

Center of Excellence in Chromosome Biology

There is nothing static about the contents of a cell's nucleus. The incredibly dynamic nature of chromatin within the nucleus is becoming clear through research at the Center for Cancer Research. A group of committed CCR scientists has formed the Center of Excellence in Chromosome Biology to consolidate expertise and pursue the impact of their observations about the workings of chromatin.

Chromatin in Motion

How are genomes organized in space and time within the cell nucleus and how do cellular gene expression machines such as transcription complexes interact with chromatin in living cells? Dr. Tom Misteli is addressing these questions and obtaining fascinating answers using live cell imaging and computer simulations.

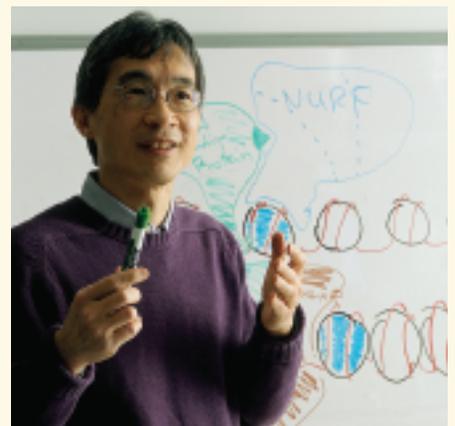


Dr. Thomas Misteli. Photo credit: Rhoda Baer

In two landmark papers, Misteli's group visualized a set of mouse chromosomes and analyzed their positions. His team provided evidence that the 3-dimensional spatial relationship among chromosomes is far from random. In fact, chromosomes actually cluster into distinct neighborhoods, depending on the cell type. Misteli's group found that chromosomes 5 and 6, which are frequently involved in translocations in liver cancers, were physically near one another in normal liver cells as well. They further showed that the gene loci involved in translocations in human lymphoma localized close by to each other in the nuclei of lymphoblastic cells in culture. This research provides important insights into the translocations between specific chromosomes seen in cancers.

The Nucleosome Slide

In the 1990s, scientists suspected that the architecture of chromatin had to be reconfigured for gene expression to occur, but they did not know how it took place at the molecular level. In 1994, Dr. Carl Wu, newly elected member of the National Academy of Sciences, found the answer by devising an



Dr. Carl Wu. Photo credit: Rhoda Baer

elegant assay that captured the changes that occur in chromatin when a transcription factor binds to a gene promoter. Promoters are DNA sequences that are recognized by the enzyme RNA polymerase, the protein workhorse of transcription, and demarcate which genes should be transcribed and, therefore, which proteins the cell will ultimately manufacture. Dr. Wu then tackled another unsolved problem. When nucleosomes change their position so that chromatin has a more open configuration, energy from a molecule called adenosine triphosphate (ATP) is required. He knew that ATP did not bind to the transcription factor directly. Instead, his lab showed that a novel, multiprotein complex he called NURF (nucleosome remodeling factor) is what utilizes the ATP. NURF reshapes the chromatin using the energy provided by ATP. This complex physically slides the nucleosomes aside so that RNA polymerase can bind and enable gene expression. This pioneering series of experiments was cited as a milestone in the field of transcription analysis in a December 2005 *Nature* supplement.

Deoxyribonucleic acid (DNA) is a molecule with a mission—to pass on instructions the cell uses to build proteins essential to life. The complete supply of DNA—all the genes and spaces in between—are packaged as macromolecules called chromosomes, and the sum total of all the chromosomes of a species is called its genome. Each chromosome houses many working units called genes, and each gene sits within tightly coiled DNA strands that are wrapped around eight histones in a package called a nucleosome. Chromatin is the full collection of these nucleosomes. All this is packed into the nucleus of the cell.



Dr. Michael Bustin. Photo credit: Rhoda Baer

Ever Changing Chromatin Fiber

The chromatin fiber is metabolically active. This dynamic and flexible structure continuously changes in response to a wide range of biological signals. Dr. Michael Bustin studies proteins that are the major agents of this structural change, the high-mobility group (HMG) proteins and H1 histones. H1 histones stabilize chromatin structure and decrease access to the DNA, while HMG proteins open the chromatin fiber and increase access of DNA to regulatory molecules. Dr. Bustin, together with Dr. Misteli, discovered that HMG and H1 histones are highly mobile and do not remain attached to a specific site of the chromatin throughout the cell cycle, as previously thought. His team demonstrated that HMG proteins compete with H1 histones for binding to chromatin and form a network of dynamic interactions to regulate a

cell's response to various signals. Today, using genetically engineered mice, the Bustin team is sorting out the exact functions of these mobile proteins and their putative roles in cancer and other genetic diseases.

Dr. Bustin pioneered the use of immunochemical approaches to the study of the structure and functions of histones and chromatin, providing unique reagents to the broad scientific community before any were available commercially.

Putting the Brakes on DNA Breaks

Dr. Andre Nussenzweig studies a type of blood cancer called B-cell lymphoma, which is linked to chromosomal translocations between an antibody gene and a cancer-causing oncogene called c-myc. Normal B cells routinely make breaks in DNA to recombine genetic information and build proteins that confer specific antibody shapes. But these DNA breaks increase the risk for chromosomal translocations. The Nussenzweig group asked how normal B cells protect themselves against accumulating too many of these DNA breaks and unwanted translocations. They discovered a powerful trio of proteins—ATM, p53, and p19—that apply the brakes in a cell when necessary, keeping deregulated B cells from progressing toward malignant growth.

When breaks accumulate in B-cell DNA, ATM activates the brakes, the p53 tumor suppressor. When the cancer-causing c-myc's protein calls for overactive growth, p53 again is activated. And when ATM is not present to signal for p53, p19 protein steps in to activate p53. The researchers also found that primary B lymphocytes that lack these three proteins have a high level of chromosomal translocations. Either ATM-p53 or p19-p53 complexes seem able to provide braking action, keeping deregulated B cells from progressing toward malignant growth.

Purposeful Repair

DNA also undergoes purposeful nicks to relax its shape during replication to make new DNA strands as part of the larger cycle of cell growth and division. If unwanted damage to DNA occurs during replication, the cell cycle stops for repairs, and then replication starts up again. Signaling molecules must be able to distinguish purposeful from accidental nicks in DNA. Dr. Michael Lichten is figuring out how the cell discriminates between these two types of nicks.

He found that, when both strands of DNA incur a break, cohesin, a protein that holds the pieces together until mitosis, is quickly recruited to the region around the breaks. The cell phosphorylates a histone called H2AX in the nearby nucleosomes that sends a signal for cohesin to arrive. Interestingly, phosphorylated H2AX and cohesin are absent immediately near the break to leave space for repair proteins and various chromatin-remodeling complexes. Throughout this flurry of repair activity, large remodeling complexes move about the nucleus to sites of DNA damage and the cell cycle temporarily stops. When phosphates are removed from H2AX in the regions that flank the double-strand breaks in DNA, the cell cycle restarts.

