the signal produced is from the full thickness of the specimen which does not allow most of it to be in focus to the observer. The confocal microscope eliminates this out-of-focus information by means of a confocal "pinhole" situated in front of the image plane which acts as a spatial filter and allows only the in-focus portion of the light to be imaged. Light from above and below the plane of focus of the object is eliminated from the final image.
Editorial Note

In this issue, we carry an article which we invited Prof. Marvin Minsky to write about his invention of the confocal scanning microscope. This is not a question of recognizing priority for a scientific insight or discovery. It is much more a question of raising the problem of how it can be possible that such an immensely important idea can go unrecognized for such a very long period. It may possibly be the case that after more research we find that yet another person discovered the same idea. That does not matter. The fact is that Minsky invented such a microscope identical with the concept later developed extensively by Egger and Davidovits at Yale and by Shepherd and Wilson in Oxford and Brakenhoff and colleagues in Amsterdam etc. The circumstances are also remarkable in that Minsky only published his invention as a patent. Yet he not only built a microscope and made it work and it was the kind of prototype of which we would be proud but he showed it to a number of people who went away impressed but nevertheless failed to adopt the concept.

We have also secured a copy of Minsky’s original letter to his patent agent which we reproduce verbatim to indicate the clarity with which he was able to describe the concept and the future potential. The original patent is also excellent reading, but that is quite freely available. We have only copied the figures from that publication.

A. Boyde
Memoir on Inventing the Confocal Scanning Microscope
Marvin Minsky

This is what I remember about inventing the confocal scanning microscope in 1955. It happened while I was making a transition between two other theoretical preoccupations ..........

The story actually begins in childhood, for my father was an ophthalmologist and our home was simply full of lenses, prisms, and diaphragms. I took all his instruments apart, and he quietly put them together again. Later, when I was an undergraduate at Harvard in the class of 1950, there were new wonders every day. I studied mathematics with Andrew Gleason, neurophysiology with John Welsh, neuroanatomy with Marcus Singer, psychology with George Miller, and classical mechanics with Herbert Goldstein. But perhaps the most amazing experience of all was in a laboratory course wherein a student had to reproduce great physics experiments of the past.
An ideal microscope would examine each point of the specimen and measure the amount of light scattered or absorbed by that point. But if we try to make many such measurements at the same time then every focal image point will be clouded by aberrant rays of scattered light deflected points of the specimen that are not the point you're looking at. Most of those extra rays would be gone if we could illuminate only one specimen point at a time. There is no way to eliminate every possible such ray, because of multiple scattering, but it is easy to remove all rays not initially aimed at the focal point; just use a second microscope (instead of a condenser lens) to image a pinhole aperture on a single point of the specimen. This reduces the amount of light in the specimen by orders of magnitude without reducing the focal brightness at all. Still, some of the initially focused light will be scattered by out-of-focus specimen points onto other points in the image plane. But we can reject those rays, as well, by placing a second pinhole aperture in the image plane that lies beyond the exit side of the objective lens. We end up with an elegant, symmetrical geometry: a pinhole and an objective lens on each side of the specimen.
The price of single-point illumination is being able to measure only one point at a time. This is why a confocal microscope must scan the specimen, point by point and that can take a long time because we must add all the time intervals it takes to collect enough light to measure each image point. That amount of time could be reduced by using a brighter light - but there were no lasers in those days. I began by using a carbon arc, the brightest source available. Maintaining this was such a chore that I had to replace it by a second best source: zirconium arcs, though less intense, were a great deal more dependable. The output was measured with a low noise photomultiplier circuit that Francis Pipkin helped me design. Finally, the image was reconstructed on the screen of a military surplus long-persistence radar scope. The image remained visible for about ten seconds, which was also how long it took to make each scan.

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This is what I remember now, and it may not all be accurate. I've never had much conscious sense of making careful, long range plans, but have simply worked from day to day without keeping notes or schedules, or writing down the things I did. I never published anything about that earliest learning machine, or about the micromanipulator, or even about that robot arm. In the case of the scanning microscope, it was fortunate that my brother in law, Morton Amster, not only liked the instrument but also happened to be a patent attorney. Otherwise I might have never documented it at all. The learning machine and the micromanipulator disappeared long ago but, only today, while writing this, I managed to find the microscope, encrusted with thirty years of rust. I cleaned it up, took this photograph, and started to write an appropriate caption - but then found the right thing in a carbon copy of a letter to Amster dated November 18, 1955.
The most serious design problem was choosing between moving the specimen or moving the beam. So far as I know, all modern confocal microscopes use moving mirrors or scanning disks. ..........., decided that it would be easier to keep the optics fixed and move the stage. I also was reluctant to use the single-lens reflected light scheme because of wanting to "see" the image right away! ....... A more patient scientist would have accepted longer exposure times and assembled the pictures as photographs - which would have produced permanent records rather than transient subjective impressions. In retrospect it occurs to me that this concern for real-time speed may have been what delayed the use of this scheme for almost thirty years. I demonstrated the confocal microscope to many visitors, but they never seemed very much impressed with what they saw on that radar screen. Only later did I realize that it is not enough for an instrument merely to have a high resolving power; one must also make the image look sharp. Perhaps the human brain requires a certain degree of foveal compression in order to engage its foremost visual abilities. In any case, I should have used film - or at least have installed a smaller screen!
Two investigators at Cambridge, Brad Amos and John White were attempting to look at the mitotic divisions in the first few divisions in embryos of C. elegans. They were doing antitubulin immunofluorescence and were trying to determine the cleavage planes of the cells, but were frustrated in their attempt in that the majority of the fluorescence they observed was out of focus no matter how much they adjusted the focus. They looked at the technique called confocal imaging which was first proposed by Nipkow and pioneered by a postdoc at Harvard named Minsky who made the first stage scanning confocal microscope in 1957. His microscope was commercially unfeasable because the technology needed to produce useful images was not available at the time. In 1986-87, a confocal microscope with the capabilities of producing very useful images could be built by combining the technologies of the laser, the computer, and microelectronics. Amos and White built the first prototype incorporating the technologies and obtained much better in-focus confocal images of the C. elegans embryos.
The laser scanning confocal microscope (LSCM) is currently the most widely used confocal variation for biomedical research applications.

http://www.microscopyu.com/articles/confocal/confocalintrobasics.html
Single-beam scanning is currently the most popular, and is the method used in the LSCM. Here the scanning of the beam is most commonly achieved by use of computer-controlled mirrors driven by galvanometers at a rate of one frame per second. To achieve faster scanning, at near video frame rates, some systems use an acousto-optical device or oscillating mirrors. The alternative method uses two beams to scan at near real time, and usually relies on using some form of **spinning Nipkow disk**. Such systems have been derived from the tandem scanning microscope (TSM), with improvements to make them more efficient for collecting images from fluorescently labeled specimens. Figure 5 illustrates one such improved system that employs dual Nipkow disks and microlenses to enhance the detection of the low fluorescence levels of real time image collection.
1831: Joseph Henry's and Michael Faraday's work with electromagnetism jumpstarts the era of electronic communication.

1862 First Still Image Transferred: Abbe Giovanna Caselli invents his Pantelegraph and becomes the first person to transmit a still image over wires.

1873: Scientists May and Smith experiment with selenium and light, this reveals the possibility for inventors to transform images into electronic signals.

Eugen Goldstein coins the term "cathode rays" to describe the light emitted when an electric current was forced through a vacuum tube.

1880: Inventors Alexander Graham Bell and Thomas Edison theorize about telephone devices that transmit image as well as sound. Bell's Photophone used light to transmit sound and he wanted to advance his device for image sending.

1884: 8 Lines of Resolution: Paul Nipkow sends images over wires using a rotating metal disk technology calling it the electric telescope with 18 lines of resolution.

1900 And We Called It Television
At the World's Fair in Paris, the first International Congress of Electricity was held. That is where Russian Constantin Perskyi made the first known use of the word "television."

http://inventors.about.com/od/tstartinventions/a/Television_Time.htm
The Nipkow disk was a device which its inventor, Paul Nipkow, thought could be used to transmit pictures by wire. The disk had a spiral of holes cut into it. These holes were positioned so that they could scan every part of an image in turn as the disk spun around. The light coming from each point would then be turned into in electrical current.

This electrical signal would light up a second light at the other end of the wire. The second light would flicker because the amount of current it received would depend on the brightness of the image being scanned. The light from this light bulb passing through a second disk spinning at the same speed, would then project the picture onto a screen.
Inventors
Paul Nipkow 1860 – 1940

German engineering student, Paul Nipkow proposed and patented the world's first electromechanical television system in 1884. Paul Nipkow devised the notion of dissecting the image and transmitting it sequentially. To do this he designed the first television scanning device.

Paul Nipkow was the first person to discover television's scanning principle, in which the light intensities of small portions of an image are successively analyzed and transmitted. In 1873 the photoconductive properties of the element selenium were discovered, the fact that selenium's electrical conduction varied with the amount of illumination it received. Paul Nipkow created a rotating scanning disk camera called the Nipkow disk, a device for picture analysis that consisted of a rapidly rotating disk placed between a scene and a light sensitive selenium element. The image had only 18 lines of resolution.
"lines of resolution" refers to the limit of visually resolvable lines per picture height (i.e. TVL/ph = TV Lines per Picture Height). In other words, it is measured by counting the number of horizontal or vertical black and white lines that can be distinguished on an area that is as wide as the picture is high.

Pixels and Resolution

The image that is displayed on the screen is composed of thousands (or millions) of small dots; these are called pixels; the word is a contraction of the phrase "picture element". A pixel represents the smallest piece of the screen that can be controlled individually. Each one can be set to a different color and intensity (brightness).

The number of pixels that can be displayed on the screen is referred to as the resolution of the image; this is normally displayed as a pair of numbers, such as 640x480. The first is the number of pixels that can be displayed horizontally on the screen, and the second how many can be displayed vertically. The higher the resolution, the more pixels that can be displayed and therefore the more that can be shown on the monitor at once, however, pixels are smaller at high resolution and detail can be hard to make out on smaller screens. Resolutions generally fall into predefined standard sets; only a few different resolutions are used by most PCs.