

It Starts with a Choice: Cancer Cells and Their Decisions to Replicate

A Senior Investigator in CCR's Laboratory of Molecular Pharmacology (LMP), Mirit Aladjem, Ph.D., has a long-held fascination with the choices cells make when DNA replication goes awry. She realized that the use of alternative signaling pathways lies at the heart of cancer's survival mechanisms—cells that choose to replicate unstable DNA and then divide can seed tumors, while those that choose to self-destruct by apoptosis can impede tumor growth.

Aladjem established her career with research showing that DNA sequences called “replication origins” genetically coordinate replication in mammalian cells. Today, she studies how these intricate signals orchestrate the mysterious process of copying DNA strands. Aladjem's work both advances basic science in cell biology and sets the stage for translational research that can develop therapies to halt the division of malignant cells.

Born and raised in Israel, Aladjem completed her Ph.D. in 1992 at Tel Aviv University, where her studies focused on chemical carcinogenesis. From there, she moved to the Weizmann Institute of Science, in Rehovot, Israel, for a short stint studying protein chemistry, before going to the Salk Institute, in La Jolla, Calif., for a postdoctoral fellowship that set the stage for her research today.

It was at the Salk that Aladjem confronted a contentious dispute in cell biology. On the one hand, many scientists suspected that DNA replication in mammals starts randomly, and not at predetermined genetic sites as had been discovered in yeast cells. Other scientists suspected that DNA replication could not start randomly on chromatin, which is the dense mass of DNA and proteins packed tightly into the nucleus. These researchers held that authentic replication origins in mammalian cells had simply not yet been discovered.

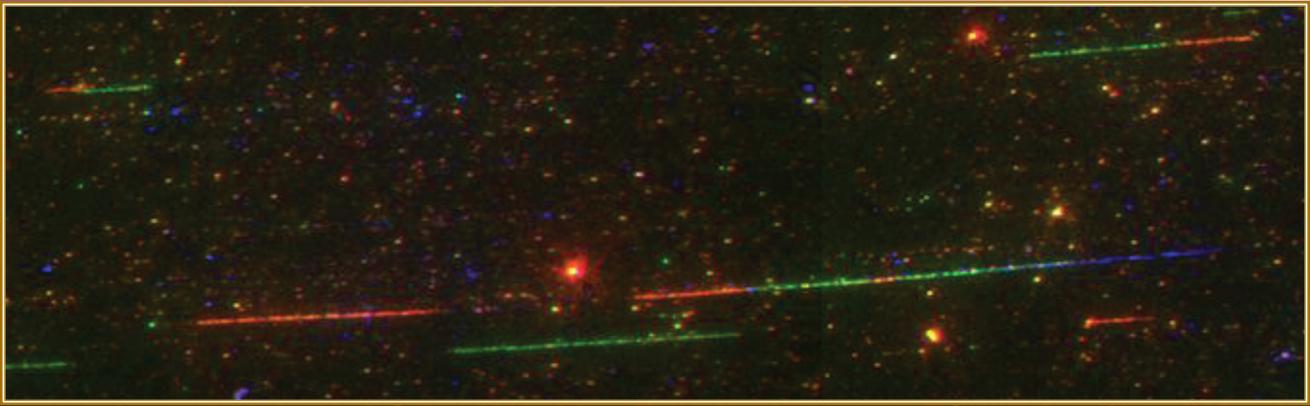


(Photo: R. Borer)

Mirit Aladjem, Ph.D.

Much of what scientists knew at the time about the replication machinery came from studies in yeast. Those investigations had revealed that origins recruit and then bind a suite of proteins known collectively as the “origin recognition complex (ORC)”, and that the union of those entities—i.e., the origin,

ORC, and some other proteins—creates a “pre-replication complex” that sits on the chromosome and launches replication once activated. A member of the pre-replication complex, known as helicase, starts the replication process when it wedges itself between the helical strands of DNA and splits them



DNA replication in human cancer cells. DNA replication can be directly followed by labeling the replicating genome with fluorescent dyes (green and red) and visualizing the DNA strands on microscope slides.

... human replication origins have direct effects on chromatin and its packaging in the nucleus.

apart. Each of those strands then becomes a template for newly created DNA.

Through meticulous experimentation in a genomic region called the human beta globin locus (a five-gene cluster involved in the production of hemoglobin, which has been extensively sequenced), Aladjem and her advisor, Geoff Wahl, Ph.D., at the Salk Institute, confirmed for the first time that origins also coordinate replication in mammalian cells. By moving specific sequences around in chromatin, Aladjem and Wahl showed that these origins could also initiate replication at different genomic sites.

Using New Tools to Study Replication Networks

Aladjem came to CCR in 1999, to build on what scientists increasingly recognize as the important role that aberrant DNA replication plays in cancer. Throughout her time here, she has used genetic, biochemical, and bioinformatic tools to study the cell networks that signal to and from chromatin during replication. Part of the challenge, she explains,

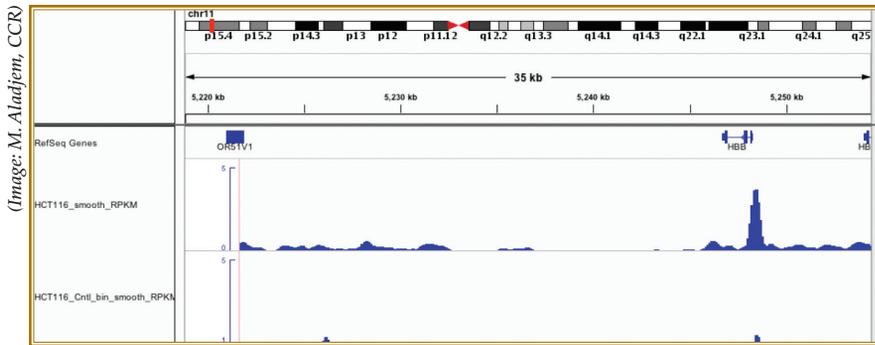
is to link what scientists glean from studies in yeast with what she and others are learning about replication in mammalian cells. Most of the yeast proteins involved in DNA replication are also encountered in human cells—suggesting their importance because they have been conserved through evolution. However, human origins are more varied than those encountered in yeast, as are the proteins that bind them. Identifying all the mammalian replication proteins and describing the nature of their interactions with chromatin is what drives Aladjem’s research today.

At the NCI, Aladjem has shown that human replication origins have direct effects on chromatin and its packaging in the nucleus. Focusing mainly on the human beta globin locus (Aladjem muses that this is her favorite genomic region), she and her research team identified the precise sequences upon which origin activity depends, and then they set out to identify the proteins that bind human origins in sequence-specific ways. Her hope, Aladjem says, is that these proteins facilitate whether replication starts or stops

in response to cell cycle signals, which are unregulated in cancer. Should that prove to be true then the aberrant proteins and/or their signaling partners might be targeted with drugs.

But a number of hurdles must still be overcome: Aladjem points out that while she and her colleagues know where origins exist in the human beta globin locus, their locations elsewhere on the chromosome are still being determined. “So we do not know how relevant our findings concerning the human beta globin locus origins will be in other genomic locations,” she explained. To broaden their perspective, Aladjem’s research team has recently mapped replication origins in whole genome sequences from cancer cells. Mining that database will allow them to study the cell’s entire origins population, rather than just a few in the beta globin locus.

Her genomic mapping studies recently showed that human origins differ from those in yeast in an important way. Yeast replication origins are typically dominated by adenine-thymine (AT) sequences, which are more loosely connected to each other than sequences made up of cytosine-guanine (CG) base pairing. The flexibility afforded by the AT bond allows helicase proteins in yeast to come between the DNA strands.



Direct measurement of the locations of replication start sites (replication origins) throughout the whole genome by sequencing short newly replicated DNA. Replication patterns at the beta globin locus on human chromosome 11 (shown at the top track) is shown. Genomic regions that start DNA replication appear as peaks in the bottom track.

The yeast cell’s ORC proteins clamp down tightly on AT-rich sequences. But last year in *Genome Research*, Aladjem reported that in mammalian DNA, replication depends on both AT- and CG-rich sequences. Her study showed that replication starts preferentially in methylated CG regions. Methylation is an epigenetic signal that modifies chromatin in a way that might taper transcription and put helicase into action.

That same study also revealed that DNA replication occurs in areas with low levels of gene transcription, but where gene transcription

levels are high, DNA replication is diminished. That finding addressed another ongoing debate in the field: previously, some scientists had claimed that replication represses transcription, while others had reported that transcription and replication occur hand-in-hand. “We found that everyone is right,” Aladjem said. “When transcription goes from zero to low levels, it encourages replication; but when transcription goes from low levels to high, it prevents replication.” Aladjem speculates that this occurs because both processes require

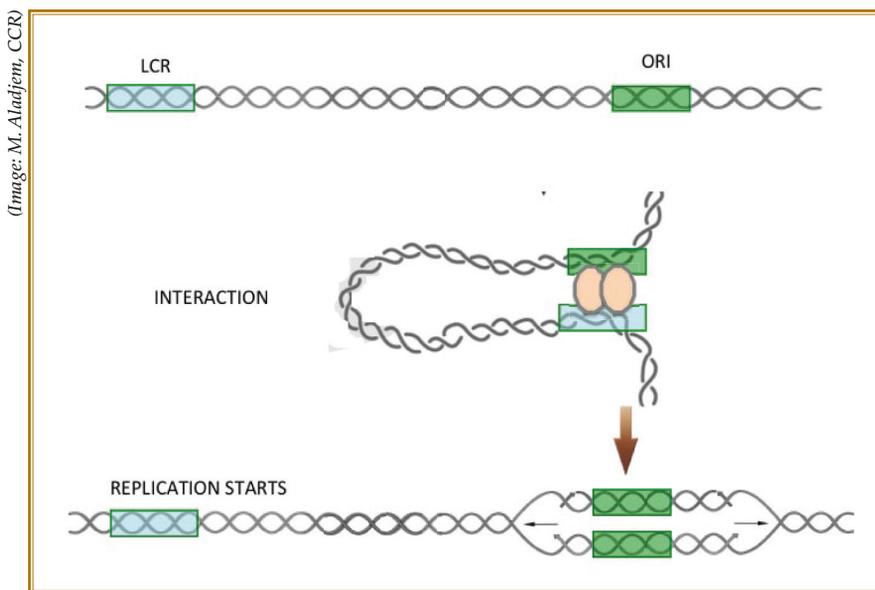
large protein complexes—the pre-replication complex and the transcription initiation complex—which compete for space on chromatin. “These complexes do not like to sit next to each other,” she explained.

Biological activity at replication origins ultimately distills to whether cells will replicate DNA and pass through checkpoints that govern cell division. This is vitally important in the context of cancer. When confronted with potentially harmful mutations, or with conditions that might mutate replicating DNA, cells typically arrest at the early (G1) phase of the cell cycle—before replication occurs—or they pause in the middle of synthesis (called S-phase) until the DNA damage or conditions that might cause it are addressed. “Cancer cells do not exhibit the same active cell-cycle checkpoint pathways found in noncancerous cells,” Aladjem said. “So we study how cell-cycle regulatory pathways interact with chromatin to activate checkpoints when needed, and how these interactions vary in cancer cells.”

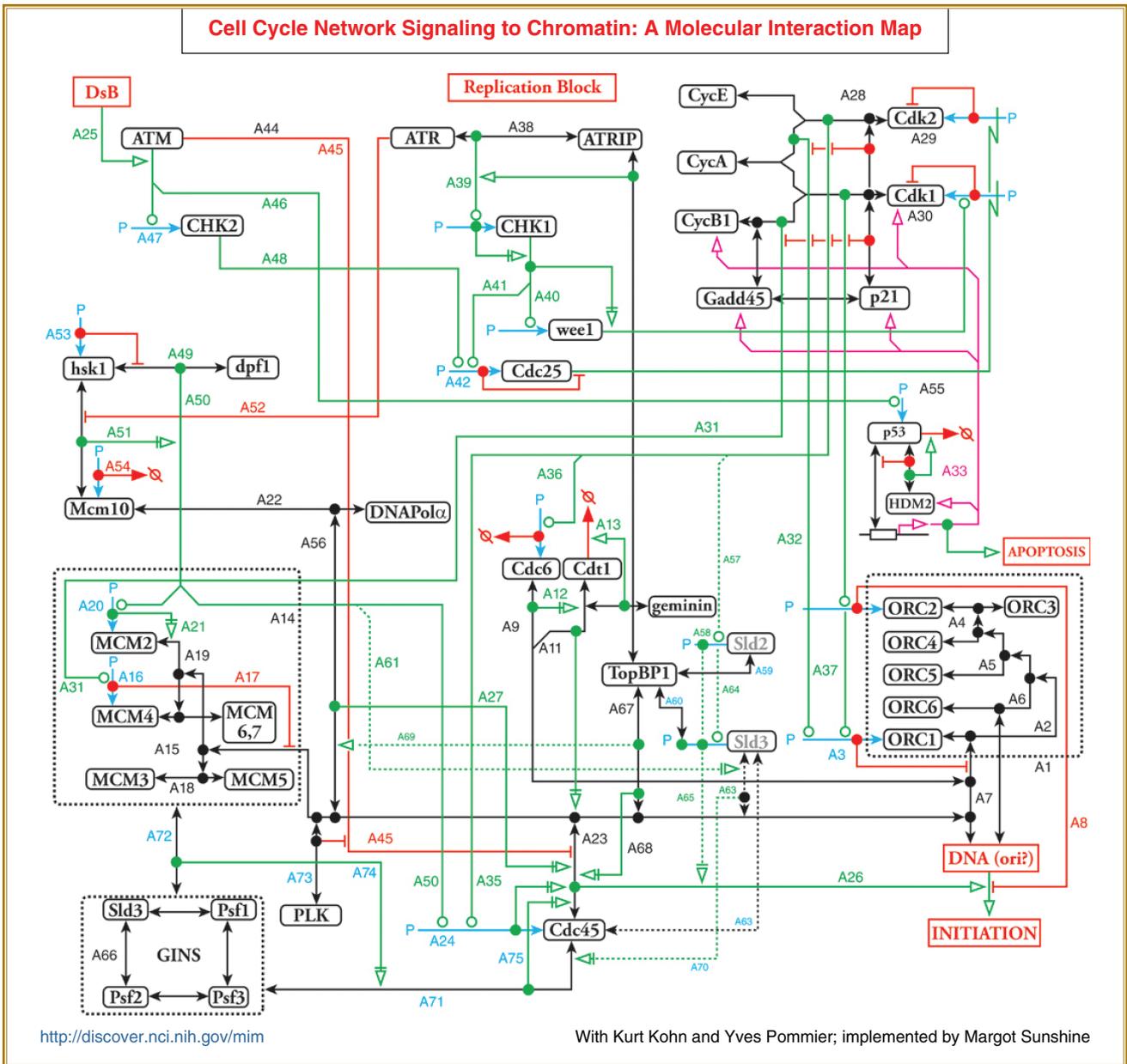
Replication as a Target in Drug Development

Now, those investigations are generating translational opportunities because interfering with replication offers a new mechanism for cancer therapy. Yves Pommier, M.D., Ph.D., Chief of LMP, collaborates with Aladjem on applications for her research in drug development. “If we can find differences in how cancer and normal cells replicate DNA, and find fragile points in the cancer cell’s replication initiation program, then we can rationalize therapeutic approaches to selectively kill these cells,” he said.

Pommier’s research has shown that a class of drugs that inhibit topoisomerase (an enzyme that



The locations of replication origins are determined by protein complexes that promote interactions between distant regions on chromatin.



A molecular interaction map of the events leading to replication. For details, see <http://discover.nci.nih.gov/mim>

regulates the over- or under-winding of DNA) has a “strong effect” on replication origins. These effects are especially pronounced in cancer cells. Through his collaborations with Aladjem, Pommier found that cancer cells have trouble adjusting replication programs in response to topoisomerase inhibitors, so they undergo apoptosis, while normal cells simply pause during division until the drug washes out from the cells. “Dr. Aladjem has the molecular

biology know-how we need for this research, while my laboratory has more expertise on the drug side; this is how our work is complementary,” he said.

Basic discoveries made by the Aladjem team are beginning to reap rewards for translational research. As Aladjem explains, “Our findings about human replication origins are prompting scientists to rethink replication complexes as determinants of cell-growth

responses; this is becoming more and more important to the development of new targeted therapies. The scientific community has begun to unravel the mystery of mammalian replication origins.”

To learn more about Dr. Aladjem's research, please visit her CCR Web site at <http://ccr.cancer.gov/staff/staff.asp?name=aladjem>.