Mapping the Cell's Nucleus

Biologists are learning that the nuclear matrix—a mostly unexplored mix of insoluble molecules—may hold keys to how genes turn off and on and how they code for working proteins

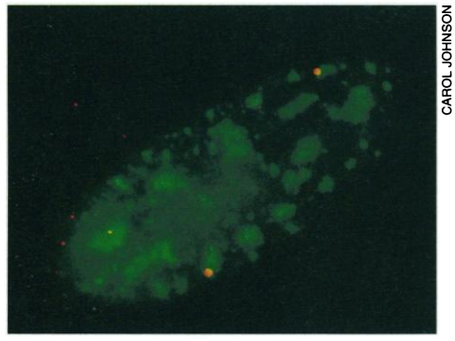
When James Davie started investigating the inner workings of the chromosomes, which carry the cell's genetic information, in the mid-1970s, he tossed some important clues down the drain. Davie, a biochemist at the University of Manitoba in Winnipeg, was studying chromatin—the DNA and protein mixture that forms the chromosomes. In the course of that work, he would extract most of the DNA and soluble proteins from the nucleus, where the chromosomes reside, and keep that material. Then, following traditional practice, he poured the insoluble fraction that remained down the sink. "We ignored that fraction for the longest time because we figured it's just nuclear debris," he recalls. "That was the feeling of the day."

But the day, and the feeling, have changed. Since the late 1980s, Davie and many other researchers who want to understand how the information coded in the genes gets converted into proteins needed to carry out the activities of the cell, have discovered that many of the molecules they're interested in were going down the drain along with that insoluble fraction. Now they view this material—a largely unexplored mix of insoluble proteins and RNA known as the nuclear matrix—as a treasure-trove of clues to the way genes work. Instead of throwing it out, they've begun sifting through it with painstaking care. "I got into the nuclear matrix because I couldn't leave it alone any longer," Davie says.

A growing number of cell and molecular biologists share this sentiment. One indication of the growing interest was a recent Keystone symposium,* the first international meeting ever devoted solely to the nuclear matrix. There, 150 attendees heard about ways that matrix-associated molecules appear to organize the transcription of active genes into RNA copies, the first step in gene expression, as well as about how matrix molecules prepare those RNA messages for the ultimate step, the actual synthesis of proteins. Other new research suggests that matrix molecules may even play a role in inactivating one of the two X chromosomes in mammalian females. Indeed, says cell biologist Gary Stein of the University of Massachusetts Medical Center in Worcester, "There's some elegant new work providing insights about the relationship between [the matrix] and gene expression."

Regulating gene activity

Matrix-associated molecules, in fact, appear to act at all stages of gene expression, beginning at the beginning, as they help turn genes on and off. At the meeting, Davie reported new results indicating that acetyltransferase and deacetylase, two enzymes located in the matrix, seem to play a crucial role in regulating gene expression by altering chromatin structure.



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Making a messenger. RNAs transcribed from some active genes *(red)* reproducibly localize to the periphery of discrete nuclear domains *(green)* enriched in proteins that splice out noncoding intron sequences.

In the chromatin, the DNA is coiled up with proteins called histones, which prevent it from being copied into the messenger RNAs (mRNAs) that direct protein synthesis. But Davie found that acetyltransferase helps the DNA unwind by catalyzing the addition of acetyl groups to certain histones in the chromatin. With extra acetyl groups attached, the chromatin resists being coiled by another histone called H1; as a result, the chromatin loosens up so that genes can be copied. Deacetylase, in contrast, removes acetyl groups from the chromatin, thereby making it easier for H1 to coil DNA and thus harder for genes to be expressed.

There are other players in the matrix besides enzymes, of course, and some of them also seem to be involved in regulating gene activity. One of the most intriguing is a particular type of RNA that's never translated into protein. Most nuclear RNAs are thought to be messenger molecules, which leave the nucleus and travel out to organelles called ribosomes where the proteins are synthesized. But some RNA remains in the nucleus, and some of this is also retained in the matrix fraction.

A team led by Jeanne Lawrence of the University of Massachusetts Medical Center in Worcester reported evidence implicating one such RNA, which is produced by the *XIST* gene, in the inactivation of the second X chromosome in the cells of mammalian females. One indication that it's playing this role, Lawrence says, is that "it's the only gene that's expressed on the inactive X and turned off on the active X." Another is the finding by Lawrence and Christine Clemson, also of the University of Massachusetts Medical Center, and Hunt Willard of Case Western Reserve University in Cleveland that the *XIST* RNA "basically paints the inactive X chromosome." Draped around the chromosome, they hypothesize, the RNA condenses and inactivates it. "*XIST* would be a precedent for a novel nuclear RNA with a role in chromatin structure," Lawrence notes. "It's a class of RNA that really hasn't been described before."

The finding is, however, consistent with previous discoveries about RNA and chromatin structure, says Massachusetts Institute of Technology (MIT) cell biologist Jeffrey Nickerson: "It's actually been known for quite a while that if you inhibit RNA synthesis, the chromatin in the nucleus collapses, suggesting that RNA may play some sort of structural role in the nucleus." What's more, he notes, the Lawrence team's finding suggests a function for some of the so-called "junk DNA," the great majority of the mammalian genome that is never translated into protein and has no other known function. Because much of this junk DNA is transcribed into RNA, Nickerson says, "we're proposing that some of that RNA is in fact structural and might have something to do with the architectural organization of chromatin."

Getting the message out

In addition to regulating gene activity, matrix molecules may also be involved in organizing the synthesis and splicing of RNA messages destined to be transported to the cytoplasm. When one of these mRNAs is first synthesized, it is a complete copy of a gene, including both the protein-coding se-

* The Keystone Symposium on "The Nuclear Matrix: Involvement in Replication, Transcription, Gene Splicing, and Cellular Regulation," was held from 4 to 10 April on Hilton Head Island, South Carolina.

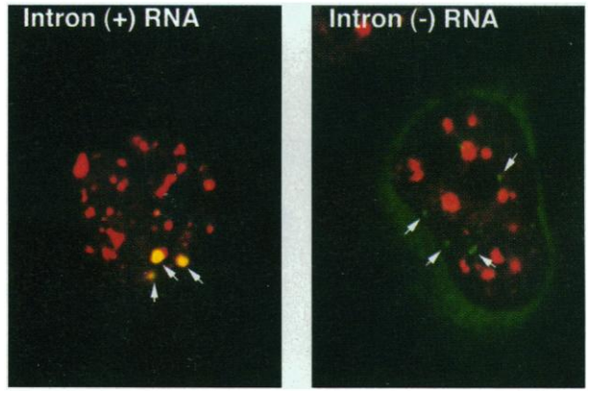
quences called exons and the noncoding ones called introns which must be spliced out before the mRNA reaches the protein-making machinery.

In 1993, Lawrence's lab found evidence suggesting that some synthesis and splicing take place in discrete regions of the nucleus that she called "transcript domains" (*Science*, 26 February 1993, p. 1330). Several groups have found that the domain material remains in the matrix fraction. And Lawrence and her colleagues also noted that the transcript domains seem to overlap with structures called interchromatin granule clusters (IGCs) that other groups have identified by electron microscopy. The idea that IGCs might be associated with transcript domains has been controversial, however: Other researchers have found little or no transcription in the IGCs, which are thought to be storage sites for proteins needed for splicing.

Despite this seeming discrepancy, Lawrence's team has continued to probe, and at the meeting she reported additional evidence for her hypothesis. Transcript domains, she's found, actually consist of two regions: A core area highly enriched in splicing components and a rim region that's crowded with newly synthesized RNA. And those rims are closely tied to the location of some active genes. Indeed, Lawrence's team, including Carol V. Johnson and Phil Moen at the University of Massachusetts Medical Center and Yigong Xing, now at Harvard, has examined the locations of 10 active genes and found that seven of them, along with their mRNAs, are associated with the domain rims. The team also looked at inactive genes and found no connection to domain rims. This has led Lawrence to suggest that transcription and splicing of some specific genes occurs in a rim around an inner core, stocked with splicing components, which likely corresponds to the IGCs.

A somewhat different view of these structures comes from work by David Spector and his colleagues at Cold Spring Harbor Laboratory on Long Island. Using electron microscopy to reveal fine structural details, they too found that the domains seem to encompass two different structures: the IGCs and other nearby masses known as "perichromatin fibrils" that may be transcription sites. Spector hypothesizes that, when a gene gets turned on, splicing proteins are recruited from a storage site such as an IGC and brought to transcription sites such as these fibrils. Then splicing takes place at or near that site, he believes.

Further evidence that gene transcription and RNA splicing are closely coordinated comes from Sui Huang, a postdoc in Spector's lab. When she transferred a gene containing introns into cultured cells, she found that 90% of the time splicing proteins localized at the sites where the gene was transcribed into RNA. "You'd expect that since those RNAs have to get spliced," Spector notes. In contrast, when she transferred an intronless gene into the cells, 85% of the time no splicing factors clustered at the gene's transcription sites. "This suggests to us that there's a very high degree of organization in the nucleus with regard to the distribution of RNA and factors involved in its splicing," Spector says. "That's an extremely exciting finding."



Splicing to go. In the cell at left, an RNA containing introns (sequences that don't code for proteins) groups with splicing factors *(gold regions)* that may remove the introns; at right, an RNA lacking these noncoding sequences *(green)* does not cluster with the splicing factors.

Adding to the excitement, an important family of these splicing factors has turned up in the matrix fraction. Phillip Sharp and Benjamin Blencowe of MIT, working with MIT colleagues Sheldon Penman and Jeffrey Nickerson, have isolated from the matrix fraction three of these proteins, known as SR proteins, that seem to be associated with mRNA splicing. At the meeting, Blencowe reported new results showing that these proteins appear to bind specifically to protein-coding exon sequences. One possibility, he says, is that during mRNA splicing, these proteins hold the exons in place so that they can be joined in the proper order as the introns are clipped out.

Searching for structure

Even though these results suggest that matrix proteins might be involved in organizing mRNA transcription and splicing, many questions about the matrix and gene expression remain unanswered. One particularly thorny issue concerns a proposal first made 20 years ago by MIT's Penman. Based on electron micrographs made by his team that revealed images of a filamentous web, he and Nickerson advocate the notion that a scaffold of matrix proteins serves as a nuclear infrastructure, keeping various molecules in their places and perhaps providing an architecture that organizes mRNA synthesis, splicing, and other functions such as DNA replication.

Early critics, however, dismissed Penman's images as artifacts of the harsh extraction methods used to prepare the matrix samples for electron microscopy. And even today, Penman and Nickerson agree that none of the protein or RNA components of those filaments have yet been identified.

That's led cell biologist Joseph Gall at the Carnegie Institution of Washington to argue that the matrix fraction may not even be involved in structural organization. While it makes sense that functions in the nucleus are organized, he says, he sees no evidence that the organizing principle resides in the matrix. "There are plenty of people who still wince when they hear the word matrix," he says.

Penman has heard such views many times before. The field has survived "an enormous amount of antagonism over the years in which an awful lot of people say there is no such thing as a matrix," says Penman. "I've listened to people curse the idea of the nuclear matrix from speakers' platforms." He firmly believes his thousands of micrographs of the matrix are clear proof of structure, even though they have not converted all nonbelievers.

Another nuclear matrix pioneer, Ronald Berezney of the State University of New York, Buffalo, believes that whatever the nuclear structure looks like, it must be fluid to serve the changing needs of the cell during development and replication. "I'm committed to nuclear organization but not to a fixed structure of any kind," says Berezney. "My idea has always been that the nuclear matrix is very dynamic and that what we isolate is something fixed in time, stabilized through the isolation."

Many newcomers to the matrix field have chosen to remain out of the fray, reserving judgment about nuclear structure for now. "The nuclear matrix is a material that you see when you biochemically extract something," says Sharp. "I remain to be convinced that it's the functional organizational unit of the nucleus. That's prudent skepticism." The question of structure will be harder to ignore when researchers are able to isolate filament proteins and their genes, mutate these genes, and look for changes in organization or activities inside the nucleus, he says. Until then, he and others plan to continue searching for functional molecules in the fraction, mining the matrix for clues to gene expression. For now, that's prudent, too.

–Yvonne Baskin

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