



RESEARCH PROFILE

David L. Spector

A journey into the nucleus

Molecular biology is a young science — so young that many of today’s leading researchers have had a hand in the discoveries that shape the areas in which they specialize. Professor David L. Spector of Cold Spring Harbor Laboratory is one such scientist.

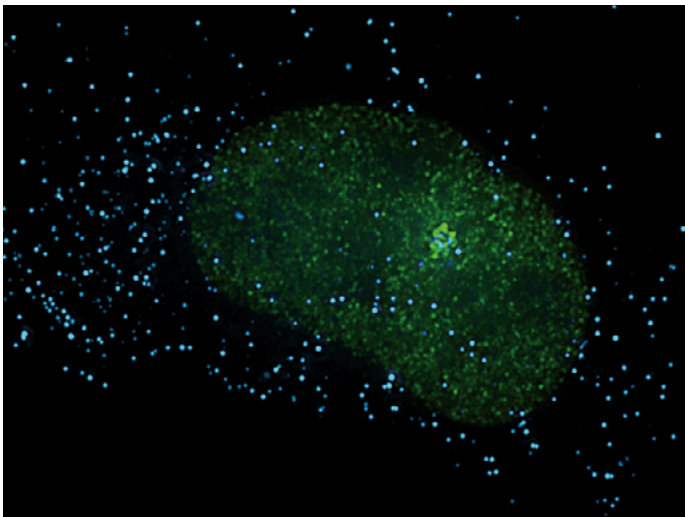
Spector is an enthusiast. To hear him relate a story of discovery in which he and his team have been involved is to be swept up in a tale of suspense, for he tells these stories as if they were mystery thrillers. And, although he probably doesn’t realize it, he has the winning custom of discussing the various molecular actors in experiments as if they were people — *dramatis personae* — rather than submicroscopic proteins and nucleic acids.

Spector, who in addition to heading his own lab group holds the demanding position of Director of Research at CSHL, is one of the foremost authorities on the cell nucleus. As depicted on the facing page, the nucleus is almost like a cell within a cell. It’s the command center where the genetic material — DNA, the stuff of the genome — resides, and where perhaps the most important activity in all of biology takes place. It’s where a variety of tiny molecular machines copy information encoded in the double helix to single-stranded “messenger” molecules made of RNA.

Many of these messenger-RNAs exit the nucleus through tiny pores and migrate to structures in the cell called ribosomes, where they serve as templates for the manufacture of proteins. Proteins are the incredibly diverse class of molecules that do the work and, indeed, form the very structures of the cells and organs of living things.

Spector’s lab was the first to visualize this central process — referred to rather dauntingly as “the central dogma of molecular biology” — as it actually unfolds in live cells. In 2004 they released time-lapse movies to the world showing how the expression of a single gene cluster led to the production of RNAs, which, upon leaving the nucleus, were processed by ribosomes to generate proteins that migrated to cellular organelles called peroxisomes.

At the time it was a technological *tour de force*, and over the years, with updates reflecting new technologies, the system he and his team developed to make it possible



Visualizing gene expression: active genes (green cluster) spur production of proteins targeting peroxisomes (blue).

has continued to be useful in labs around the world. But the pace of discovery in biology is relentless, and today, less than a decade later, both Spector and his field have moved, in a real sense, a world beyond the central dogma. The world of their science now reveals how that dogma (DNA → RNA → protein) does not, after all, describe the fate of many expressed genes in the nuclei of our cells. Alas, many RNAs are *not* messages carrying instructions for protein synthesis. But what *do* they do?

A busy, beautiful world

“What most non-scientists don’t know,” Spector says, “is that there’s a lot more to the cell nucleus than DNA.” Much of his career has been devoted to characterizing and learning about how various structures — “nuclear bodies” — discovered inside the nucleus perform a variety of functions. As Spector’s career has progressed, the nucleus gradually has been revealed, in part by his own research, as a world within the world of the cell — complex, beautiful, dynamic, and very, very busy.

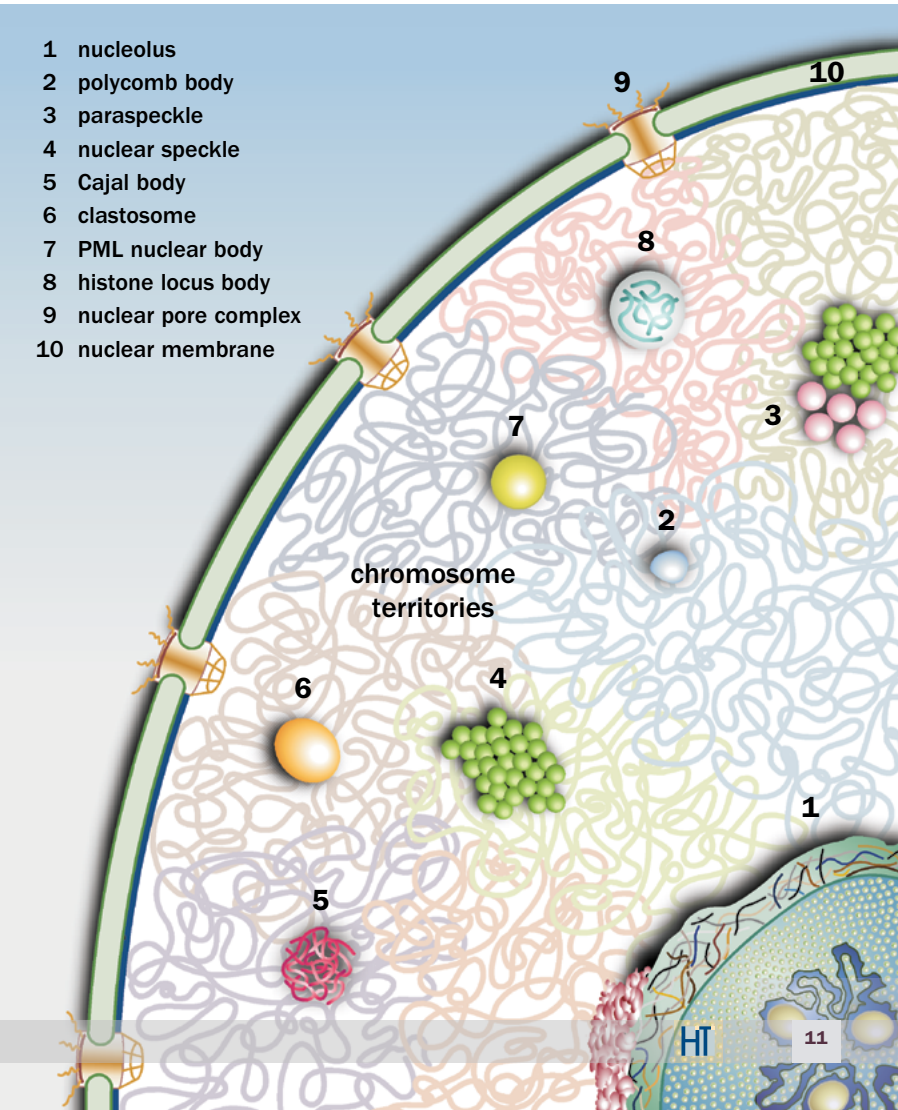
A native New Yorker whose interest in biology was piqued by a City College professor who taught him how to use an electron microscope, Spector became fascinated in a kind of algae called dinoflagellates. By the time he finished his doctoral research at Rutgers University, was nearly ready to publish what proved the first textbook on the tiny marine organisms, responsible for red tides and harmful algal “blooms.” By the early 1980s Spector had transferred his attention to mammalian cells, and specifically, to the process by which RNA messages carrying the genetic code are spliced — edited — by fleets of specialized proteins inside the cell nucleus.

At the Baylor College of Medicine, Spector in his first academic appointment headed a group looking at these proteins, called splicing factors. Initially, using antibodies derived from

people with autoimmune disorders, it was possible to examine how splicing factors localized in the nucleus and to study their relationship to specific nuclear bodies. “For me, this was the *entrée* to trying to correlate structures in the nucleus with their functions,” Spector explains. Ever since, this has been the central theme of his work. He carries this part of his work forward using super-resolution imaging approaches.

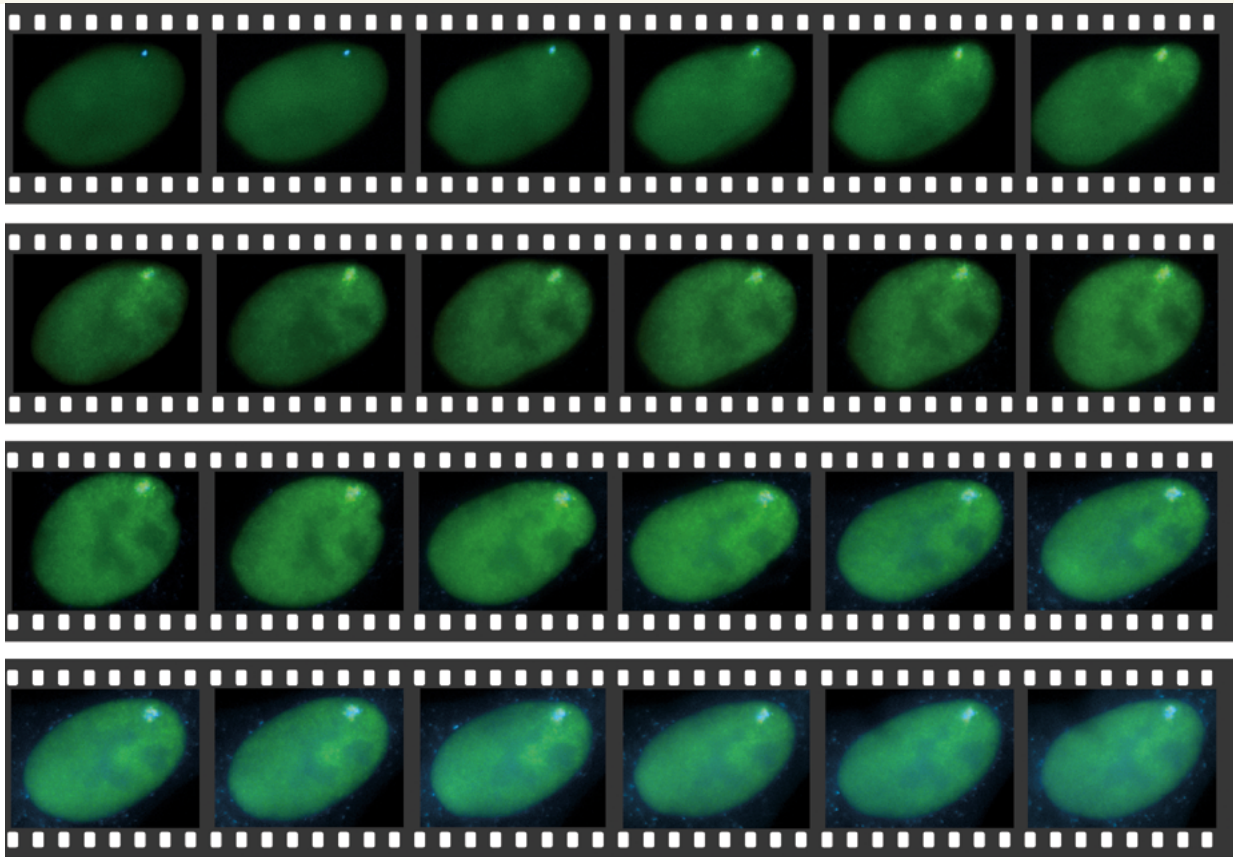
Not long after arriving at Cold Spring Harbor Laboratory in 1985 — hired over a luncheon with then-Director Jim Watson (“who ended the meal by asking, ‘When can you start?,’ which I have to say was pretty cool!”) — Spector soon collaborated with another new hire, Adrian Krainer, and future Nobelist Richard Roberts on projects relating

The cell nucleus is a world within the larger world of the cell (whose watery cytoplasm, here shaded blue, surrounds it). The nucleus is bound by a double membrane and contains, at various times in the cell cycle, up to 10 nuclear bodies. But most of the nucleus is a ‘sea of chromatin’ — chromosomes containing the genome and the proteins that pack them. Chromosome territories often overlap to facilitate gene expression.





Spector's real-time visualization system clearly marks 'locus' of a switched-on gene (blue dot). Locus grows in size as the DNA 'decompacts,' so as to be accessible to machinery that copies its 'message' to RNA. Green background color changes as proteins are recruited from the nucleus to take part in the process.



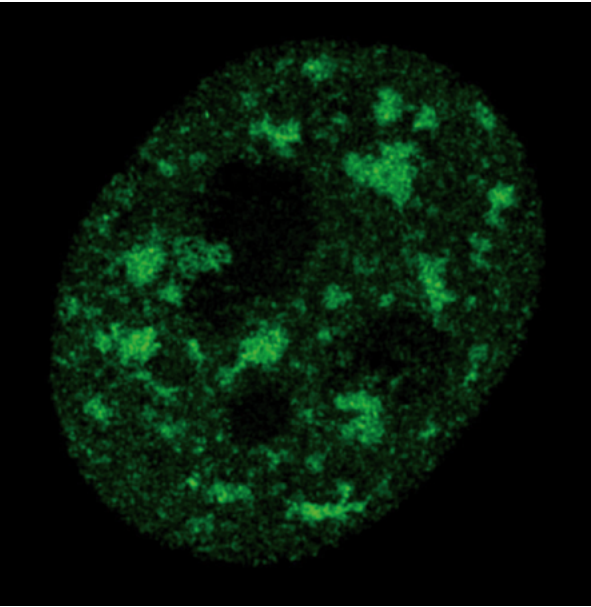
to the splicing machinery. Gradually, Spector's focus turned to the broader machinery involved in gene expression.

Two technological advances, the introduction of GFP, or green fluorescent protein, and the use of a bacterial DNA sequence called the lac operator array, made it possible by the late '90s for Spector to make visible both genes and their products, in a novel way. The visualization systems his lab designed were "inducible": by providing a drug, one could switch on what amounted to "a designer gene," and watch what happens in real time.

### Movies 'worth a million words'

One thing that happens is a process called decompaction, in which tightly bundled DNA becomes much more loosely packed, making it possible for specialized protein complexes to attach to the DNA and initiate the process of gene expression. Spector, who also directs the Microscopy Shared Resource at CSHL, has always maintained a keen interest in techniques of visualization, reminding us that "you will find things in continuous imaging, i.e., in 'movie' form, that you can't detect and often cannot imagine from fixed-cell studies." If a static picture is worth a thousand words, he says, "a movie is worth a million."

Spector's live-cell imaging work helped him realize that there is a good deal more plasticity in the structure and positioning of nuclear components than he was at first willing



Green areas show distribution of nuclear speckles in a single nucleus. These bodies store and assemble splicing factors that 'edit' RNA messages.

to believe. This proved "one of the most unexpected findings in my career," reminding him how "plasticity is actually a good thing, enabling biological systems to respond to a wider range of conditions than if they were rigid or fixed and providing them with an evolutionary advantage."

Research in the years since the "gene expression" movie was made has shed light on the varied functions that nuclear bodies perform. Spector has become keenly interested in the possibility that they may serve as prognostic or diagnostic markers in disease. His interest in nuclear bodies, moreover, intersects with an interest he shares with other CSHL lab leaders including Gregory Hannon and Thomas Gingeras: in types of RNAs that *don't* serve as templates for protein synthesis. "One current project in my lab focuses on a specific non-coding RNA (ncRNA) that localizes to bodies in the nucleus called nuclear speckles," Spector notes. "We're studying its role in breast cancer metastasis."

Another line of work linking the lab's interest in the nucleus with disease has grown out of inquiries relating the spatial position of nuclear bodies to the manner in which genes are expressed and regulated. Among the critical facts that have come to light: sometimes a protein will migrate from a nuclear body to "find" a gene sequence in the nucleus, to which it binds to perform a specific role — say, help switch the gene on or off. Other times, it appears that genes or RNAs actually *move to* areas occupied by certain nuclear bodies, so as to avail themselves of something inside needed for their regulation.

In contrast to what Spector and others long believed, it now appears that chromosomes occupy "neighborhoods" in the nucleus which overlap to some extent. This facilitates interactions between genes located on different chromosomes and provides the basis for gene interactions and translocations that can result in various forms of cancer.

It's not far-fetched, Spector acknowledges, to think of the nucleus as a sea of chromatin — the protein-plus-DNA bundles that "pack" the chromosomes into the tiny nuclear space; and at various locations in that chromatin sea are 10 or more types of nuclear bodies, whose contents and position in nuclear space at a given moment facilitate the critical processes by which: genes are expressed; RNAs are traf-

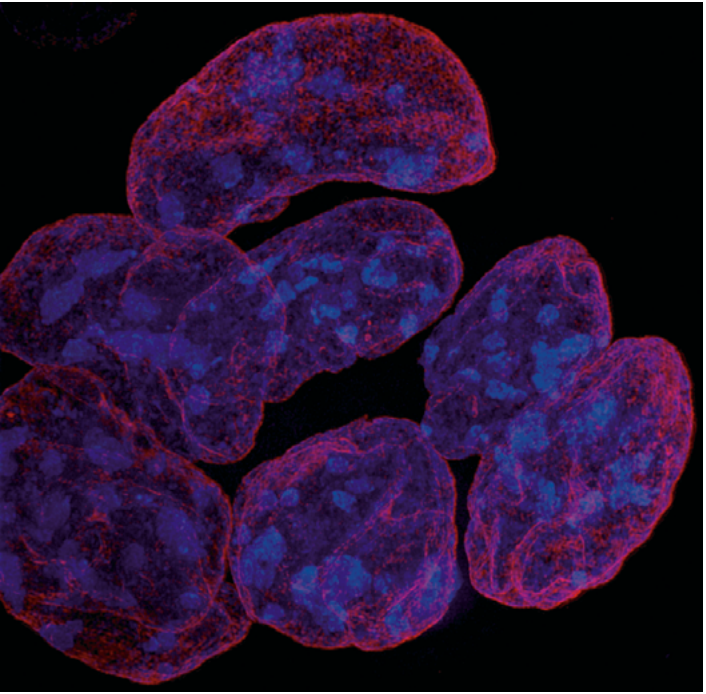
ficked; and proteins move in and out of nuclear structures. A busy picture, indeed.

### Insights into disease

A recent area of excitement in the Spector lab centers around studies utilizing mouse embryonic stem cells. "We're very interested in understanding the regulatory mechanisms that are engaged to keep these remarkable cells in the pluripotent state as well as those influencing their differentiation to different cell types." Studies focusing on gene positioning and expression levels in the nucleus, as well as differential expression of ncRNAs, are providing exciting new hints as to how these processes may be regulated.

In turn, says Spector, this raises the larger question of "how do genes 'know' they're making RNA at the right level?" It's a question of great importance across the full gamut of diseases that are caused in whole or part by gene misregulation, from cancer to diabetes to disorders of the brain. It is, too, a superb example of the value of basic research. For Spector and his team, a specific interest in the microscopic world of the cell nucleus has led to inquiries of this kind, with the broadest of consequences for human health.

Peter Tarr



Mouse embryonic stem cells (nuclei shown here) may hold clues to causes and means of addressing genetic illness.