How the Nucleus Gets It Together

Cytological and biochemical studies both indicate that the synthesis of finished messenger RNAs requires a high degree of coordination among reactions in the nucleus

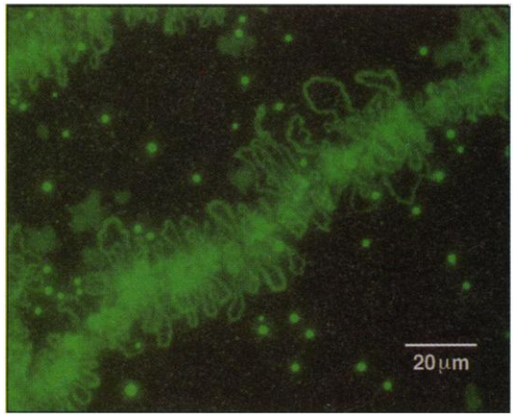
Much like a train station at rush hour, the nucleus seems chaotic at first glance—packed with a jumble of chromosomes, RNA molecules, and proteins. Yet, just as careful observation of a train station reveals an orderly flow of commuters to platforms at particular times, research over the past few years has revealed that the molecular movements needed to carry out one of the nucleus' main functions—the conversion of the information carried by the DNA of the genes into the messenger RNAs (mRNAs) that direct protein synthesis—are far more coordinated than had been suspected.

To produce an mRNA, a gene first has to be transcribed into a raw copy called a pre-mRNA. But to make the finished products, pre-mRNAs have to undergo certain "processing" reactions, such as removal of the noncoding sequences, called introns, that were copied directly from the gene. At one time, molecular biologists thought that these reactions could take place anywhere in the nucleus, wherever the RNAs and their associated molecules happen to meet up. Now, two converging lines of evidence are making it clear that the production and processing of RNA are closely linked in time and space.

Cytological studies, in which researchers observe cells to track the movements of the molecules involved, show that gene transcription occurs in very specific places, with intron removal and other processing reactions apparently taking place either at the same spots or very close by. And while there are still uncertainties about exactly how the transcribing and processing machinery get together, test-tube studies are bolstering the cellular observations by uncovering biochemical connections between key components of the two types of reaction. In particular, they suggest that RNA polymerase II (Pol II), the enzyme that copies genes into pre-mRNAs, also plays a critical role in seeing that the machinery that splices introns out of the pre-RNAs is assembled appropriately.

"What's really coming to the fore is [the need for] viewing mRNA synthesis as a whole," says David Bentley, a molecular biologist at the Amgen Institute, which is funded by the biotech company Amgen in Toronto. "It seems to be a highly integrated process." Indeed, the high level of coordination and organization prompts cell biologist Alan Wolffe of the National Institute of Child Health and Human Development to describe the nucleus as "very much like a Swiss watch."

Understanding how the gears in this nuclear "watch" mesh is essential, not just for understanding how mRNAs are formed but also for understanding the functioning of the cell as a whole. Recently, for example, researchers have shown that mechanical forces transmitted through the protein filaments of the cell cytoskeleton alter nuclear structures, possibly leading to changes in gene expression (*Science*, 2 May, p. 678). Because the proteins involved in RNA synthesis and processing may be among the structures rearranged, figuring out where and when they do their jobs may help provide a clearer view of how that cytoskeletal network works.



JOSEPH GALL

In the loop. Splicing factors (green) occur in granules and along chromosomal loops—the sites of transcription and, presumably, splicing.

What's more, there are indications that disorganization of certain nuclear proteins, including those involved in RNA processing, can lead to diseases such as a leukemia and spinal muscular atrophy, an often fatal genetic disease in which muscles waste away and the spinal cord degenerates. "From the point of view of cell biology and disease, it's important to understand the relationship between nuclear structure and function," concludes Angus Lamond, a biochemist from the University of Dundee in Scotland.

On to splicing

The "Swiss watch" picture of the nucleus began developing in cytological studies performed in the early 1980s. In one experiment, cell biologist Jeanne Lawrence and her colleagues at the University of Massachusetts Medical Center in Worcester found a link between gene transcription and the splicing of RNAs. They observed what appeared to be mRNAs within domains that correspond to small structures called interchromatin granular clusters (IGCs).

Those clusters had already been implicated in RNA processing, as previous research had shown that these clusters are packed full of splicing factors, proteins needed to cut the introns out of newly synthesized RNAs. Some newly made RNA was known to exist along the rim of these clusters, entwined with molecular tangles called perichromatin fibrils, but these researchers were the first to see it inside clusters.

The Lawrence team also went a step further. Using fluorescent-labeled nucleic acids to tag both a gene and its newly made RNA, they showed that the gene and the clusters are juxtaposed, and that transcription apparently takes place during this liaison (*Science*, 26 February 1993, pp. 1326 and 1330).

Results from cell biologist David Spector and his colleagues at Cold Spring Harbor Laboratory in New York have also fingered the IGCs, in particular their associated fibrils, as important sites for transcription and splicing. These researchers have a different picture of how the processes are coordinated, however. They think the clusters keep their distance from the genes, instead serving as storage depots and assembly sites for splicing factors that move to transcription sites to work on newly made RNA.

Spector and his postdocs Luis Jiménez-García and Sui Huang came to that conclusion in 1993 after putting RNA, either with or without introns, into cells with splicing factors labeled with fluorescing antibodies. When they then localized the RNAs by tagging them with fluorescent stains, they found that the splicing factors gathered around the intron-containing RNA but not around RNA lacking introns. "The splicing factors seem to be dynamic," Spector explains—capable of making their way from the IGCs to the genes.

Developmental biologist Joseph Gall of the Carnegie Institution in Baltimore saw a similar picture in the nucleus of the unfer-

tilized eggs of various amphibians, including the toad *Xenopus laevis*. Because these eggs are very large compared to the ordinary somatic cells other researchers are studying, it's much easier to distinguish one site from another in the nucleus, Gall says. "What people who study somatic nuclei argue endlessly about, we can answer with a quick look," he maintains.

Under the microscope, the oocyte's chromatin looks like a bottle brush in which the "bristles" are loops of DNA that extend out from the chromosome axis. And Gall says that when he tags the molecules he wants to track with labeled antibodies, he can see that both transcription and the RNA-processing components occur along these loops. Thus, he thinks RNA processing takes place there, not near the clusters, which are some distance away.

But perhaps the best evidence for splicing-factor migration comes from Spector and his postdoctoral fellow Tom Misteli, who have actually followed the movements of a splicing factor in living cells, not just in the dead, fixed cells used in previous experiments. For this work, the researchers merged the gene for green fluorescent protein with the gene for a splicing factor and then put this genetic construct into living cells. The fusion protein it produced glowed green, enabling the researchers to film the actual splicing-factor movements.

These images revealed that clusters basically stay put. But as the Spector team's previous experiments had indicated, splicing factors move out from the clusters and head to a transcription site, identified by subsequent studies in cells fixed right after these observations were made. The fluorescent green arms could be seen "extending out [from the IGCs], twisting and contorting," Spector explains. (These results appear in the 29 May issue of *Nature*.)

The Lawrence team, too, finds that certain genes don't move to the clusters. Last December at the annual meeting of the American Society for Cell Biology, Lawrence's collaborator Phillip Moen Jr., now at NEN Life Science Products Inc. in Boston, described how the team studied 14 genes to see whether they are transcribed at the site of a cluster, and they find that the answer varies from gene to gene. For example, the gene for the myosin heavy chain, which becomes active only in mature muscle cells, apparently moves close to the clusters of splicing factors as the immature muscle cell precursors differentiate. "Its position in relationship with the [clusters] changed in correlation with its activation," says Moen.

The story is different for the gene for dystrophin, the muscle protein that's defective in muscular dystrophy. Even though its RNA requires splicing, the gene "is a very dramatic case of something that appears to avoid the [clusters] like the plague," says Moen. The splicing factors must somehow move to its location. Moen does not know why the two genes differ in this regard. But the lesson from all this, Wolffe concludes, is that "what everyone is seeing may be true. It just depends on the particular gene."

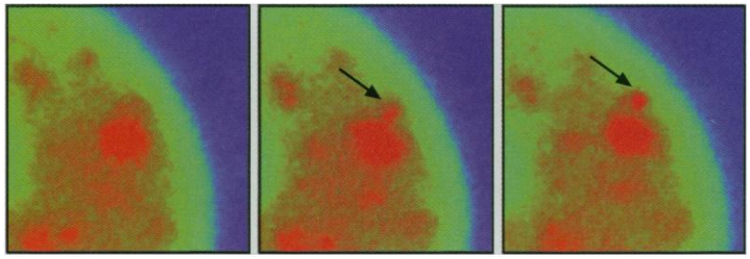
Linchpin polymerase

In spite of these differences about just how RNA synthesis and processing are choreographed, the conclusion that these events are linked is gaining further support from another quarter: biochemical data on the proteins involved. One is the RNA-synthesizing enzyme Pol II. Until a few years ago, most researchers had not considered any role for the enzyme beyond transcription. But in 1990, biochemist Jeffrey Corden of the Johns Hopkins University School of Medicine proposed that Pol II might be a key actor in RNA processing as well as in transcription. He noted that the last several hundred amino acids on the carboxyl end of the enzyme, a sequence known as the carboxyl terminal repeat domain (CTD), might be a loading site for proteins involved in processing RNA.

The CTD is rich in serine and threonine, amino acids that are potential targets for the addition of phosphate groups. In 1993, biochemist Arno Greenleaf of Duke University Medical Center in Durham, North Carolina, suggested that by taking on several phosphates, the CTD would become negatively charged and attractive to splicing factors that happen to be rich in positively charged arginine amino acids. Once attached, these factors would then have access to the newly formed RNA the enzyme was producing.

By 1995, Steve Warren, now a cell biologist at NeXstar Pharmaceuticals Inc. in Boulder, Colorado, had evidence supporting this picture. Using antibodies to tag both phosphorylated Pol II and the IGC proteins, he showed that much of Pol II and these proteins collected in large, rounded clusters when the cell was not actively transcribing genes. But these clusters seemed to break up when the nucleus became active, and Pol II and the proteins dispersed. These data suggested that Pol II and these proteins were working in concert, Warren says.

Likewise, Spector and Misteli had observed that in cells that are actively transcribing DNA, splicing factors are found both in the IGCs and at the transcription sites, suggesting they moved between these two places. But when they treated cells with a specific Pol II inhibitor, they could no longer see arms extending from the clusters of splicing factors, an indication that the transport of splicing factors had stopped. While Spector hesitates to assign Pol II a role in shuttling splicing factors, it's clear that the arms are important to their movement, he notes.



Nuclear dynamics. From left to right, these images show splicing factors (false-colored red) moving from a storage site (large dot) to a transcription site (small dot, arrow) over a period of 6 minutes.

T. MISTELI AND D. SPECTOR/COLD SPRING HARBOR LABORATORY

Other researchers have evidence that phosphorylation of the Pol II CTD helps the enzyme associate with splicing factors. In July of last year, for example, Corden and his colleagues reported that they had used a yeast screen to fish out molecules that interact with the CTD and had found four proteins with structures that suggested they were splicing factors. Then, when they used antibodies against the phosphorylated form of Pol II to extract the enzyme from cells, the enzyme came out in a complex with these proteins. These biochemical studies suggested these proteins and Pol II interacted in cells.

That suggestion was borne out a month later by Ronald Berezney of the State University of New York, Buffalo, and his colleagues. They used fluorescing antibodies to label both these proteins and the phosphate-laden Pol II in rat kidney cells and found that the two colocalized. And the association apparently does have functional consequences, because Corden's team found that antibodies against either the CTD or the splicing proteins inhibit splicing reactions in a test-tube assay.

All these data point to the CTD end of Pol II as having a role in the nucleus very similar to that of a dispatcher at a train station. It may help coordinate the movements necessary for transcription and RNA processing to occur. And just as commuters might eventually be able to find their way to the right train without being told where to go, splicing factors may still link up with their target RNA without Pol II. "But what [Pol II] may do is greatly accelerate the rate of splicing," Corden suggests.

He and his colleagues all agree that much more needs to be learned about the traffic patterns in the nucleus and about the interactions of the many proteins involved in

making RNA. But the convergence of the biochemical and cellular results is finally making sense of the cellular train station.

–Elizabeth Pennisi

Additional Reading

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PLANT SCIENCE

Making Plants Aluminum Tolerant

Among all the high-profile pests and blights that plague the world's agriculture, there is a culprit less well known: aluminum. The most common metal in soils, aluminum is a problem on 30% to 40% of the world's arable lands, where acid soil releases aluminum ions into the ground water. Indeed, for some important crops, such as corn, it is second only to drought as an impediment to crop yields, reducing production by up to 80%. Now, Mexican researchers have come up with a possible genetic-engineering fix.

On page 1566, molecular biologist Luis Herrera-Estrella and his team at the Center for Research and Advanced Studies of the National Polytechnic Institute in Irapuato, Mexico, report that they were able to make tobacco and papaya plants aluminum tolerant. They did so by genetically engineering them to pump out citric acid from their roots. This organic acid ties up aluminum ions in the soil, preventing them from entering and damaging the plants' roots.

Crop researchers using traditional plant-breeding methods have boosted the aluminum tolerance of some food crops, notably wheat. But they would like to have a gene for aluminum tolerance that they could introduce into a wide variety of crop strains already bred for high yield and pest resistance. Herrera-Estrella's work is "a powerful first step" toward that goal, says Leon Kochian, who studies aluminum tolerance in crop plants at the U.S. Department of Agriculture's (USDA's) Agricultural Research Service laboratory in Ithaca, New York.

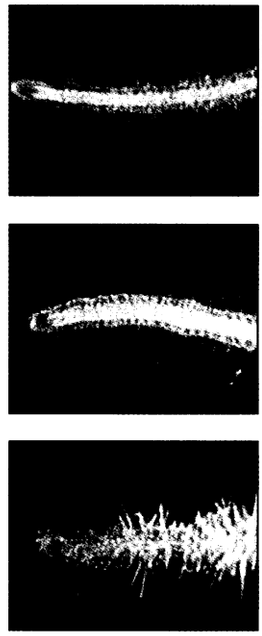
Farmers around the world would reap the benefits of such a gene, but the payoff would be especially high in developing countries. Although aluminum is present virtually everywhere, in nonacid soils it is locked up in insoluble compounds. But in acidic soils, which are most common in the tropics (where heavy rains leach alkaline materials from the land), the aluminum becomes soluble. It can slip into the cells of plant roots, where it poisons cell metabolism and prevents healthy root growth.

One solution is to plow lime into the soil, but lime must be added every few years, can treat only the top layer of soil, and is too expensive for many farmers in developing countries, says plant geneticist Shivaji Pandey, who directs the aluminum-tolerance breeding program for corn at the International Research Institute for Breeding of Maize and Wheat in Mexico City. As a result, farmers in much of the world settle for poor crop yields on acidic soils. The toll is huge: Strains of corn that would yield 10 tons per hectare in neutral soil may produce only 2 tons in acidic soils, says Pandey.

In their effort to create aluminum-tolerant crops that could boost these yields without extra cost, Herrera-Estrella and his colleagues built on prior work by Emmanuel Delhaize and his colleagues at the Commonwealth Scientific and Industrial Research Organization in Canberra, Australia. Delhaize's team found that some naturally aluminum-resistant plant strains have roots that secrete citric or malic acid, which binds to aluminum and prevents it from entering the roots. Several labs are chasing down the mutant gene responsible for the acid secretion, but Herrera-Estrella decided to take a different approach. "It occurred to us that because organic acid biosynthesis is a general phenomenon," he says, "we could use genes from other organisms to produce organic acids in plants."

The organism the researchers turned to was the bacterium *Pseudomonas aeruginosa*. They introduced the bacterial gene for citrate synthase, the enzyme that makes citric acid, into two plant species: tobacco, a popular plant for laboratory work because it is easy to transform with foreign DNA, and papaya, an important crop in tropical Mexico that is highly sensitive to aluminum.

The gene transfers had the desired effect. The plants carrying the citrate synthase gene secreted five to six times more citrate from their roots than control plants did. And that extra citrate translated into aluminum tolerance: The citrate-producing plants could grow well in aluminum concentrations 10-fold higher than those tolerated by control plants. "That is a significant increase," says Kochian.



LUIS HERRERA-ESTRELLA

Invisible shield. In an aluminum concentration that causes malformed roots in wild-type tobacco plants *(bottom)*, the root of a genetically engineered plant *(middle)* grows as normally as one not exposed to aluminum *(top)*.

This degree of tolerance could well allow some crop plants to be planted where they couldn't grow before. For example, Mexico's papaya crop, estimated at $97 million a year, comes from 20,000 hectares of land in the tropics, says Jose Garzon of the National Institute of Forestry, Livestock, and Agricultural Research in Celaya, Mexico. That crop could be expanded, he adds, if the new aluminum-tolerant plants could be grown on some of the 3 million hectares of tropical Mexican land where aluminum toxicity has prevented papaya cultivation.

With tobacco and papaya as a first step, Herrera-Estrella's team has recently moved on to put the citrate synthase gene into two major food crops: rice and corn. They found that the engineered plants make extra citrate, although the results aren't in yet on whether they are aluminum tolerant.

Still, all the work so far has been in the lab, and Kochian and plant physiologist Michael Grusak of the USDA laboratory in Houston caution that the transferred gene may impose physiological costs on the recipient plants; those could show up in the field and offset some of the benefit. All that extra citrate the plants are churning out means that "fixed carbon ... is lost from the plant," Kochian says. And that, he says, will impose an extra energy demand that could reduce the plants' productivity. "It is a trade-off," says Grusak, but one that might prove well worthwhile in areas where aluminum takes a serious toll.

–Marcia Barinaga