Anything Goes at the Cell Biology Meeting

These days, cell biology is pretty much an umbrella term describing anything that can happen inside or outside a cell. And perhaps nothing illustrates that point better than the annual meeting of the American Society for Cell Biology, which drew more than 7000 biologists to Boston last month to hear talks on topics ranging from cellular architecture and embryonic development to neurobiology and immunology. The following summarizes some highlights of the meeting.

New Evidence Found for a Nuclear Matrix

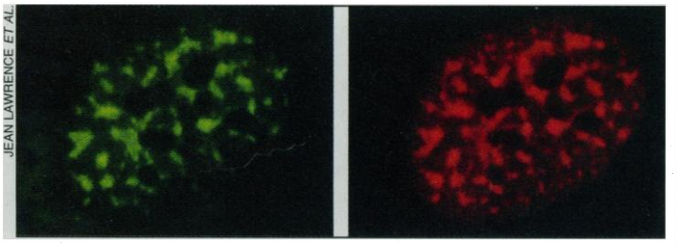
Is it real, or is it artifact? That's a question many cell biologists have been asking since the mid-1980s about "the nuclear matrix," a network of interlaced protein filaments thought to provide a sort of internal skeleton for the nuclei of the cells of higher organisms. Recently, however, evidence in favor of a nuclear support system has started to come in from several researchers, including Jean Lawrence and her colleagues at the University of Massachusetts Medical School in Worcester, who presented their new data in a talk and several posters in Boston.

If they're right, it would mean that the conventional wisdom about how the genetic materials DNA and RNA are synthesized would have to be revised. The current view is that these synthesis reactions occur anywhere within the aqueous medium that fills the nucleus. But if there is a matrix, the nucleus would be more highly organized and the reactions would likely take place at discrete sites within the cell's nucleus, possibly while the synthetic machinery is attached to the matrix itself. Such a situation would alter the current perception of the geometry and hence of the very mechanism and regulation of nucleic acid synthesis.

The doubts about the existence of a nuclear matrix stem from the way the early experiments purporting to show its existence were done. In those experiments, done independently in several labs, researchers treated the nucleus with degradative enzymes called nucleases that remove all of the nucleic acids and also with detergents and salts that remove almost all of the proteins. When all those cellular elements were cleared away, a meshwork of protein filaments—the nuclear matrix—still remained. But the treatments used were so harsh, say disbelievers, that they might have artificially caused nuclear proteins to bind together in what appears to be a meshwork, even though no such thing actually exists in nature. What's more, because the putative matrix proteins are extremely insoluble, no one has been able to isolate and characterize them. In addition, every cell type seems to have a distinct set of proteins making up its matrix.

The trick then has been to demonstrate a regular nuclear architecture, without knowing exactly what proteins are involved and under conditions that do not disturb whatever structures may be there. To perform that feat, the Lawrence group made use of a technique they developed for quite a different purpose. In 1987 the researchers worked out a scheme to locate the exact position of a gene on a specific chromosome. They found they could pinpoint the gene's position more precisely if they tagged it with a fluorescent probe during interphase, the part of the cell cycle when the chromosomes are least tightly wound.

Lawrence and her colleagues subsequently showed that the technique, which is relatively gentle, could also be used to locate sites of active gene expression, where DNA is being copied into RNA. During that work, they noticed that the active genes are limited to the inner 50% of the nuclear volume, which suggested that there was a defined place within the nucleus for transcription and hence a defined nuclear architecture to support it.



JEAN LAWRENCE *ET AL.*

Located together. *The same cell stained for RNA* (left) *and snRNPs, which are part of the RNA splicing machinery* (right).

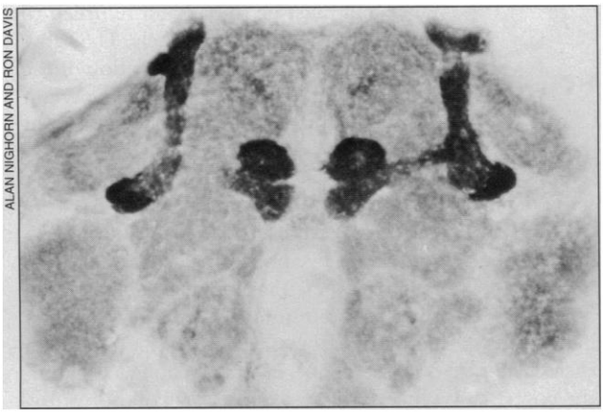
In their most recent work, members of the Lawrence lab have found further evidence that RNA is contained within specific compartments in the nucleus. They see recently synthesized RNA only within small, discrete islands they call "transcription domains." The domains also appear to contain the machinery for snipping noncoding sequences out of the RNAs and performing the other modifications necessary before they are exported out of the nucleus. In contrast, the group finds that DNA synthesis, particularly of DNA that replicates later in the cell cycle, is sequestered in very different regions of the nucleus, which means that something is physically separating the DNA and RNA compartments.

In further support of a nuclear matrix, the Lawrence lab traced the fate of newly synthesized RNA molecules, reasoning that a nuclear architecture should include a conduit system for exporting these molecules to the cytoplasm, where they ultimately direct protein synthesis. And in fact, that's exactly what they saw. Tagging newly transcribed RNA molecules gives tracks radiating to the nuclear membrane. That work, says Ken Carter, a postdoc in the Lawrence lab, had nuclear matrix proponents, "jumping out of their skin." All in all, the experimental foundation for a nuclear matrix is becoming much more solid.

Fruit Fly Learning Research Mushrooms

When researchers started to investigate learning and memory in the fruit fly *Drosophila melanogaster* a few years ago, it seemed like a match made in heaven, the perfect marriage between genetics and physiology. Initially, however, the matchmaking didn't go so well. The genetics were there, but like a shy bride, the brain's locus for learning was not showing itself. And without knowing where in the brain learning takes place, it would be impossible to correlate physical changes with alterations in the genes associated with *Drosophila* learning. But all that has begun to change as a result of recent work by Ron Davis and his colleagues at Cold Spring Harbor Laboratory. They've found that a brain structure known as the mushroom body because of its shape is the primary site for learning and memory in *Drosophila,* and have begun to trace out the candidate memory genes that are active there. And at the meeting, Davis outlined some of the work leading up to this conclusion as well as some new work that strengthens it.

The first clue to the mushroom body's importance came early this year, when Davis found that *dunce*, a mutant gene associated with learning defects in the fruit fly, is extremely active in that brain region (see



ALAN NIGHORN AND RON DAVIS

Seat of learning. *The dark areas show the mushroom bodies stained with an antibody to the* dunce*-encoded enzyme.*

Science, 27 September 1991, p. 1486). *Drosophila* can't do calculus problems, but the flies can be taught to discriminate smells and remember what they've learned for as long as 24 hours. Unless, that is, they have mutations in some key genes. And *dunce* is probably the best described of these. The Davis lab has been accumulating evidence since 1986 that it encodes an enzyme called cyclic AMP (cAMP) phosphodiesterase, which destroys cAMP. That was considered an encouraging finding because some of the neurotransmitters through which nerve cells communicate work through cAMP, and so a mutation in an enzyme that helps control its concentration might well interfere with learning.

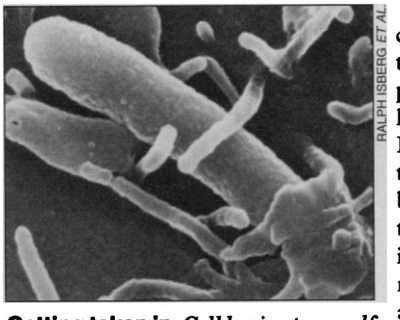
In their more recent and still unpublished work, Davis and his colleagues have gone on to provide further evidence that the mushroom body is the seat of learning in the fruit fly. They've found that another learning-associated gene, this one called *rutabaga*, is also extremely active there. This was much tougher than the *dunce* work. The Cold Spring Harbor group had already cloned the *dunce* gene, and used it as a probe to see where in the brain the gene is expressed. But because the protein product of *rutabaga* hadn't been identified with certainty, the Davis group was forced to take a different tack. They attached a "reporter gene" that can turn cells in which it's expressed blue onto a transposon, a genetic entity that can arbitrarily insert itself into genes, thereby interrupting their coding sequences and producing mutant proteins. After allowing the transposon with its reporter gene to jump into fruit fly chromosomes, the researchers screened the flies to see which had blue areas in the mushroom body, indicating that the gene was expressed there, and were also learning mutants.

Among the fifty mutations they created this way, several proved to be in the *rutabaga* gene. The Davis group teamed up with Randy Reed and colleagues at Johns Hopkins University who had previously cloned a gene for adenylate cyclase, an enzyme that makes cAMP. As it turned out, *rutabaga* mutations caused decreased expression of this gene. Taken together the two labs showed that the *rutabaga* protein product is adenylate cyclase. "Up until now, there was no certain evidence that *rutabaga* was the structural gene for adenylate cyclase," says Davis.

In his recent work, Davis has also identified at least 10 previously undiscovered *Drosophila* genes that are expressed in mushroom bodies, and preliminary evidence suggests that several of these, when mutated, give rise to learning or memory defects. All of which suggests that the marriage between mushroom bodies and learning has taken place at last.

New Clues to How Bacteria Get Into Cells

All that most disease-causing bacteria have to do to make people sick is get inside the body, replicate, and secrete some nasty toxins. But a few species—such as members of the *Chlamydia* group, which cause genital and eye infections, and the pathogen that causes typhoid fever—have to cross an additional barrier before they can do their dirty work: They have to get inside cells. Exactly how they accomplish that task at the molecular level has long been a mystery, but recent work by several groups has begun to tease out the molecular interactions between an invading bacterium and its chosen host. And at the cell biology meeting, Ralph Isberg, a microbiologist at the Howard Hughes Medical Institute at Tufts University Medical School in Boston, presented new evidence that further clarifies that interaction—information that may help in designing new drugs to combat infection by intracellular bacteria.



RALPH ISBERG *ET AL*

Getting taken in. *Cell begins to engulf an* E. coli *bacterium making invasin.*

Microbiologists have known for years that intracellular bacteria coax their host cells to take them in by phagocytosis, a process in which cells send out projections to surround and eventually engulf the bacteria. But researchers have been confronted by a conundrum: How can bacteria trigger phagocytosis in cells that are not normally phagocytic, such as the epithelial cells that line body surfaces. About 7 years ago, however, Isberg, who was then a postdoc with Stanley Falkow at Stanford University School of Medicine, got the first insight into this conundrum when he discovered that *Yersenia pseudotuberculosis*, an intracellular bacterium that causes intestinal diseases in mice, makes a surface protein called "invasin." All it takes for the bacterial cells to seduce their unwitting hosts into taking them in, Isberg found, is that they be appropriately dressed in invasin. Indeed, even *Escherichia coli*, a normally extracellular bacterium, can trick a normally nonphagocytic epithelial cell into ingesting it if the bacterium has been genetically engineered to express invasin on its surface.

Last year, Isberg took the work a step further when he showed that invasin attaches bacteria to the host cells by binding to cell surface proteins known as integrins. But that still left a big mystery. Integrins are adhesion molecules used by cells to bind to extracellular materials, such as basement membranes and the connective tissue that hold cells together. The mystery? Fibronectin, one of the extracellular materials to which the integrins normally bind, does not cause epithelial cells to become phagocytic. Now, in more recent work, Guy Tran Van Nhieu, a postdoc in Isberg's lab, has begun to resolve that paradox.

At first, Tran Van Nhieu thought that invasin and fibronectin might bind different segments of the integrin molecule and so induce different host cell responses. But that theory has not been borne out. Instead, Tran Van Nhieu found that invasin simply binds much more tightly to integrin than fibronectin does, and this difference in binding affinity causes the different responses, Isberg says.

The question then, of course, is why does the tighter binding triggers phagocytosis when the looser binding doesn't. Isberg doesn't yet know the answer. "One possibility," he says, "is that the increased affinity of invasin for integrin makes it more effective at recruiting a sufficient number of integrin molecules to the potential entry site, which for reasons we still don't understand is an important first step in phagocytosis." Whatever the mechanism ultimately turns out to be, one thing is for sure: Only the best dressed pathogens will gain admittance into the body's most exclusive cells.

■ MICHELLE HOFFMAN