The Cell's Nucleus Shapes Up

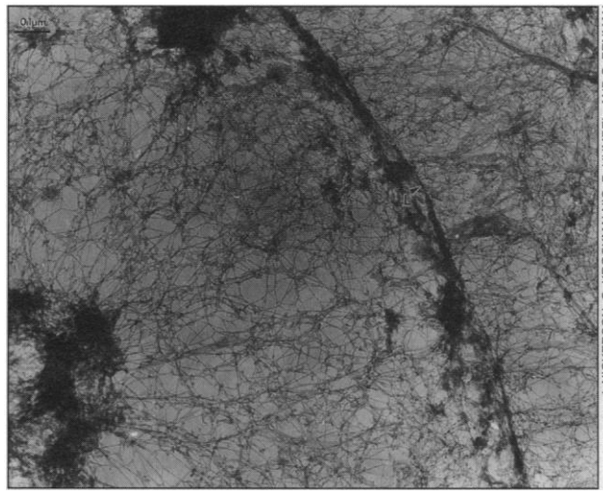
Cell biologists are getting a better view of how the internal structure of the nucleus may influence gene replication and activity

In high school—or even college—biology, few of us thought much about the complexity of the cell nucleus. Of course, we knew that as the container for the gene-carrying chromosomes, it serves as "control central," not only directing the cell's day-to-day activities but also having the task of duplicating the genes before cells divide so that each daughter inherits an intact set. But that's pretty much what we thought the nucleus was—a container. Quietly, over the past 20 years, however, cell biologists have come to recognize that it's a great deal more than just a sac of chromosomes and enzymes sloshing around in a watery solution. Its structure is highly organized, with every molecule having its own place. It even has its own skeleton, a network of insoluble protein fibers known as the nuclear matrix. And that's not all. As researchers take the work one step further, they are also finding that this complex internal organization may well influence gene activity and the other critical biochemical reactions that take place inside the nucleus, as two papers published in this issue of *Science* illustrate.

When a gene is turned on the information it encodes first has to be copied into a molecule of messenger RNA (mRNA), which in turn directs the synthesis of the gene's protein product. On pages 1326 and 1330, a team led by Jeanne Lawrence of the University of Massachusetts Medical Center in Worcester provides the first three-dimensional view of just where in the nucleus mRNAs are synthesized and made ready for export to the protein-synthesizing machinery in the cytoplasm. Says cell biologist Jeffrey Nickerson of the Massachusetts Institute of Technology (MIT): "Jeanne Lawrence's results allow us to see features of nuclear organization that in the past we could only speculate about. Her lab has experimentally confirmed that RNA metabolism is architecturally organized in the nucleus. This result is very exciting to those studying nuclear architecture—as it should be to those studying RNA metabolism."

Why the excitement? Because cell biologists expect this and other work defining the organization of the molecular structure of the nucleus not only to go a long way toward explaining how mammalian genomes are organized, but also to provide a framework for understanding what controls gene expression in different cell types—one of the central issues in molecular biology today. Yes, molecular biologists have already learned a great deal about the basic molecular mechanics of gene expression. But virtually all that work has been done in simple test-tube systems, ignoring the possible influences of the nuclear matrix and other aspects of nuclear structure on the reactions. Indeed, says physicist-turned-cell biologist Sheldon Penman of MIT, an early proponent of the existence of a nuclear matrix, "there's a strong prejudice to believe in soluble biochemistry."

And defining the role of the cell's nuclear structure may have implications beyond its impact on the theoretical underpinnings of cellular biology. The research may have practical applications as well. It turns out that the nuclear matrix proteins of cancer cells differ from those of their normal counterpart, a fact that may prove useful in developing new diagnostic tests for cancer (see story on page 1258).



J. NICKERSON, G. KROCKMALNIC, B. HUMBEL, AND S. PENMAN

Branching out. This view of a segment of a HeLa cell nucleus shows the fine branched network of internal nuclear matrix filaments attached to the nuclear lamina.

This recognition of the importance of the nuclear structure was a long time coming, however. Indeed, for years many cell biologists didn't believe the nuclear matrix existed at all.

The idea dates back to work done in the mid-1970s by Ronald Berezney, who was then a postdoc in Donald Coffey's lab at John Hopkins University School of Medicine. At the time, the general view was that the nucleus is a "bag of chromatin floating in a sea of nucleoplasm," as Berezney recalls the official line. Even so, there had already been hints that nuclear structure was considerably more complex than thought; electron micrographs made in the 1960s showed fibrous and granular structures throughout the nucleus. And then Berezney also began finding evidence for nuclear structure.

He noticed, for example, that if he punched holes in the nuclear membrane, allowing its contents to pour out, the nucleus retained its spherical shape. "I started to think that something might be holding the nucleus together," Berezney recalls. Together with Coffey, Berezney went on to show that if all the nucleic acids were digested away with enzymes and the more soluble proteins were removed from the nucleus with detergents and salts, a meshwork of fibrous proteins, similar to those in the early electron micrographs, was left behind. The network, which Berezney and Coffey called the nuclear matrix, might therefore be the "something" holding the nucleus together.

But even though other groups also found evidence for a protein network in the nucleus, the general scientific public remained unconvinced, says Penman. The main problem was that the matrix still could not be seen very well in electron micrographs of whole cells. The prevailing attitude was that if it couldn't be seen clearly, it wasn't there, Penman explains. By the mid-1980s, Penman helped resolve the impasse by developing new techniques that allowed much better resolution of nuclear structures, the matrix included. In the intervening years Penman's group has made what Nickerson describes as "hundreds—perhaps thousands" of micrographs of the nuclear matrix.

Meanwhile, evidence was already building that nuclear structures are key participants in one important biochemical reaction that takes place in the nucleus—the replication of the DNA. From the work of researchers, including Berezney, Coffey, Drew Pardoll and Bert Vogelstein, also at Johns Hopkins, as well as Fredrich Wanka's group at the University of Nijmegen, the Netherlands, and Peter Cook's at Oxford University in England, a picture gradually emerged showing that replicating DNA forms repeating

loop structures, called replicons, that are attached to the matrix at the loop bases. The replication machinery is located at the attachment sites, and when the DNA is copied the loops are pulled through the machinery.

But the matrix's influence on nuclear DNA organization apparently doesn't stop at the replicon level. The 50,000 replicons in the average mammalian genome are duplicated in clusters of 10 to 100, and recent work by Berezney's group, now at the State University of New York at Buffalo, and by Hiromu Nakamura and his colleagues at the Aichi Cancer Center in Nagoya, Japan, indicates that the nuclear matrix is needed to organize the replicon clusters. Berezney is also now able to pinpoint the coordinates for every single replication site. This may be useful, he says, in understanding how the nuclear architecture of normal and cancer cells differ, and how this may be related to the increased proliferation of cancer cells.

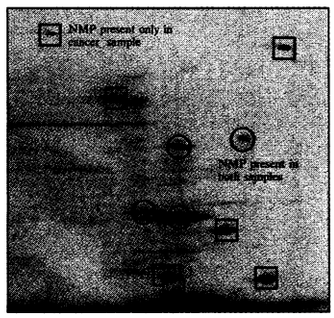
Impact beyond DNA

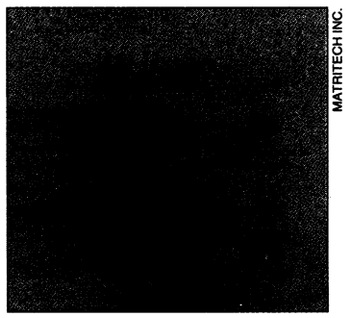
Of course, DNA replication is only one of the major biochemical reactions that takes place in the nucleus. The DNA of the genes also has to be copied into messenger RNAs, the necessary first step in protein synthesis. And since genes contain sequences that do not code for protein structure, these noncoding segments, called introns, must be spliced out of the mRNA molecules before they move out of the nucleus into the cell cytoplasm where protein synthesis actually takes place. And that's where the new work by the Lawrence group comes in, as it points to the locations in the nucleus where those reactions take place.

For reasons that aren't understood, after mRNAs are synthesized a long sequence of adenine nucleotides known as the poly(A) tail is added to one end. (Nucleotides are the building blocks of nucleic acids.) Since other forms of nucleic acids don't have a poly(A) tail, Lawrence and her colleagues reasoned that if they could find the nuclear localization of all the poly(A) tail-containing molecules, they would better understand the arrangement of mRNAs and the genes that encode them in the nucleus.

Looking for Cancer in Nuclear Matrix Proteins

Within the past decade, cell biologists have begun to work out the functional implications of the nuclear matrix, a network of structural proteins that permeates the nucleus and helps to maintain its shape. It also plays a role, they are finding, in regulating key reactions in the nucleus, such as gene activation (see main story). And further study of the matrix may lead to new methods of cancer detection and treatment as well. At least that's the optimistic view of officials at Matritech Inc., a small biotech company in Cambridge, Massachusetts.





MATRITECH INC.

Cancerous difference. Two-dimensional electrophoresis finds proteins in cancer cells *(left)* not seen in normal cells.

Already in the Matritech's pipeline are assays for detecting bladder, breast, colon, cervical, and several other cancers, which are based on findings that the protein composition of the nuclear matrix differs between cancer cells and their normal counterparts. The medical market for such assays is estimated at more than $1 billion per year, so it may seem surprising that, to date, Matritech has had the commercial nuclear matrix field mostly to itself. Part of the explanation for this is that for years researchers had essentially ignored nuclear matrix proteins (NMPs) because the insoluble proteins are extremely difficult to isolate and work with. "Until recently, most biochemists had thrown this stuff down the drain," says Graham Lidgard, vice president of product development at Matritech.

But while scientists have had trouble breaking the matrix down into its component parts, nature apparently achieves this with ease. In test-tube studies over the past few years, company researchers have been startled to find that as cells die, their nuclear matrix disappears and the matrix proteins are released into the surrounding fluids, whether this be in lab culture or in living animals.

This discovery opened up another line of inquiry: Researchers could compare the NMPs released by distinct types of cells to see if they were all the same or whether different cell types have their own distinct variants. Using monoclonal antibodies on cells in lab culture, Matritech has identified more than a dozen NMPs, many of them cell-specific. More important to Matritech's financial future, NMPs released by cancer cells differ from those released by normal cells of the same type. Indeed, in the latest issue of *Cancer Research*, a team from John Hopkins University School of Medicine, including Alan Partin and Donald Coffey, reports that prostate cancer cells carry a nuclear matrix protein not seen in normal prostate cells or even in "hyperplastic" prostate tissue that shows excessive cell growth but is still benign. Matritech hopes to detect proteins like that in simple blood or urine tests that use such standard lab procedures as two-dimensional electrophoresis to separate proteins according to their size and charge.

Cancer diagnostics based on that principle should begin clinical trials this year, according to Matritech officials, who believe their assays could be much more sensitive and specific than the few current tests on the market, many of which look for proteins found on a cancer cell's surface. Those assays, says Lidgard, often give too many false-positives, because the proteins they detect are released in conditions other than cancer. For example, one popular serum test to detect prostate cancer often turns up benign hyperplasia. Another cancer screening test, the Pap smear for cervical cancer, suffers from the opposite problem; the rate of false negatives is estimated at 25%. To tackle that problem, Matritech is developing an antibody assay for matrix proteins to supplement the traditional test that depends simply on observations of structural changes in cervical cells to diagnose potential cancers.

And that's not all: The company has just set up a new research group to begin exploring whether their nuclear matrix work could provide any new therapeutic strategies for cancer. It's still too early to know what those strategies might be, company researchers say, but they wouldn't be investing the money if they weren't optimistic. On the other hand, Matritech's scientific and financial success is far from assured. Cautions Shelia Taube, chief of the Cancer Diagnostic Branch at the National Cancer Institute: "I think [their tests] are reasonable approaches to try, but I don't think we have sufficient data at this point to say they will work."

–John Travis

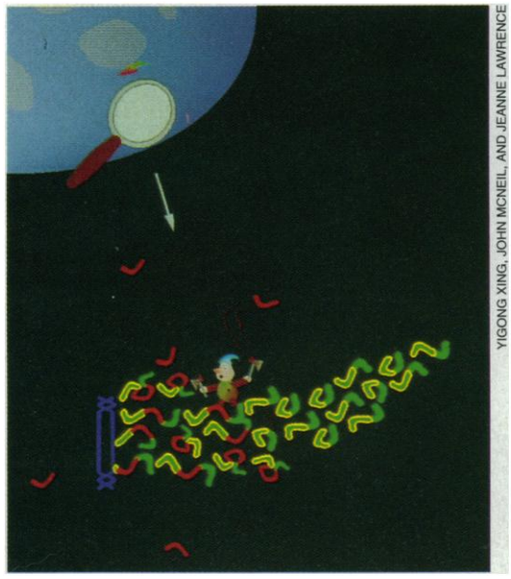
Their previous work had shown that poly(A) RNAs concentrate in discrete regions of the nucleus that also contain components of the mRNA-splicing machinery. Now, Lawrence and her colleagues, including Kenneth Carter and Fredric Fay, also at the University of Massachusetts Medical Center, have determined the three-dimensional arrangement of those regions, which they call "transcript domains," in the nucleus (also see page 1330). They proved to be arrayed, Lawrence says, in centers that lie in a single plane, just below the midline of the nucleus and some distance from the nuclear envelope. And what made that finding particularly significant was the identity of another molecule that the Lawrence group identified in the transcript domains.

Located in the core of each was a protein called SC35 that Tom Maniatis' group at Harvard University has found to be required for the assembly of the molecular machinery that splices the introns out of mRNA. Components of the splicing machinery had been shown by several groups, including David Spector's at Cold Spring Harbor Laboratory on Long Island and Nils Ringertz's at the Karolinska Institute in Stockholm, Sweden, to occupy discrete nuclear locations. But this is the first time that mRNA transcripts have been shown to be present at the exact same sites. The finding strongly suggests, Lawrence says, that the splicing machinery is assembled very near or in the same place where splicing actually takes place.

Suggests is one thing, of course; showing is another. Enter Yigong Xing, who with Lawrence and colleagues took a crucial second step, described in the Lawrence group's report on page 1326. In phase two, the researchers followed the path taken through the nucleus by the messenger RNA for a particular cell protein, called fibronectin, to determine where it was first transcribed and then spliced. The Lawrence group had previously shown that a viral mRNA made long tracks from the center of the nucleus to the periphery, and the researchers wanted to know if the cellular mRNA would make similar tracks. It did, although the tracks were somewhat less dramatic than those of the viral mRNA. What's more, in almost 90 % of the cases, the tracks either overlapped the transcript domains, or at least were in contact with them.

To ask directly whether the mRNA tracks form at the site where the fibronectin gene is transcribed, the researchers used probes that allowed them simultaneously to find the locations of both the fibronectin gene and its mRNA transcript. The result: The relatively tiny gene overlaps the mRNA track, usually near one end, showing that the mRNA was synthesized in one part of the track.

Then, the researchers asked whether the mRNA was also spliced along the track. To look at this, they used probes that could distinguish between the mRNA's introns and exons. By following the distribution of both, they were able to show that the exons are present along the entire track, whereas the introns are present only during part of it. "It is evident," says Lawrence, "that splicing must have occurred somewhere along the track." While more work is needed to define the precise connection between the tracks and the domains, it's clear that the mRNA metabolic machinery is integrated with nuclear structure, says Lawrence.



YIGONG XING, JOHN MCNEIL, AND JEANNE LAWRENCE

Cut-up. The upper left of the cartoon shows the nucleus with a transcript domain *(bright spot)* and an adjacent mRNA track. Below is a magnified view of the track where the axman is cutting out introns from the messenger.

Tomorrow's research

As elegant as these experiments are, they have merely posed a new question for cell biologists: Do the nuclear matrix or other aspects of nuclear architecture influence the organization or activities of the transcript domains seen by the Lawrence group? If so, then the architecture may play a role in regulating gene activation and mRNA synthesis, as it apparently does for DNA synthesis.

One clue that it might comes from neurobiologist Laura Manuelidis at Yale University School of Medicine. She has shown that the nuclear DNA is highly organized in nerve cells, even when they are "resting" and not getting ready to divide. If she tags a particular site of a chromosome, for example, she finds that it always occupies the same location in the nucleus at a given stage of development. She also finds that this organization changes in predictable ways as nerve cells mature. That suggests, Manuelidis says, that the architectural alterations may help control which genes are active at the different stages of nerve cell life, although at this stage of the research it's not known whether the matrix or some other factor is responsible.

Evidence suggesting that the nuclear matrix may influence what genes are turned on in different cell types comes, however, from Gary Stein's group, which is also at the University of Massachusetts Medical School. Genes are generally turned on by a complex of several protein "transcription factors" that have to act together. But many of the proteins that activate expression of the cell-type specific genes are found in extremely low concentrations in the cell, Stein points out. That means, he explains, that if they were distributed randomly, they would never meet each other to bind and form the required gene-activating complex. The matrix, however, could serve to concentrate and localize all the DNA to be transcribed as well as the proteins necessary for tissue-specific gene expression so that they can initiate gene expression.

In support of his view, he and colleagues Jane Lian, Janet Stein, and their associates have evidence, which appears in the 15 February issue of the *Proceedings of the National Academy of Sciences*, that a transcription factor needed for activation of the gene for osteocalcin, a bone-specific protein, becomes associated with the nuclear matrix of immature bone cells at just the time they turn the gene on. The finding suggests, Stein says, that attachment to the matrix may be important for the gene expression. In that event, the nature of the matrix-bound components would have to change throughout development, as Manuelidis and others have proposed. Stein is quick to point out, however, that matrix attachment may not be required for all gene expression. For some genes, the factors contributing to transcription may be found in such abundance that the services of the matrix may not be required.

Still, researchers are building a case that nuclear structure will have to be taken into account when it comes to understanding gene replication and expression, especially when gene control is complicated. During embryonic development, for example, 100,000 or more genes may have to be turned on or off. "We're pretty good at thinking about how individual genes are turned on and off," says Lawrence. "We're not as good at thinking about how the whole genome is coordinated." But that may well improve as the full picture showing how reactions taking place in the nucleus are linked to three-dimensional nuclear structure comes into sharper focus.

–Michelle Hoffman

Michelle Hoffman is an associate editor at American Scientist.