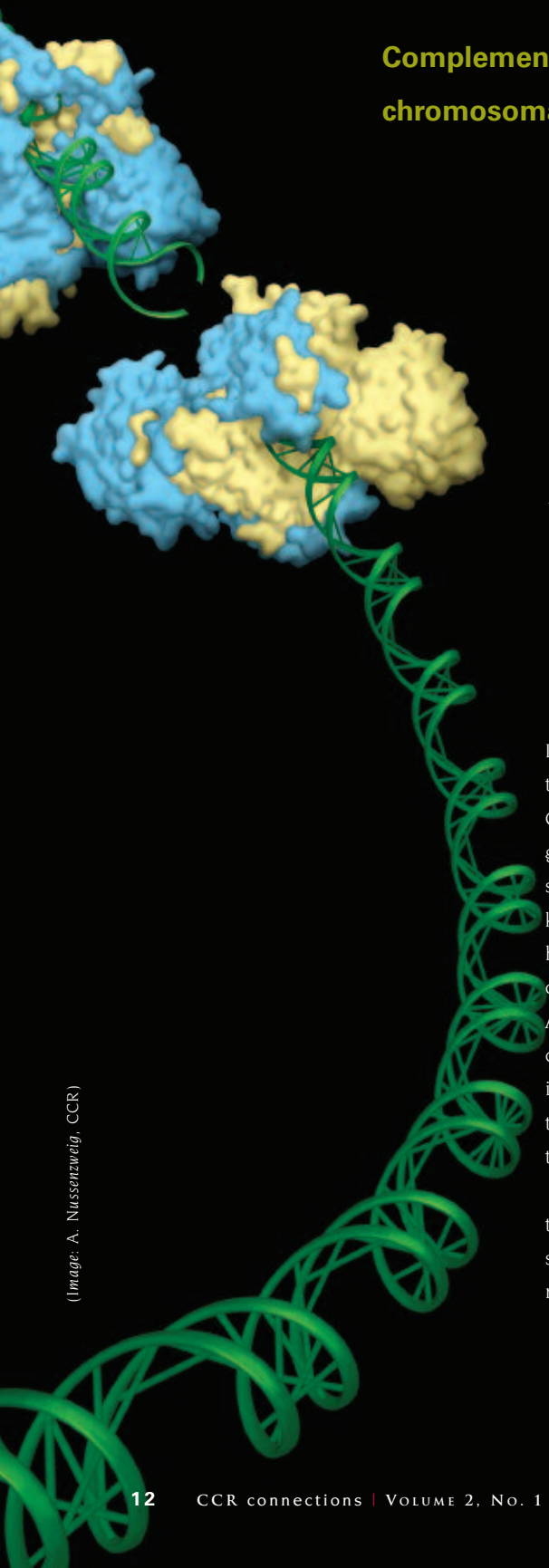
Joining Forces

Complementary approaches are piecing together the mysteries of chromosomal translocations and cancer



(*Image: A. Nussenzweig, CCR*)

It has been almost 50 years since a "minute chromosome" was first identified in patients with chronic myelogenous leukemia (CML). This genetic abnormality, named the Philadelphia chromosome for where it was discovered, was the first genetic defect linked to cancer (Figure 1). Investigators initially believed that the Philadelphia chromosome resulted from the loss of genetic material. However, advances in cytogenetics over the next decade made it possible to view the true nature of this abnormality —the genetic material "missing" from chromosome 22 was not lost but "translocated" to chromosome 9.

It is now known that this specific translocation, found in 95 percent of patients with CML, fuses a proto-oncogene (a normal gene with oncogenic potential) on chromosome 9 (*c-ABL*) to a site on chromosome 22 known as a breakpoint cluster region. This hybrid oncogene, *BCR-ABL*, produces a constantly activated mutant protein (BCR-ABL), which wreaks the genomic havoc in the cell that ultimately causes CML but which also is the target of imatinib mesylate (Gleevec®), the first FDA-approved treatment to target a translocation-specific fusion protein.

Since the 1970s, chromosomal translocations have been associated with several types of blood cancers, and they are recognized increasingly as key players in solid tumors as well. Today, cytogenetic testing is performed frequently in the clinic to determine which chromosomal translocations are present in patient samples, helping facilitate diagnosis as well as treatment planning. But although we have come a long way in 50 years, we have yet to fully understand the molecular mechanisms behind these deadly chromosomal rearrangements and why they happen so often in the same chromosomal locations. Instead of treating the resulting cancer, can we prevent them from occurring in the first place? Researchers in the Center of Excellence in Chromosome Biology at CCR are putting together answers to these questions.

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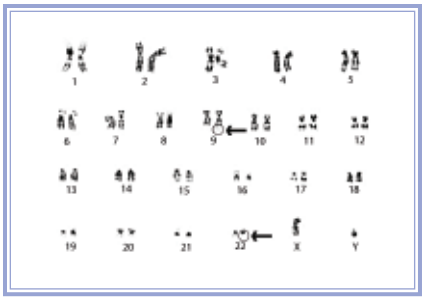
Taking a Global View

Tom Misteli, Ph.D., Head of the Cell Biology of Genomes Group in CCR's Laboratory of Receptor Biology and Gene Expression, arrived at NCI nine years ago with the task of building an imaging program. Within a few years, he gradually began to apply *in vivo* imaging techniques to chromosome biology and specifically to understanding how genome organization affects genome regulation (see "The Right Place at the Right Time"). He and his CCR colleagues are now using high resolution microscopy, live-cell imaging, and computer simulation to study the positioning of entire chromosomes and particular gene loci within the nucleus in order to understand how these arrangements change during normal and aberrant physiological processes (Figure 2).



(*Image: Feinstein Kean Healthcare*)

Figure 1: The Philadelphia chromosome—the result of a translocation between chromosomes 9 and 22 (circles)—is often found in the cells of patients with chronic myelogenous leukemia.

Far from being randomly scattered around the nucleus, chromosomes, subchromosomal domains, and individual genes are nonrandomly organized into discrete territories, or neighborhoods, within the nucleus. Although these entities may have preferred localizations, the patterns can change in response to the cellular environment. The distinct territory occupied varies with cell type, upon cell differentiation, and when cells exit the cell cycle, suggesting a link between positioning and genome function. The positioning is believed to influence gene expression programs as cells undergo changes throughout development and differentiation.

Positioning can determine direct interactions between genes, which in turn can regulate gene expression. In naïve T-helper immune cells, for example, when a specific region on mouse chromosome 11 (TH2) directly interacts with the Ifng locus on chromosome 10, the locus is turned "off." When these cells then receive a signal to differentiate, the two regions separate, and the Ifng locus turns back "on."

Chromosomal translocations occur when direct physical interactions go wrong, and these can be deadly. DNA double-strand breaks occur frequently throughout the genome during replication or as a result of DNA-damaging agents like radiation. What remains unclear is how the broken ends of two different chromosomes—which should not be allowed to "mingle" under normal circumstances—assemble and fuse together, forming a translocation.

Spatial mapping studies by Misteli's laboratory demonstrated that the breakage sites of several common translocations (e.g., Myc-Igh, BCR-ABL, and RAR-PML) are preferentially positioned in close proximity to each other in normal B lymphocytes prior to undergoing chromosomal rearrangement. This observation suggests that proximity may be a requirement for translocation.

Taking a Closer Look

This proximity requirement suggests that broken DNA ends do not just flop around in the nucleus but are fairly limited in their freedom of movement. To better understand these findings, Misteli teamed up with Andre Nussenzweig, Ph.D., Head of the Molecular Recombination Section at CCR's Experimental Immunology Branch. Nussenzweig and his colleagues (see "Making the Right Connections") explore chromosomal translocations at the molecular level—an approach that complements Misteli's "macro" view of chromosome biology—to focus on the cellular mechanisms of genomic stability and how defects in DNA repair and cell cycle checkpoints lead to chromosomal translocations and malignancies.

Misteli and Nussenzweig designed an experimental system that allowed them to introduce a double-strand break within the genome at will and then visualize the fate of each of the damaged DNA ends in living cells in real time. They found that while the broken chromosome ends do separate slightly from each other, their long-range motion is significantly constrained. This positional stability depends upon the presence of a specific DNA binding protein known as Ku80, a component of the nonhomologous end-joining (NHEJ) DNA-repair pathway. Data from Ku80 knockout mice generated by the Nussenzweig lab demonstrate that Ku80 is important for maintaining genomic stability—it forms an asymmetric ring around the two broken ends to align them for repair. Several follow-up studies are being conducted to further characterize this protein and the role it plays in DNA repair and chromosomal translocations.

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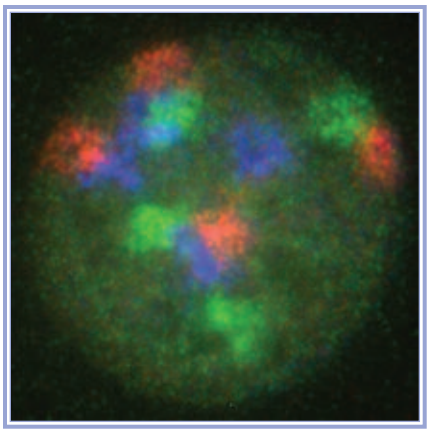
Of Recombination and Translocation

The NHEJ pathway is a key pathway in normal immune cell development through its involvement in V(D)J recombination. This normal programmed recombination event occurs early in the life of developing B lymphocytes, resulting in the generation of cell surface receptors that can accurately identify a massive diversity of intruders and mobilize the immune system to respond. During recombination, the variable (V), diversity (D), and joining (J) gene segments are selected randomly and recombined to ultimately produce an antigen receptor gene. These receptors can even recognize microbes that an individual or his/her ancestors have never encountered, which explains how some individuals are naturally immune to new infections or viral strains.

By studying the process of V(D)J recombination, Nussenzweig and colleagues hope to gain invaluable insight into the mechanisms responsible for chromosome translocations. Aberrant chromosomal translocations between antigen receptor loci and proto-oncogenes are a hallmark of lymphoid cancers. These translocations are not random—they involve only a few oncogenes with recurrent breakpoints. Mature B-cell lymphomas, for example, typically involve fusions of antigen receptor loci with *BCL1* (mantle zone lymphoma), *BCL2* (follicular lymphoma), or *MYC* (Burkitt's lymphoma).

V(D)J recombination is initiated by the RAG-1/2 endonuclease, an enzyme that introduces specific double-strand breaks within the VDJ genes. The broken DNA strands are then fused together via the NHEJ pathway to form the antigen receptor gene. The strands are prevented from joining illegitimately by ataxia-telangiectasia mutated (ATM), a key enzyme that is activated by DNA double-strand breaks. ATM also prevents the propagation of cells with broken chromosomes by activating mechanisms that lead to cell cycle arrest, DNA repair, or apoptosis (cell death) (Figure 3). Mutations within the *ATM* gene result in the rare disorder ataxia telangiectasia (from which the gene name is derived), which is characterized by immunodeficiency and predisposition to lymphoid malignancies caused by chromosomal translocations near antigen receptor genes.

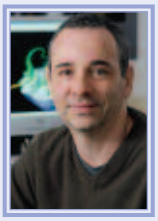
Nussenzweig's group discovered that ATM is also part of a system that prevents genetic damage from being passed on to a cell's offspring. In a recent study published in *Cell*, Nussenzweig and his brother, the Rockefeller University's Michel Nussenzwieg, M.D., Ph.D., showed that when ATM is absent, chromosomal breaks created during V(D)J recombination go unrepaired, and checkpoints that normally prevent the damaged cell from replicating are lost. Remarkably, the cell divides, matures, and maintains the breaks, which can persist for more than five generations *in vitro* and approximately two weeks *in vivo*. Since ATM is mutated in a number of lymphomas, the new finding suggests to researchers that the lymphocytes could have been living with DNA damage for a long time, and that this damage likely plays a role in later chromosomal translocations that lead to cancer. This novel form of "delayed" genomic instability, which permits more time for long-range movement of chromosomal breaks, might



(*Image*: T. Misteli, CCR)

Figure 2: The positioning of the 23 pairs of chromosomes within the nucleus (here labeled with red, green, and blue dyes) as a cell goes through the cell cycle may significantly influence gene expression.

Making the Right Connections



(*Photo*: R. Baer)

Andre Nussenzweig, Ph.D.
Head, Molecular Recombination Section, Experimental Immunology Branch

Andre Nussenzweig takes a different angle toward understanding how and why chromosomes break and rejoin and how they relate to cancer: that of a physicist (Ph.D. from Yale) and an expert in laser spectrometry. This background has, during his nearly 10 years with NCI/CCR's Experimental Immunology Branch, given him a unique perspective on the mysteries of cell nuclei. "Al Singer was crazy enough to hire me," he joked.

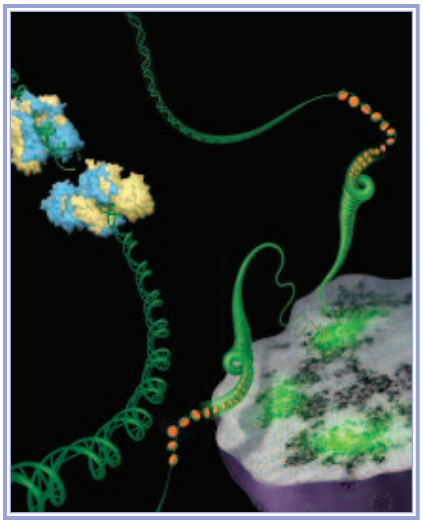
Nussenzweig "learned biology" during a postdoctoral stint at Memorial Sloan-Kettering Cancer Center, where he studied a mouse model of defective DNA repair. It was there that he first teamed up scientifically with his brother Michel, a Howard Hughes Medical Institute Investigator at the Rockefeller University. This familial collaboration continues to this day.

(*Photo*: R. Baer)

Elsa Callen, Ph.D.
Visiting Fellow

For the past three years in Nussenzweig's lab, Elsa Callen has been working to understand the possible roles of DNA repair proteins normally seen in diseases like lymphoma. Before joining NCI, Callen completed her Ph.D. at the Universitat Autonoma de Barcelona (Spain) at the end of 2004, where she studied Fanconi anemia, a devastating disease that can be caused by a set of genes involved in DNA repair. This work led her to seek further understanding of DNA repair mechanisms in Nussenzweig's lab.

"Working here has many advantages," said Callen, particularly in the availability of necessary resources and the collaborative atmosphere. "It makes it really easy to develop interesting, challenging, and high quality projects that would be almost impossible under other circumstances."



(*Image*: A. Nussenzweig, CCR)

Figure 3: If the protein ATM (blue and yellow) is mutated, double-strand DNA breaks can go unrepaired and result in genomic damage that can be passed on to daughter cells. If unchecked, this damage can promote the development of lymphomas and the rare disorder ataxia telangiectasia.

contribute to the oncogenic transformation of mature lymphocytes.

In a separate study published recently in N*ature*, a group of NCI scientists, led by Raffael Casellas, Misteli, and Nussenzweig, identified one mechanism by which ATM protects cells from passing on genetic aberrations to progeny cells. When mouse cells are exposed to a DNA damaging agent, the resulting double-strand breaks transiently inhibit RNA polymerase I (which is required for the synthesis of ribosomal RNA) via the ATM pathway. Thus, in response to chromosomal breaks, ATM shuts down ribosomal gene expression, which ultimately impacts the assembly of all cellular proteins.

This new finding suggests new avenues of research for understanding the role of translocations in early disease development. Such insights into the structural and molecular defects that happen in the earliest stages of chromosome translocations have perfectly positioned CCR researchers to develop novel strategies for the early detection of cancer and, perhaps, to even prevent these genetic abnormalities from occurring in the first place.

The Right Place at the Right Time

(*Photo*: R. Baer)

Tom Misteli, Ph.D.
Head, Cell Biology of Genomes Group, Laboratory of Receptor Biology and Gene Expression

Tom Misteli's focus when he joined NCI was on setting up a cellular imaging program, based on his pioneering efforts to visualize gene expression and protein dynamics in living cells. However, he soon started looking at the behavior of whole chromosomes within their nuclei. "I always wanted to do work on chromosome positioning, but it just wasn't fundable outside of the NIH," said Misteli.

His success has attracted numerous research fellows. "We have 10 to 12 people at any time now, all extremely good at what they do," said Misteli. "In fact, every postdoc in my lab is better than I ever was."

Misteli received his Ph.D. from the University of London and the Imperial Cancer Research Fund, London, United Kingdom.

(*Photo*: M. Spencer)

Karen Meaburn, Ph.D.
Visiting Fellow

Karen Meaburn has been doing research in the Misteli lab for the past two and a half years on understanding chromosome and gene positioning, particularly in breast cancer. Her hope is that her work will help lead to ways to detect breast cancer at its earliest stages.

According to Meaburn, being at NCI provides her with both the support she needs and intellectual stimulation beyond her immediate area of study. "There is the opportunity to get the resources to try things out," she said, "and there is always someone doing something I am interested in."

Meaburn received her Ph.D. in biological sciences in 2005 from Brunel University in London, United Kingdom.

(*Photo*: M. Spencer)

Evi Soutoglou, Ph.D.
Visiting Fellow

Evi Soutoglou has spent four years at NIH, first as an International Human Frontiers Science Program Fellow and now as a Visiting Fellow in the Misteli lab. She says that imaging work was an "obvious" choice for her, and that the capabilities and research interests at NCI attracted her, particularly in working in the Misteli lab in understanding the stability of chromosome breaks.

"Tom is not afraid to try new things and brings the energy and resources to help us address difficult but interesting questions," said Soutoglou, who also cites the collaborative atmosphere provided by the lab and by her colleagues in other labs as unique aspects of the NCI intramural program.

Soutoglou received her Ph.D. in 2002 from the University of Crete's Institute of Molecular Biology and Biotechnology.