Membrane proteins captured with a molecular hole punch

A punch biopsy is an important tool to diagnose skin disorders. A small, circular piece of tissue is removed to examine the skin cells for defects that might reveal their failing functions. In research laboratories scientists can now take far smaller punch biopsies of single cells to learn how individual proteins in the surfaces of cells function. Associate Professor Nathan Alder recently received a $1.5 million grant from the National Institutes of Health to study membrane proteins using molecular-sized punch biopsies called nanodiscs.

“The real selling point of this grant, probably the major one, was the use of nanodiscs,” Alder says. He

Genetic parasites reveal evolutionary processes

Gogarten and R. Thane Papke are turning to another kind of pond, a warm, but very salty one, to study how life evolves by a process Darwin could not have imagined.

Gogarten and Papke, along with their collaborator Uri Gophna at Tel Aviv University, were recently awarded $230,000 by the United States-Israel Binational Science Foundation for a 4-year study of the process of horizontal gene transfer among microbes living in very high salt concentrations. Genes usually pass to offspring from their parents, but microbes can also inherit genes from other microbes. The salt-loving microbes that Gogarten and colleagues will study are haloarchaea that
Hole punch

will use nanodiscs to study a multi-protein complex called TIM23 that is embedded in the membranes of mitochondria, the powerhouses inside nearly every cell of your body. TIM23 complexes move proteins newly made in other parts of a cell into the mitochondria so they can carry out their functions there.

Mitochondria are enclosed by inner and outer membranes composed of lipid molecules that repel water and contain proteins, like the TIM23 complex, that control what moves in and out of the mitochondria. Alder has discovered that lipids enhance the interactions of the component proteins of the TIM23 complex during the transport of proteins. “We’ve identified a very important role for cardiolipin which is an important phospholipid in the mitochondrial inner membrane,” explains Alder.

Since membrane-embedded proteins repel water as the lipids do, they are very difficult to isolate and study. Normally detergents are used to remove them from membranes, but in doing so they can lose their functions. “Nanodiscs give great experimental control,” Alder says. To make polymer-based nanodiscs, a polymer is mixed with mitochondrial membranes. The polymer molecules move into the membranes, surround proteins there and pop out small circular “mini-membrane” patches. Punching out membrane proteins with some of the membrane preserves the proteins’ functions. “The benefit here is that you can now test nicely the activity of the protein in the presence of its native lipids,” he says.

Using nanodiscs Alder will examine in detail the earliest steps in protein transport by the TIM23 complex. The importance of cardiolipin in this process may account for defects in cardiolipin synthesis found in human ailments like Barth syndrome, an inherited mitochondrial disorder. “If you have a defect in cardiolipin biogenesis you reduce the activity or efficiency of protein biogenesis,” Alder notes, but using nanodisc technology, “you can pinpoint what part of the process is defective by this kind of approach.”

Alder is one of the first American researchers using polymer-based nanodiscs and one of the few examining mitochondrial biogenesis. “The importance of mitochondrial biogenesis has not been emphasized by American researchers,” Alder says. NIH reviewers commented on the technological innovation aspects of Alder’s proposed work. He is combining this innovative technology with examinations using structural biology approaches, including the small angle X-ray scattering instrument newly acquired by the Institute for Materials Sciences, to generate novel hypotheses that will certainly lead to new insights into mitochondrial functions.

Genetic parasite

belong to the Archaea, the third form of life besides the Bacteria and Eukarya, which includes us.

The team will observe how a segment of DNA that codes for a protein called an intein is passed among haloarchaea by horizontal gene transfer. Inteins are very effective molecular parasites. They insert their gene into a gene encoding an essential protein using a homing endonuclease (HE) activity that is encoded with the intein. The haloarchaeal DNA polymerase is an essential gene that can be invaded by inteins. When the DNA polymerase protein is made, it forms a fusion protein with the intein and HE. The intein protein cuts itself out with the HE and fuses the two DNA polymerase segments back together. Cells cannot lose the intein gene, because its loss will likely destroy the essential DNA polymerase gene.

Gogarten notes that, “The inteins have a huge fitness cost.” That is, cells that have them cannot compete as well against cells without them. Geller’s lab showed that a haloarchaeal strain without inteins grew 7% faster than an identical strain lacking an intein.

So why have inteins been tolerated during evolution? “One of the fallacies is that we always think ‘what is it good for?’,” Gogarten explains. “The sad truth is that in nature a lot of things are not good for anything. They are not detrimental enough to be eradicated.”

Gogarten seeks to understand how an intein survives in a population of haloarchaea. “An open question for inteins is ‘how do they really survive in a population?’,” says Gogarten. Inteins are usually not found in every cell in a population. “The intein comes in, it spreads, but it never really reaches everyone in the population,” Gogarten explains.

Each member of the research team will study different aspects of intein transfer. Geller’s lab will focus on isolating haloarchaea from coastal Mediterranean salty ponds in Israel. Shannon Saucy, a Microbiology PhD student has traveled to Israel to assist in the isolation of haloarchaea with inteins. Papke’s lab will head up the genomic sequencing of the newly
MCB hosts CT Symbiosis Symposium. The Fourth Annual Connecticut Symbiosis Symposium was hosted by Asst. Prof. Jonathan Klassen at the Biology Physics Building May 12. The Symposium brought together local research groups with interests in symbiosis, microbial communities, and host-microbe interactions. Featured speakers were Dr. Geoffrey Attardo, Yale Dept. of Epidemiology of Microbial Diseases; Dr. Marcy Balunas, UConn Pharmaceutical Sciences; Mathew Fuller, Gogarten lab, MCB; Dr. Michael Smith, Illumina; Dr. Lauren Petersen, The Jackson Laboratory for Genomic Medicine; and Dr. Michael Nelson, Graf lab, MCB. Graduate students from Yale and UConn labs also presented posters for viewing between the seminars.

NSF Research Fellowship. Anne Kaplan, a graduate student in the Structural Biology, Biochemistry and Biophysics program, was recently awarded an NSF Research Fellowship award. Kaplan is a second year PhD student working in the laboratory of Prof. Andrei Alexandrescu who is determining the structure of a part of a bacterial protein, Hemolysin II, that forms cell-killing pores in red blood cells. Kaplan has found a novel structure in this protein, a finding that could lead to better understanding of its function.

Klassen awarded grant for fungus garden genomes. Assistant Professor Jonathan Klassen was awarded a Community Science Project Grant by the US Department of Energy Joint Genome Institute (JGI) for a study examining how the microbes that live in ant fungus gardens work together to resist diseases and degrade the plant material collected by the ants. JGI will sequence DNA from 92 ant garden samples, 60 individual bacterial genomes, and 3 fungus genomes. JGI will also synthesize about 100 genes found in the environmental DNA sequences as potential products that affect microbes, such as antibiotics.

New editorships. The new American Association for the Advancement of Science’s sister publication to Science, Science Advances, announced editorships for two MCB faculty. Professor Joerg Graf joins as an Associate Editor and Professor Emeritus and former Department Head Philip Yeagle was named a Deputy Editor. Science Access is AAAS’ first open access, on-line only journal that will publish work in a broad spectrum of areas of science.

isolated haloarchaea and Gogarten will lead the bioinformatics analysis and modeling of intein distribution and evolution.

“One of the things that I am fascinated by is selection acting on different levels,” Gogarten says. “You have selection acting on the fitness of the organism, but then the fitness of the organism does not improve [by intein invasion]. So maybe in the long run … there can be some group selection that benefits by the homing endonuclease being there.” Darwin struggled with idea that natural selection can work on groups as well as individuals, so perhaps, as he suggested, studies of little ponds can reveal life’s mechanisms of evolution.

MORE NOTES

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