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Editorial overview: Genome architecture and expression: The nucleus, top and bottom

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Dr Corces studied biology and biochemistry in Madrid before leaving Spain for a postdoctoral fellowship at Harvard. His research became centered on epigenetics: For more than three decades, he has been studying fruit flies to uncover secrets of DNA's arrangement and organization in the cell nucleus. He is a Howard Hughes Medical Institute Professor and created a program called RISE to motivate high school students to follow science careers.

Progress toward understanding nuclear structure and function has derived from top-down observational and bottom-up biochemical approaches. The top-down approach is as old as the microscope. The first natural philosophers to train microscope lenses on living things appreciated that cells were inhomogeneous with a lumpy substructure. The first microscopist, Antonie von Leeuvenhoek, viewing the nucleated red blood cells of salmon noted a central 'lumen'. The botanist Robert Brown coined the term nucleus (*latin*; kernel) in the 1830s although its function was unknown. Improving microscopes and the development of the natural and synthetic stains, especially Hematoxylin and Eosin (H&E) (1870s), provided perhaps the most robust and enduring means for viewing tissues and cells that revealed the spectrum of physiological and pathologic changes associated with nuclear morphology.

The bottom up approach dates to the synthesis of urea by Friedrich Wöhler [7] and the realization that animate and inanimate materials are comprised of the same stuff. Friedrich Miescher, working under Felix Hopp-Seyler seeking to explore the composition of white cells, was the first to separate nucleus from cytoplasm and recovered a phosphorous-rich, sulfur-free material they termed 'nuclein' (1870s) [3], which proved to be nucleic acid (mostly DNA reflecting treatment with alkali). Miescher was skeptical of a role for nuclein in heredity because it lacked sufficient chemical diversity, a refrain that echoed for almost a century.

The first half of the 20th Century saw developments advancing both the top down and bottom up approaches. With advent of quantum mechanics came the DeBroglie equation and the recognition that high energy electrons could probe biological materials with higher resolution than possible with conventional light microscopy. Starting from purified material, the classic experiments of Avery *et al.* [1], demonstrated that DNA rather than protein was likely to be the genetic substance and presaged the compelling proposal of the double helix; perhaps the most compelling and useful bottom up insight in the history of biology. Concurrently, by showing doubling of Feulgen stainable material (DNA) in cells during the cell cycle, Hewson Swift provided a top-down histological confirmation for DNA as the substance of heredity [5]. Perhaps more important even than the electron microscope, the invention of immunfluorescence microscopy [6] presaged the fusion of the top down and bottom up approaches.

Bottom up studies of the nucleus were advanced by the application of the tools of the metabolic chemists and biochemists who isolated the enzymes and defined the pathways of intermediary metabolism. Application of these paradigms to nuclear contents so successfully identified the enzymes, activities and components of replication, transcription, recombination and

translation that the reconstitution of these processes from soluble components became the finish-line for their characterization. The availability of recombinant proteins enormously facilitated the purification and increased the availability of proteins required for these experiments.

Though solution biochemistry was powerful for characterizing fundamental reactions, the fine regulation of the nuclear processes proved more difficult to recapitulate *in vitro*; something was missing. Much of that something turned out to be chromatin. The discovery that the genetically identified and proven transcription regulator GCN5 from yeast was a histone acetylase [2,4] proved that chromatin was more than a DNA-storage system and set off a still ongoing effort to identify and characterize the enzymes and complexes that modify, arrange and remodel chromatin. As the number of well-characterized chromatin-active complexes grew, it became apparent that they were modular. The complexes involved in gene regulation were found to share and exchange subunits, and to undergo dynamic transitions through many intermediate stages during their reaction cycles.

At the same time, the availability of recombinant DNA proteins and technology (especially polymerase chain reaction) enabled the generation of antibodies and fluorescent fusions protein as immunological and *in vivo* probes for microscopy, as well as enabling the development of nucleic acid probes for hybridization. Studies of the localization and trafficking of virtually any protein or RNA became possible, at least in principle. The top-down approach seemed to be limited only by the resolution of light microscopy, or in some cases by immunoelectron microscopy.

It is becoming more and more apparent that DNA transactions are driven by interacting supramolecular complexes that are difficult to study with solution biochemistry, similarly these same processes challenge state of the art methods for visualization within cells. Yet the development of super-resolution approaches, fluorescence energy transfer (FRET) microscopy, as well as other novel or improved methods for acquiring and processing images have made lab bench microscopy an accessible quantitative molecular approach. And advances in biochemical methods, often involving mass spectrometry as well as molecular or anatomic structure determination have empowered investigations of the structure and function dynamics of very large complexes. The regulation of the colocalization of these complexes in particular nuclear neighborhoods is a matter of importance and speculation. For example, whether transcription factories are a consequence of the folding of chromosomes and a local high concentration of transcription components that flicker in and out of existence or whether they are dedicated structures remains to be determined. The directory of nuclear neighborhoods awaits full compilation.

The confluence of thought and approaches for nuclear biology has conceptually unified the disciplines of biochemistry, molecular biology, and cell biology alongside of systems biology. Notwithstanding the convergence of the top-down and bottom up approaches, formidable barriers need to be breeched in order to harvest the intellectual and practical bounties they promise. Foremost among these obstacles is simply the scale of the data. The combinatorial complexity of multiple dynamically interacting, highly modified biochemical components paralleled by high density dynamic imaging of interacting molecules in solution or via looping and bridging interactions while bound to chromatin presents overwhelming computational issues. And even when the computational problems yield, the thorniest challenge may be to put these data into coherent schemata that inform and inspire, but do not overwhelm our comprehension.

The contributions to this volume have been arranged to roughly scale from smaller to larger nuclear hierarchies. No universal principle has been established to define and predict their arrangement; indeed parallel and overlapping principles may coexist. Chromosome territories, transcription precincts, replication parishes, heteochromatin districts, and nuclear pore neighborhoods are simultaneously and plastically delineated. The functional and architectural interplay across these multiple dimensional levels is dynamically robust. Though it is not independent, the coupling between these alternative organizations is not rigid. The net structure of the nucleus is organic.

We start with a discussion of the physical principles and constraints for packing DNA into the nucleus (Lavelle), and continue to consider the nature of the proteins — histones — that do the packing (Dalal and Volle), and of the patterns and distribution of the major covalent modification of DNA — methylation (Schübeler and Baubec). We then consider the elastic forces that twist and writhe the chromatin fiber — supercoiling (Gilbert and Allan) and some of the consequent structural perturbations of the genome (quadruplex DNA) (Balasubramanian and Murat). We next study how DNA sequences loop (Blobel and Deng), and the interplay between chromosome folding and dynamics with the machineries that activate or shutdown transcription machinery at promoters versus enhancers (Lis and Buckley, Young and Dowen, Wendt and Grosveld, Cavalli and Cheutin). Although most studies of enhancers have focused on enhancer binding proteins, cis-elements, and more recently chromatin modifications, enhancer RNAs (eRNAs) (Shiekhattar and Lai), and indeed other non-coding RNAs (Lei and Názer) are likely to emerge as important regulatory players with roles yet to be fully defined and appreciated. The chromosomal and genetic states that discriminate between physiological states must involve a myriad of enhancer and promoter interactions as well as overarching

changes in patterns of chromosome topology and action; the molecular events that discriminate between such states during cellular reprogramming are addressed by Soufi. Entropy ensures that every biological process goes awry despite often redundant repair and control systems; chromosome arrangement and folding is no exception (as considered by Hakim and Schwartz). The overlay of patterns of replication timing with genetic damage and transcription provides another albeit not fully orthogonal axis to help define a conceptual space for the interplay between chromosome geometry and function with pathology (Gilbert and Sima). Finally two essays that deal with the physiology and pathology of nuclear pore complexes and of the nuclear lamina and envelope remind us that the neat cubby holes that we use to sort molecular complexes for structure and function often breakdown as the complexes and their components play multiple roles in segregating, arranging and directing the activity of chromosomes and genes (Capelson and Pascual-Garcia, Brickner and Sood, and Reddy and colleagues).

We hope that together the pieces in this volume make a collage that invites the reader to see connections and interactions perhaps unanticipated by the authors and editors as we strive to see and appreciate the nucleus at once from the top and from the bottom.

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