

# Nuclear Biology: What's Been Most Surprising?

## Restricting Genomic Partners



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The regulatory potential of the human genome is much richer than some had anticipated. With greatly refined annotations, we now realize that each gene finds itself surrounded by a huge number of potentially regulatory elements in a very crowded nucleus. Given that many regulatory elements control genes through direct physical interaction, one can imagine that this could create a potentially risky situation in which genes get misregulated by chance encounters with inappropriate elements. So, a major question in the field of nuclear organization is how do cells ensure that genes only respond to the right regulatory elements while ignoring the hundreds of thousands of others?

Recent work has revealed a surprisingly simple strategy for matching genes to only some regulatory elements, which involves the spatial organization and folding of chromosomes inside the nucleus. In *Drosophila*, mouse, and human nuclei, chromosomes are spatially compartmentalized. Using 5C and Hi-C technologies, it has been shown that chromosomes form strings of topologically associating domains (TADs) that are each hundreds of Kb in size but are spatially insulated from neighboring TADs. As a result, a given gene lives in a relatively small neighborhood where it encounters only a small section of the genome and thus can partner with only a small number of regulatory elements. Future studies will no doubt unveil how TADs are established and how they insulate genes from the wrong crowd.

## Plasticity of Interpretation



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Advances in genomic profiling technologies combined with the realization that certain chromatin features can be effectively used to annotate *cis*-regulatory elements enabled a large number of recent epigenome mapping efforts across a myriad of cell types and organisms. The picture that emerges from these studies elucidates the astounding degree to which our genome, including the repetitive regions derived from transposon elements, appears to be dynamically utilized for the purposes of gene regulation. The human ENCODE project alone mapped nearly 400,000 distinct transcriptional enhancers, most of which showed high cell type specificity of the chromatin-marking patterns. Other studies have demonstrated that thousands of regulatory regions undergo activation or decommissioning even during transitions between closely developmentally related cell types. It seems highly likely that the information content within regulatory parts of the genome substantially exceeds that of protein-coding regions, suggesting the enormous potential for combinatorial complexity of gene expression regulation during embryogenesis.

Dynamic changes between distinct chromatin states have proven to be remarkably commonplace during differentiation. Moreover, discoveries of enzymatic activities that are responsible for removal or alteration of chromatin modifications previously thought of as relatively stable, such as methylation of histone proteins and DNA, contribute to the mechanistic explanation of the observed chromatin dynamics. Taken together, emerging views change our thinking about both the content of our genome and the plasticity of its interpretation through chromatin-mediated mechanisms.

## Surprises at the Membrane



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Nuclear biology is full of surprises because, like all biology, the underlying mechanisms result from evolution and have been selected to work, independent of how. Studying nucleocytoplasmic transport, an early eye-popping moment resulted from calculating the flux of macromolecules transported between cytoplasmic and nuclear compartments. Who would guess that transport through nuclear pore complexes (NPCs) affects millions of macromolecules every minute in mammalian cells? Transport substrates vary, but some are huge, like viruses or RNPs of up to 50 MDa, whereas passive macromolecules of >60 KDa are excluded from NPC transit. It is therefore remarkable that NPC passage per se occurs independent of energy input. Instead, import or export is driven indirectly via energy-dependent assembly or disassembly of transport-competent complexes on one side or the other of the NPC. NPC selectivity results from the properties of intrinsically disordered segments of the NPC proteins that line and occupy the transport channel and the transport receptors with which they interact. But the most dynamic aspect of nuclear biology is the complete disassembly and reassembly of the nucleus during each metazoan cell division. Here, the finding is so old that it is no longer a surprise, but we have no idea why this should happen, especially as many single-celled eukaryotes undergo mitosis with an intact nucleus.

### A Blueprint for Spatial Sequencing



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From an information theoretic standpoint, the throughput of today's sequencers dwarfs almost any other means of interrogating a biological sample. This makes it increasingly tempting to try to "translate" far-flung biological questions into the language of DNA sequence. But how well can this sort of experimental shoehorning work?

If the recent experience of nuclear biology is any guide, the answer is: better than we might have guessed. New proximity ligation methods based on the nuclear ligation assay and its intellectual descendants have made DNA sequencers the platform of choice for rapidly estimating the physical distance between genomic loci in the nucleus of a cell. As a result, "three-dimensional" DNA sequencing has begun to have a marked impact on our understanding of chromatin structure, playing a role that is highly complementary to microscopy.

Because ligation-based methods can be used to probe the distance between other cellular actors, such as RNAs and proteins, this development suggests a broader template for translating cell biology's spatial puzzles. And why limit ourselves to the cell's interior? Recent proposals have suggested mapping "connectomes" by tagging individual neurons with DNA barcodes and then ligating the tags. Today's nuclear biology might prove to be tomorrow's neuroscience.

### RNA Rewrites Central Dogma



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The pervasive role of RNA in nearly all aspects of nuclear biology is a continuing revelation. The eukaryotic nucleus is commonly perceived to be a realm in which DNA reigns supreme. Elucidation of the genetic code showed that messenger RNAs, transfer RNAs, and ribosomal RNAs transcribed in the nucleus are exported to the cytoplasm for protein synthesis. These early studies suggested that DNA gave the orders and RNA carried out the mission elsewhere. Fast forward to today and compelling evidence that an RNA-based biology predated the evolution of DNA for information storage, and one sees the nucleus in a new light: as a hotbed of RNA-mediated information management. DNA replication is initiated by RNA primers. Chromosome ends are maintained by RNA-templated telomere addition. Multiple classes of small regulatory RNAs (e.g., snRNAs, snoRNAs, and scaRNAs) are critical for messenger RNA splicing, transfer RNA maturation, ribosomal RNA processing, and RNA chemical modification by methylation or pseudouridylation. More recently, long noncoding RNAs and short RNAs (siRNAs, miRNAs, and piRNAs) have been shown to act within the nucleus to regulate cytosine methylation (e.g., plants and mammals) or histone modification (most eukaryotes). These epigenetic modifications regulate genes during development, silence transposons and retroviruses, and contribute to centromere function and accurate chromosome segregation. An emerging RNA-centric view of the nucleus represents a major paradigm shift.