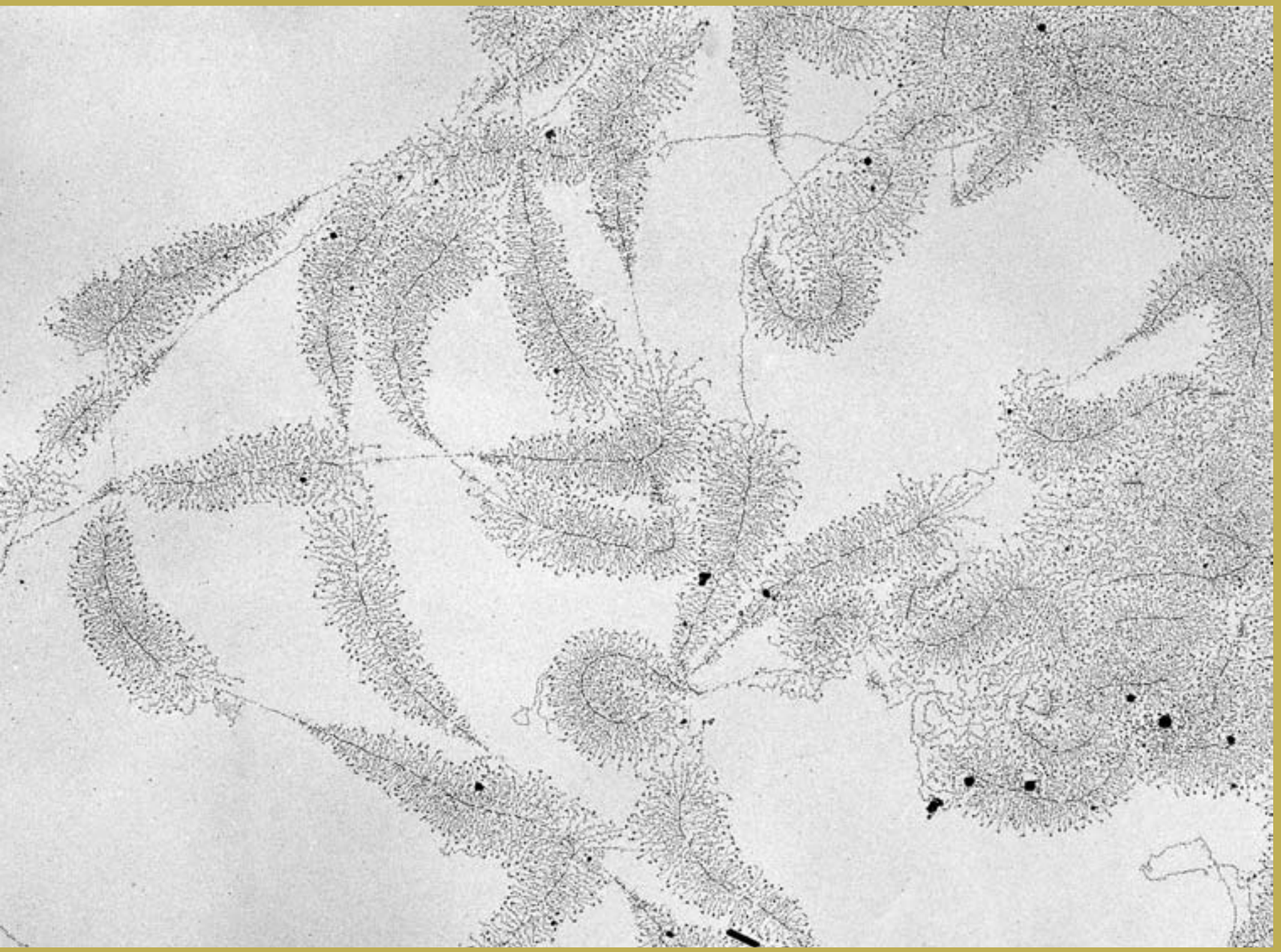

Genes coding for ribosomal RNA form the "trunks" of Christmas tree–like arrays in the first electron micrograph showing DNA in the act of transcription. The genes, isolated from the nucleus of a spotted-newt oocyte, are tandemly arranged with similar polarity along a single, unwound DNA molecule. Transcriptionally inactive DNA segments serve as spacers separating adjacent genes, which are visible because 80 to 100 RNA polymerases (black dots along each gene axis) simultaneously transcribe each gene to form rRNA precursor molecules, coated with associated proteins, which appear in successive stages of maturation (from the short chains at the "top" of the Christmas tree to the full-length chains at the end of each gene). This micrograph (27,500X magnification) appeared on the cover of *Science* on May 23, 1969, accompanying Miller, O. L., Jr., and Beatty, B. R, Visualization of nucleolar genes, 164:955–957. (Reproduced by permission of the American Association for the Advancement of Science.)

Felice Frankel

In the mid- to late 1960s, Oscar Miller and Barbara Beatty developed a technique at Oak Ridge National Laboratory to "see" genes during transcription. Their technique was later used to obtain information about structural aspects of genetic activity and genome replication in a wide variety of cell types, from human to bacterial. Dr. Miller, now Professor Emeritus in Biology at the University of Virginia, and I recently had some wonderful conversations about this remarkable visualization achieved years ago.

F. F. Before we get to the question of how you actually made the image, I wonder if you remember your reactions when you finally saw the results for the first time?

O. M. Oh yes! I can vividly remember finding the right area of the carbon film in the electron microscope and focusing on it. There they were! The genes for ribosomal RNA spread out in all their glory. I leaned back from the scope with a chilling thrill, clasped my hands on the back of my neck, closed my eyes and said to myself, "We finally did it." Then, I immediately thought, "Take a picture quickly before the film breaks. No one will ever believe we have done this unless we have pictures!" And then, of course, there was the thrill of telling Barbara to come see the genes!

F. F. So tell us, how did you make the sample?

O. M. Biochemical evidence from other labs indicated that the compact nucleoli of cell nuclei contain rRNA genes coding for the molecules that are involved in the structure of the protein-synthesizing apparatus of all cells. In our attempts to visualize genes in action, Barbara Beatty and I were fortunate to be working with the best cell type available, the amphibian oocyte—in this case, a newt oocyte. Unlike most cell types, which have one or more nucleoli attached to specific chromosomes, the very large amphibian oocyte nucleus typically also has hundreds of extrachromosomal nucleoli. To visualize the rRNA genes we first manually isolated the contents of the oocyte nucleus using jewelers' forceps, then carefully removed them with a pipette and placed them in a small drop of distilled water. The nucleoli rapidly came apart, allowing the genes to disperse from their normal compact state. Next we whirled the sample with fixative in a microcentrifugation chamber in order to deposit it on a thin carbon support film for the electron microscope specimen grid.

Two problems arose. Very thin carbon films were used to support our specimens in the EM beam. However, the surfaces of carbon films are typically very hydrophobic, so our specimens precipitated when dried on the untreated film. To prevent this, we made our support film hydrophilic. The other problem was surface-tension distortion during drying. We solved this by carefully rinsing the grid that held the partially attached, dispersed nucleolar genes (after centrifuging) with a very dilute solution of Kodak Photo-Flo, a surface-tension reducer used in photography. This allowed the genes to dry straight down and completely attach to the support film surface in two dimensions without any surface-tension distortion. Finally we stained the sample, attaching heavy-metal ions to increase its electron density in the electron beam. This was sort of the "icing on the cake" step.

F. F. Were you concerned that the process of making the various samples for these new imaging investigations might produce artifacts and that you were misinterpreting what you were seeing? Did anyone ever raise questions about your technique?

O. M. In the Dark Ages of our labors, when preparations were precipitated or distorted by surface tension, there were a number of disbelievers. But when the two "magic" steps, plus the "icing the cake" staining, were put together, the results looked so similar to what one might expect from all the biochemical evidence available that the disbelievers suddenly disappeared. Of course, as soon as one disturbs the normal living state of a cell, artifacts are always introduced. Knowing this, we very carefully monitored the results of each step with either phase-contrast light microscopy or electron microscopy as appropriate. So we felt confident that we could extrapolate from our final "artifacts" back through each preparative step to draw useful conclusions about what was going on in the nucleoli of living cells.

Felice Frankel is a science photographer and research scientist at the Massachusetts Institute of Technology and author of Envisioning Science: The Design and Craft of the Science Image. *Address: c/o* American Scientist, *P.O. Box 13975, 99 Alexander Drive, Research Triangle Park, NC 27709-3975. Internet: felicef@mit.edu*

Resource: Making Science and Engineering Pictures: A Practical Guide to Presenting Your Work

Felice Frankel