The Shape of Heredity

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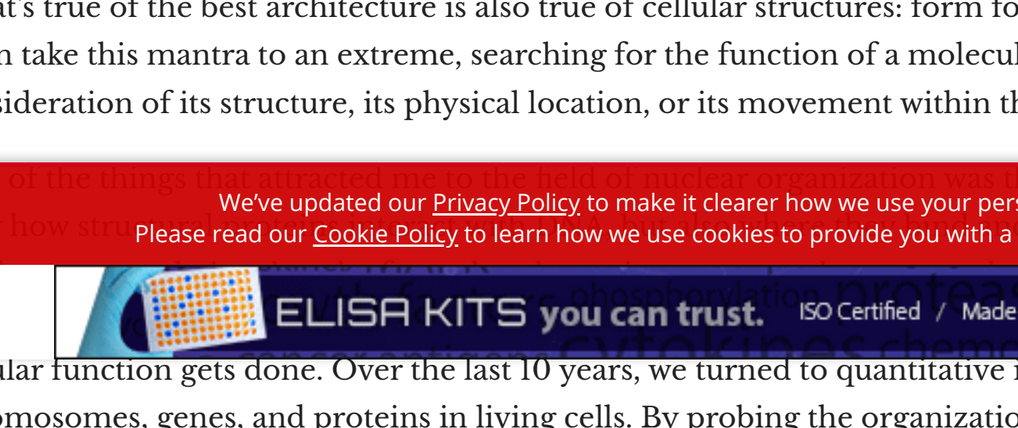

What's true of the best architecture is also true of cellular structures: form follows function. We biologists often take this mantra to an extreme, searching for the function of a molecule or gene without much consideration of its structure, its physical location, or its movement within the cell.

One of the things that attracted me to the field of nuclear organization was the need to understand not only how stru[...] interested in [...]

cellular function gets done. Over the last 10 years, we turned to quantitative imaging of GFP-tagged chromosomes, genes, and proteins in living cells. By probing the organization and dynamics of the genome within the nucleus, we have been able to discern much about the elegant choreography that links form to function.

In 1983, when I started working in the field, chromatin research was relatively sleepy. It had been a decade since the nucleosome had been shown to be the basic unit of genome organization—a building block containing eight histones around which 147 bp of DNA is coiled. We also knew that the basic nucleosomal fiber persisted throughout the cell cycle, from metaphase through interphase, yet most scientists thought histones were nondescript—identical units without function beyond that of rendering DNA generally less accessible than it is in a bacterium. We proposed that histones served as "general repressors" of DNA-based functions. At the time, only a few scientists pursued the arcane idea that modification of histone proteins, then mostly acetylation, might be functionally significant. In the early 1980s, talks on histone modifications were in late evening sessions; few thought it cutting-edge work.

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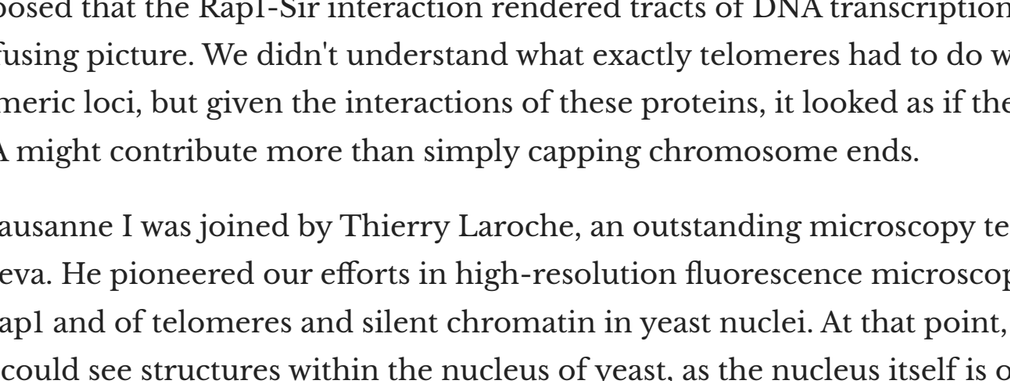
Yet histone modification eventually captured everyone's imagination. Once researchers showed that mutations that removed N-terminal tails or changed specific residues could provoke striking phenotypes in yeast, few could contest the importance of these proteins and their modifications. Not only did mutations in histones change the folding of the DNA, but they could affect which genes were being transcribed and when cell division occurred.

Today almost everyone who works on transcription studies chromatin. Researchers have made antibodies to nearly every modification found on histones, both in cores and tails. The field has come to accept that there exists a histone code (although it is more like a series of signposts than a code per se) that influences genome function. But the structure of the genetic material within the nucleus is controlled by much more than histones. I've spent much of my career working out the other players that give this essential organelle its form—and, subsequently, its function.

My interest in the structure of the nucleus began when I started as a postdoc in Ulrich Laemmli's lab at the University of Geneva in 1983. As young college graduates, my husband and I had decided to try something other than the usual hop from the halls of the University of Chicago to another graduate school in the United States. We moved to Switzerland, where my husband had roots, to pursue our PhDs; his was in logic and mine in molecular biology. This took me to Jeff Schatz's laboratory at the Biozentrum of the University of Basel, a place teeming with excitement and excellent science. After my PhD, I joined Laemmli's lab, which was known for its work on mammalian metaphase chromosomes, as well as phage assembly and the invention of the SDS polyacrylamide gel. In his lab, I started looking at how the nucleus organizes DNA by seeking proteins, other than histones, that were important in packaging the genome.

The structural proteins of the nucleus are distinctly different from those of the cytoplasm. Neither actin filaments nor microtubules give the nucleus or the genome its shape. For that reason we disliked the term "nucleoskeleton," which implied a cytoskeleton-like organization. Laemmli was interested in finding proteins that helped package DNA into loops that would be transcribed in interphase nuclei, but then further compacted to form metaphase chromosomes. We looked for factors that might help organize enhancers, promoters, genes, origins of replication and other control elements, indexing the nucleus to facilitate its function. Our goal was to find out how the genome was spatially—and not just linearly—organized.

In Laemmli's lab, I developed the biochemical skills that I later put to use when I started my own lab 3 years later at the Swiss Institute of Experimental Cancer Research (ISREC) in Lausanne, up the lake from Geneva. To carve out my own niche, I returned to the organism I'd worked on with Schatz—budding yeast—and applied to it the techniques I'd used on *Drosophila* and HeLa cells as a postdoc. We started by isolating the DNA fragments that were bound to structural proteins after extraction of histones. We reasoned that if we could find the tracts of DNA that were tightly associated with nonhistone proteins, we might get our hands on both proteins and DNA with structural or organizational roles. By working backwards from these sequences to the proteins that bound them, we felt we could identify and then disrupt the genes for nonhistone proteins that might be involved in genome folding.

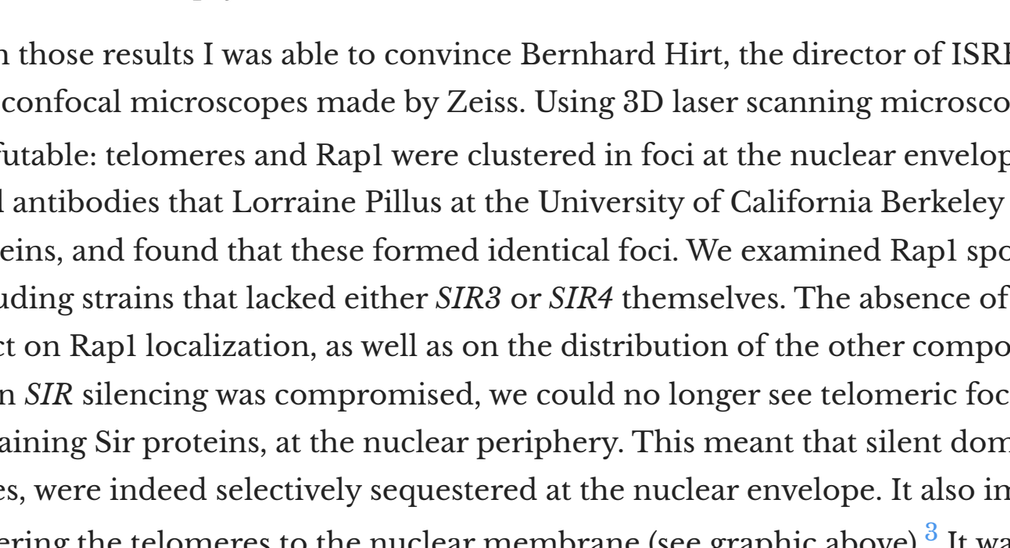


We tagged one chromosomal locus—not located near telomeres—and used a confocal microscope to watch its movement in 5 second increments within the stained perimeter of the nucleus.

One protein that emerged in large quantities from these searches was the repressor activator protein 1 (Rap1)—a protein that David Shore had just identified in Kim Nasmyth's laboratory at the Medical Research Council in Cambridge, as a silencer and promoter binding factor. It was unclear how Rap1 worked, but we soon showed that Rap1 could bind the repetitive telomeric DNA, which caps the ends of the chromosome.

David Shore showed that Rap1 interacted with the silent information regulators Sir3 and Sir4, and proposed that the Rap1-Sir interaction rendered tracts of DNA transcriptionally silent. But it was still a confusing picture. We didn't understand what exactly telomeres had to do with silencing at a non-telomeric loci, but given the interactions of these proteins, it looked as if these relatively inert repeats of DNA might contribute more than simply capping chromosome ends.

In Lausanne I was joined by Thierry Laroche, an outstanding microscopy technician, whom I had met in Geneva. He pioneered our efforts in high-resolution fluorescence microscopy to examine the distribution of Rap1 and of telomeres and silent chromatin in yeast nuclei. At that point, no one really believed that you could see structures within the nucleus of yeast, as the nucleus itself is only 2 microns in diameter. But with affinity-purified antibodies and a lot of patience, we could discern discrete spots of Rap1 staining at the rim of the yeast nucleus. Given the abundance of Rap1 binding sites in the telomeric repeats, we deduced that the Rap1 foci were clusters of telomeres. This was exciting because it suggested to us that the interphase nucleus wasn't just a jumble of DNA. While it didn't have membrane-bound organelles, it was beginning to look like the nucleus might have domains that performed very specific functions.



While the Sir4 protein was the first protein we found to tether telomeres to the nuclear envelope, the Ku protein turned out to be an even more critical anchor. Ku tethers telomeres by binding Mps3 which spans the inner nuclear envelope, allowing telomeres to cluster. Ku also recruits Sir4 to the chromosomal ends. Together with Sir2 and Sir3, Sir4 forms a complex that spreads along the proximal chromatin by binding histones and DNA. The condensed chromatin stays at the nuclear envelope through an interaction of Sir4 with Enhancer of silent chromatin 1 (Esc1).
Source: Biochim Biophys Acta, 1677:120-31, 2004

With those results I was able to convince Bernhard Hirt, the director of ISREC, to purchase one of the first confocal microscopes made by Zeiss. Using 3D laser scanning microscopy, our conclusions were irrefutable: telomeres and Rap1 were clustered in foci at the nuclear envelope.[1,2] At the same time, we used antibodies that Lorraine Pillus at the University of California Berkeley had raised to the yeast Sir proteins, and found that these formed identical foci. We examined Rap1 spots in a range of mutants, including strains that lacked either *SIR3* or *SIR4* themselves. The absence of Sir proteins had a profound effect on Rap1 localization, as well as on the distribution of the other components of silent chromatin: when *SIR* silencing was compromised, we could no longer see telomeric foci, visualized by Rap1 and the remaining Sir proteins, at the nuclear periphery. This meant that silent domains, notably subtelomeric genes, were indeed selectively sequestered at the nuclear envelope. It also implicated Sir proteins in tethering the telomeres to the nuclear membrane (see graphic above).[3] It was exciting to see how microscopy could validate concepts we had discussed and tested biochemically for many years: we could see a chromatin domain, decipher its structure, and visualize the location of particular proteins. High-resolution microscopy provided an open door and a powerful tool for analysis—as useful as Laemmli's gels had been in the 1980s.

To determine whether the telomeres were actually tethered to the nuclear membrane rather than simply excluded from the nuclear center, we combined genetics and biochemistry, and cross-checked results by microscopy. We fished for other proteins that could interact with Rap1, Sir factors, and also to origins of replication, which were also tightly bound to an insoluble fraction of histone-depleted yeast nuclei. Over the years, we have developed a range of assays that allow us to determine accurately the location of proteins and sequences within intact nuclei. Using these, we dissected the pathways that anchor DNA to the membrane and to nuclear pores, which allow proteins and RNA to pass through the double lipid bilayer of the nuclear envelope.

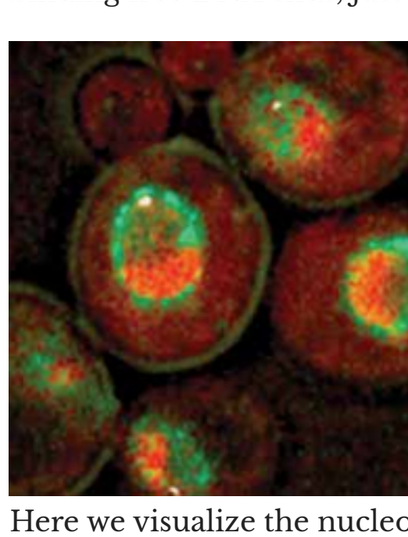
By probing the organization and dynamics of the genome within the nucleus, we have been able to discern much about the elegant choreography that links form to function.

An intriguing picture was beginning to emerge in the early 1990s. Repetitive DNA domains like telomeres seemed to play key roles in genome organization, as well as in setting up local domains of transcriptional repression. We knew that telomeres were capable of silencing genes directly adjacent to their repetitive regions, but we started to think that a spatial proximity to telomeres—and not only linear proximity—might promote repression. Amanda Fisher in London had similar thoughts about the repression of genes in proximity to centromeric repeats—the centers of the X-shaped chromosome—during B-cell development. Her work showed that the developmentally regulated genes were often brought near centromeric repeats when they were repressed. Collectively, our work showed how nontranscribed repetitive DNA—such as that found at telomeres and centromeres—can influence nuclear organization and gene expression.[4]

To return to the role of histone modifications in this repression, we teamed up with Michael Grunstein's lab at the University of California, Los Angeles, who had been testing the effect of histone tail mutants on Sir3 and Sir4 binding in vitro. A postdoc in his laboratory, Andreas Hecht, had shown that a point mutation on the tail of histone 4 affected silencing by impairing Sir3 binding. Thierry and I then showed that the same point mutation caused the dispersion of Sir proteins and Rap1 from the telomeres. This suggested that the Sir proteins were involved in tethering telomeres, and that the deacetylated histone tails were important for the binding and spreading of Sir proteins.[5]

At that point, we felt that we had a firm understanding of the sequestration of Rap1 and Sir proteins by telomeres, and the fact that the foci helped nucleate Sir-mediated repression, which then was propagated by contact with deacetylated histone tails. We found that mutations that interfered with the binding of Rap1 to Sir4, and/or mutations of the histone tail modification sites, disrupted both nuclear organization and silencing.

Yet we had one curious finding from our immunofluorescence studies of *sir* mutations that we couldn't quite explain. In cells lacking Sir proteins, only about 50% of the telomeres released their hold, suggesting that other proteins that we hadn't yet found were also involved in tethering. Over the next several years, my lab sought to identify additional candidates that served as anchors for telomeres at the nuclear membrane. A breakthrough came when we invited Edward Louis, then at the Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, to give a seminar. Ed had a curious finding of his own. He had found that yeast telomeres did not recombine efficiently with internal sequences, although they recombined among themselves just fine. During that visit, we suggested that telomeric tethering might restrict the freedom of movement that telomeres would need to interact with internal sequences, and at the same time might favor exchange with other telomeres. Ed had performed a screen for mutations that released telomeres and isolated genes encoding the yKu70 and yKu80 proteins. This was surprising but also tantalizing: the Ku protein plays a key role in nonhomologous end-joining in all eukaryotes by binding free DNA ends, just like one finds at telomeres.



Here we visualize the nucleolus (red), the yeast spindle pole body (white) and the nuclear envelope (green ring) and track GFP-tagged telomere (brighter green spot) with live imaging. The cells shown here are at different stages of the cell cycle and illustrate the organization within the nucleus.

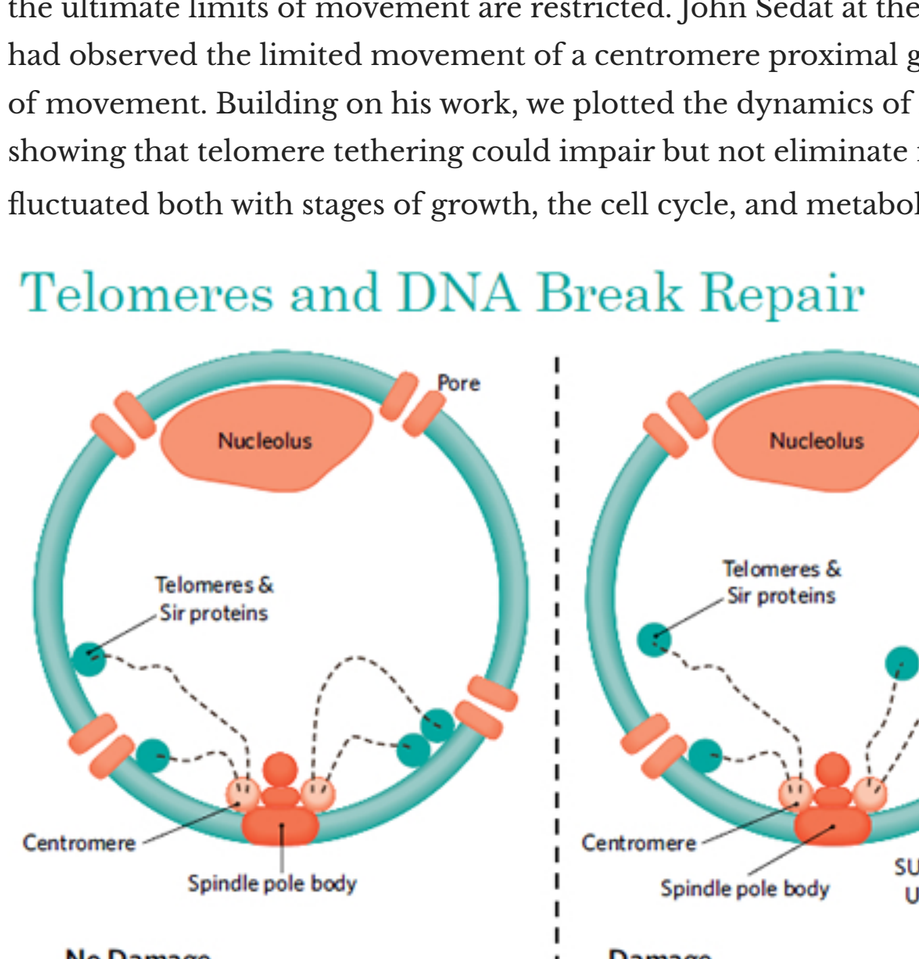
We immediately tested telomere positioning in mutants that lacked the *yku70* and *yku80* genes and sure enough, telomeres released their grip on the nuclear envelope and Sir proteins were dispersed from the telomeres.[6] A series of papers were published in 1998, implicating yKu in telomere biology, and like so many discoveries, many labs came upon yKu as a key telomeric factor at once. It also made us curious as to the relationship of telomeres to double-strand break (DSB) repair. We found it hard to imagine that yKu could both protect telomeres from recombination by sequestering the chromosomal end and promote end joining, but it does just that.

To figure out whether there was any overlap in mechanisms used to heal breaks and those that tether telomeres, we induced DSBs in a strain in which we could follow Sir proteins by chromatin immunoprecipitation (IP) and live imaging. Amazingly, upon inducing a break, Sir3 and Sir4 were released from telomeres, as was yKu, and both bound near the break. The kinetics of recruitment were very different, however: yKu bound immediately and transiently to the DSB, while Sir proteins accumulated only after 2 to 4 hours, which was so long (on a yeast scale) that we figured that the cell had probably given up on trying to repair the cut (see The Silent Chromatin Anchors graphic).[7] Telomere-bound yKu and Sir proteins were also released when the cell arrested its progression to mitosis and it was shown by others to be provoked by other types of genomic stress as well. This led to the model that the telomere serves as a reservoir for proteins that could—under appropriate conditions—be released to function elsewhere. Recently, we have pursued this concept further, and have shown that Sir proteins "released" from telomeres can still function. They repress promiscuously at internal genes, altering patterns of gene expression,[8] as part of a survival mechanism. Indeed, the redirected Sir proteins repress genes involved in ribosome biogenesis.

We could see the nuclear core was full of rapidly moving chromatin, a riot of movement that could be described biophysically as a constrained random walk.

Our studies of telomere and Sir protein foci in *yku* mutants showed that yKu might provide an anchor for telomeres even in the absence of silencing. This was nailed in a series of papers in which we monitored telomere position by live microscopy. We came to the conclusion that there are partially redundant pathways, involving yKu and Sir4, that promote telomere tethering at the nuclear rim both in the absence and presence of silent chromatin. The yeast nucleus now seemed to be divided into zones of concentrated Sir proteins, which both promoted their own attachment or helped yKu anchor them, and regions depleted of Sir proteins, in which promoters were more likely to be transcriptionally active.

Filming the movement of telomeres and other tagged loci in real time was pioneered by a graduate student in my lab, Patrick Heun.[9] Under the careful tutelage of Thierry Laroche, the two watched and filmed, optimizing conditions so as not to perturb cell growth or induce damage by the imaging itself. We, of course, confirmed that telomeres were enriched at the nuclear envelope, but could also see telomeres stray away, albeit never for very long. The biggest surprise came from studying loci that were located at the center of the nucleus in transcriptionally active zones: We could see the nuclear core was full of rapidly moving chromatin (see graphic on p. 34 and 35, a riot of movement that could be described biophysically as a constrained random walk. This means that movement goes in random directions, yet the ultimate limits of movement are restricted. John Sedat at the University of California, San Francisco had observed the limited movement of a centromere proximal gene in yeast, and had proposed this kind of movement. Building on his work, we plotted the dynamics of many loci under various conditions, showing that telomere tethering could impair but not eliminate movement, and that chromatin dynamics fluctuated both with stages of growth, the cell cycle, and metabolic state.[9]



When the nucleus detects a DNA double-strand break, the Ku and Sir proteins are partially released from telomeric chromatin (aqua), which leads to a partial release of the telomeres themselves. The Ku protein is rapidly recruited to the breakpoint (not shown), but soon—if the damage cannot be repaired by end–joining or recombination— the DNA shifts to the nuclear pore. There, the StUBL protein helps channel the DNA to an alternative pathway of repair.

We used this tool to further our study of double-strand breaks. A postdoc, Karine Dubrana, GFP-tagged a site at which a double-strand break could be induced, and saw that after a while these cut sites traveled to the periphery.[10] However, they did not shift to telomeric foci as we expected. (The telomeric environment might, after all, have helped heal or protect the DNA ends from exonuclease or recombination.) Rather, the irreparable DSB ended up at nuclear pores, where they were worked on by a bizarre new enzyme complex called a StUBL. The StUBL recognizes one post-translational modification on proteins (SUMO) and adds another (UB) to target modified proteins for degradation. We inferred that at pores, StUBL targets one or more proteins for degradation, since the proteasome—the cell's degradation and recycling machine—is also recruited to irreparable DSBs at the pore (see graphic above). Genetic analysis showed that StUBL was working on the same pathway as factors for DSB repair, and might even be in a shared complex. Based on our results, our best guess of how DSBs are trafficked in the nucleus is that they shift to the nuclear envelope for processing and are then channeled either into a pathway of recovery or degradation.

DNA thus seems to have a reason for moving. The nucleus is clearly as compartmentalized as the cytoplasm despite an absence of membrane-bound organelles. With sites along the nuclear envelope for silencing, DNA repair, and gene expression, we can imagine it like a ballet studio, with some dancers warming up on the bars at the periphery, waiting for their turn to dance, while those at the center sweep across the floor in tune with their music. A strained muscle, and the dancer is back to the edge for repair. Intriguing are genes that dance at different tempos—perhaps we will find that they listen to histone modifications, as well.

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