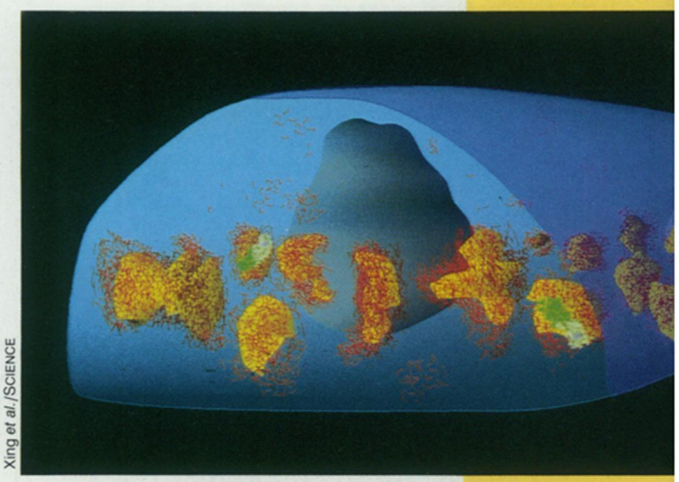
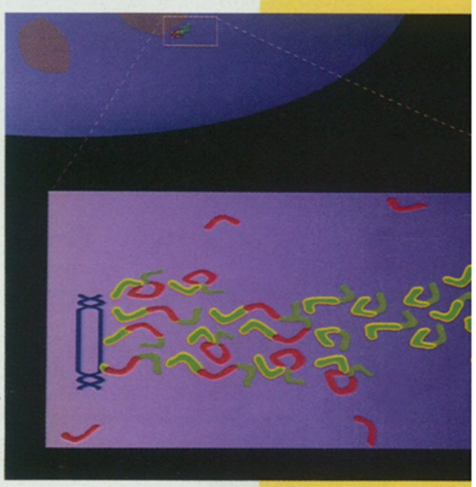
Tracking R-N-A

Scientists finally glimpse the sites of RNA processing

By ELIZABETH PENNISI



Xing *et al.*/SCIENCE



Carter *et al.*/SCIENCE

Deep within a cell lies the nucleus, the forum for the legislation of life. Here, information-laden DNA issues dicta about how the cell should develop and function. Then a supporting staff of proteins and other molecules carries out, vetoes, amplifies, or even rewrites these orders. It's a closed-door meeting, however, and the participants reveal little about the particulars of the process.

Yet just as reporters probe secret legislative events, biochemists over several decades have gleaned important insights into the chemistry of these nuclear sessions. Now, with the help of a variety of sophisticated imaging techniques, cell biologists can glimpse DNA and its staff in action. What biologists have begun to realize is that seemingly haphazard interactions among these molecules are actually carefully staged events that may help determine what genes get expressed in a given cell.

"There was a feeling that everything is soluble and floats around freely [inside the nucleus]," recalls Donald S. Coffey, a biochemist at Johns Hopkins University School of Medicine. "It turns out that things are quite organized."

For several years, researchers at the University of Massachusetts Medical Center in Worcester have been sneaking peeks at RNA, single strands of nucleic acids that relay genetic information encoded in the DNA to the rest of the cell. "RNA is the manifestation of gene expression," says Jeanne Bentley Lawrence, the geneticist who heads this research group. All human cells contain the same complement of genes, but only about 5 percent of these genes are active in a particular cell. Lawrence suspects that spatial arrangements inside the nucleus may help determine what genes a cell expresses, or uses.

Biochemists have known for some years that inside the nucleus a section of the double-stranded DNA untwists and unzips to expose a gene's code. The unzipped sequence of DNA then serves as a template for building a matching strand of RNA. The RNA assembles along the open section and then peels off in a process called transcription. That raw RNA then undergoes several transformations. A tail of adenine bases attaches to one end of it. Enzymes cut out nonessential codings (introns) and splice together the remaining bits of vital information (exons). The linked exons make their way as messenger RNA to the edge of the nucleus and exit through pores in the nuclear envelope.

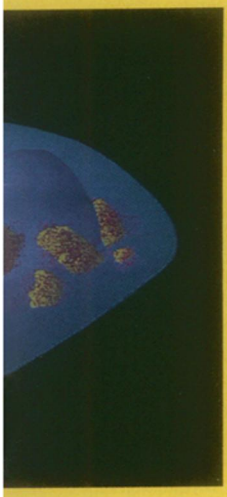
Lawrence's lab has filled in some details of this process for a particular type of RNA. In two reports in the Feb. 26 SCIENCE, the researchers confirm that RNA processing occurs at discrete locations within the nucleus and describe how they pinpointed this activity by using a variety of fluorescent tags for RNA, DNA, and certain proteins.

In one report, cell biologist Kenneth C. Carter and his colleagues at the University of Massachusetts Medical Center used a technique called digital imaging microscopy to generate a three-dimensional picture of the nucleus. They confirmed the existence of discrete RNA processing centers, which they call transcript domains. In a second report, graduate student Yigong Xing and her colleagues describe several experiments in which they tracked RNA made by two genes, one carrying the code for a nerve-cell chemical called neurotensin and the other coding for a structural component of cells called fibronectin.

Their images show scientists where RNA processing occurs. "It is certainly a gigantic step in understanding the highly organized nature [of the nucleus]," comments Coffey. "It's not a swimming pool of spaghetti; it's a highly organized machine."

Earlier work had suggested the existence of organization in the nucleus. During the 1970s, Coffey's group found evidence that the nucleus contains an internal skeleton that might help arrange other nuclear components. Other researchers observed that certain fluorescing antibodies create distinctive patterns of speckles inside a nucleus, indicating the existence of clusters of certain proteins. While some researchers argued that the speckle patterns arose only because of the staining and preserving processes used during experiments, others interpreted the patterns as indications of internal structure. A few scientists exploring the inner cell thought that active genes — and the RNA they make — concentrate at the inner edge of the nucleus' envelope; from there, it would be just a short hop to the outside for RNA. Later, David L. Spector and Sui Huang, cell biologists at Cold Spring Harbor (N.Y.) Laboratory, discovered that messenger RNA from a gene called *cfos* tended to be associated with these patterns of speckles.

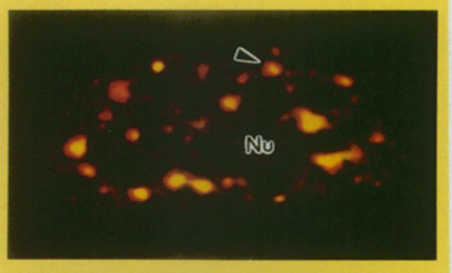
Lawrence's group decided to look more closely at the speckles. To do this, they worked with Fredric S. Fay, also of the University of Massachusetts Medical Center. Fay is an expert in digital imaging microscopy, which depends on mathematical tricks to get a three-dimensional image. The research team used the mi-

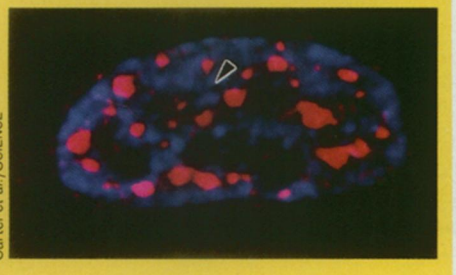


Computer graphic shows how RNA (red) gathers near transcript domains that include SC-35 proteins (yellow). The exons (green) and introns (white) of RNA and the gene (lavender) that codes for that RNA also occur near these domains. Also seen, the nucleolus (dark gray/purple).

Computer model of RNA-processing tracks close up: DNA (blue) codes for both introns (red) and exons (green with yellow outline) in RNA, but introns are later spliced out.

Restored images reveal placement of fluorescing labels along a 1-micrometer-wide plane through the nucleus. Top: RNA with adenine tail (red) overlaps with SC-35 at yellow spots. Bottom: In places, labeled DNA (blue) overlaps (pink) with this same RNA (red). (Nu shows nucleolus positions.)





Carter *et al.*/SCIENCE

croscope to observe specific molecules bearing fluorescent tags.

The scientists attached the tags in two ways. To track a key protein called SC-35, they treated the cells with an antibody that seeks out only that protein. The antibody carries with it a molecule that, in turn, glows only when exposed to a specific wavelength of light.

The researchers lit up RNA and DNA by means of a process called *in situ* hybridization. This technique takes advantage of the tendency of DNA and RNA to "zip" with matching sequences of nucleic-acid bases. By putting into the nucleus fluorescing sequences that pair up with the DNA or RNA sequences they wanted to study, the researchers could make those sequences visible. Various chemical tricks enabled them to label just the DNA, just the RNA, or even just the RNA during a particular stage of its processing.

When Carter labeled a type of RNA called polyadenylate-RNA and the SC-35 proteins, he not only saw speckles – the RNA-processing transcript domains – but he also could identify their precise locations. "It's a very in-depth, three-dimensional study of how the transcript domains are distributed in the nucleus," Lawrence says.

Between 20 and 40 clusters arrange in a horizontal plane just below the midline of the nucleus. They do not touch the edge of the nucleus, nor do they connect directly to one another. Thus, the results suggest that, contrary to previous suggestions, these speckles do not help transport RNA to the edge of the nucleus. Nor do they represent a continuous structure that stretches across the nucleus, Lawrence says.

At the same time Carter was developing this global picture of RNA's distribution in the nucleus, Xing was focusing on much smaller structures within the nucleus, called tracks. In 1989, Lawrence and her colleagues had observed that viral RNA in a cell nucleus aggregated into specific patterns, or tracks, that resembled bolts of lightning. She, Xing, and their Massachusetts colleague Carol V. Johnson then sought and found tracks for the cell's own RNA. Although the tracks proved less dramatic than those seen for viral RNA, they were nevertheless distinctive, says Lawrence. Somewhat linear patterns formed with fibronectin's RNA and more shapeless ones with neurotensin's RNA.

The researchers then looked more closely at these clusterings by tagging the fibronectin DNA and various forms of RNA with different fluorescing labels. They used one color for the exons and another for the introns. These tags also made unprocessed RNA – which contains both introns and exons – visible in either color.

To monitor the positions of these various RNA forms and the DNA, Lawrence's group used special filters that enabled them to see two colors at the same time in their fluorescence microscope. In addition, they took pictures with a special camera.

The exon tag lit up a longer track than the intron tag. This result both surprised and pleased the researchers. "In this track, there was a spatial separation of the intron-containing RNA and the exons," Lawrence says. "We conclude that splicing – removal of the introns – is occurring there."

Next, the team tagged the gene and the RNA with different labels. In almost 90 percent of the cells studied, they observed the gene as a pinpoint of fluorescence at the same place as the track, Lawrence says. They also saw that individual tracks lay close to the larger transcript domains.

"It shows where an individual gene and the RNA coming off that gene are, relative to other fundamental structures in the nucleus," Carter says. Thus the tracks appear to be molecular assembly lines. "It's both the site of transcription and the site of splicing of introns and exons," he adds. "It's a sequence of steps that occur bang, bang, bang – all in one big complex."

All these results may hint at the mechanism by which just a few genes get expressed at any one time. Scientists want to understand how and why RNA from a particular gene gets made, processed, and then shipped out of the nucleus, because that RNA helps give each cell type – nerve, skin, muscle, or bone cells, for example – its particular identity. "Some broader implications have to do with how these [RNA-processing] centers relate to the functional organization of the genome," Carter says. "Perhaps in some way the gene has to be physically next to these centers to be expressed."

However, both Lawrence and Spector caution that researchers must still determine whether all, or even most, RNA is processed this way. There may be multiple processing routes, they note. Preliminary data from Lawrence's group already bear out this idea. In fact, the team has begun looking at other types of RNA. "Some of them, but not all of them, associate with the domains," Lawrence says.

The researchers at the University of Massachusetts Medical Center are quite pleased with their progress thus far. "It wasn't easy data to come by, by a long shot," says Carter. "To have all the mechanics of the microscope all work at the same time, all the mathematics work at the same time, and all the visualization techniques working at the same time, and to do it on enough cells to have really significant data – it was terribly technically frustrating at times.

"It's not for the weak-hearted." □