



Richard
Kolodner

Photo by Stewart Marciano

THE MUTATOR HUNTER

Richard Kolodner has made landmark discoveries on DNA repair and its link to cancer. Now he has unearthed a passel of genes that stabilize the genome, and are often defective in tumors.

There are many reasons to become a scientist, and not doing what your dad does is probably as good as any. Just ask Ludwig San Diego Director Richard Kolodner, who hopscotched from city to city in his childhood as his father, a gifted mathematician, took jobs at institutes and universities across the US. “I became interested in science in part because I didn’t want to be a mathematician,” he says, chuckling.

Mathematics’ loss, it turns out, was a big win for biology. Over the last four decades, Kolodner has methodically probed the molecular mechanisms by which living things—from plants to yeast to humans—guard the integrity of their genetic information, making landmark discoveries in plant, microbial and cancer genetics. In 2016, he added another chapter to his storied career with a paper in *Nature Communications* describing the results of a study he led with Ludwig San Diego’s Christopher Putnam and Ludwig alum Sandro de Souza, a professor of Bioinformatics at the Federal University of Rio Grande do Norte in Brazil. Kolodner and his colleagues describe in their paper how they developed and applied a novel method to suss out the full spectrum of genetic pathways that ensure the stability of the genome. When compromised, these pathways cause massive genomic

rearrangements that can fuel a wide variety of malignancies.

DNA DETECTIVE

After finishing high school in Pittsburgh, Pennsylvania, where his father was then chair of the mathematics department at Carnegie Mellon, Kolodner joined a bachelor’s program in Biological Sciences at the University of California, Irvine, and then the Biological Sciences graduate program there, completing his bachelor’s and PhD in a little over 6 years. Characterizing the DNA of chloroplasts, the bean-like organelle in which plant cells turn sunlight into food, Kolodner helped pioneer plant molecular biology as a graduate student. In 1976, he began a post-doctoral fellowship in Charles Richardson’s lab at Harvard Medical School, learning how to purify viral proteins and studying DNA replication in the T7 bacteriophage, a virus that infects bacteria.

Hired to the medical school’s faculty following his post-doc, Kolodner started a lab at the Dana-Farber Cancer Institute and began exploring bacterial recombination—a bacterial version of sex that generates genetic diversity. He also started looking into a phenomenon that would occupy him for the next few decades: how a couple (or a few) erroneously paired bases in



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replicating DNA are repaired by cells to prevent mutations. This work led to the development of some of the earliest assays for bacterial mismatch repair (MMR), many of which are still in use today.

By the mid-1980s, Kolodner had shown that yeast too can detect and fix mismatched bases in their DNA and had identified mutations in the known yeast mismatch repair gene, PMS1. This set off a search in his lab for additional yeast MMR genes, resulting in the identification of the MSH1 and MSH2 genes, among others. Kolodner noticed that the effects of MSH1 mutations resembled some features of cellular defects in human genetic diseases known as mitochondrial myopathies. The observation set off a new hunt in Kolodner's lab for the human homologues of yeast MMR genes.

By late 1993, Kolodner and his lab were in a race to identify the human MMR gene responsible for a relatively common form of inherited colon cancer known today as Lynch syndrome. They were uniquely suited to the task. Researchers, including a team lead by Ludwig Johns Hopkins Co-director Bert Vogelstein, had reported that summer the presence of mutations in the Lynch genome that looked an awful lot like the mutations Kolodner had seen in yeast with defective MMR genes.

Within six months, Kolodner and his collaborators had cloned the human MSH2 gene and established that an inherited mutation of the gene caused Lynch syndrome in a patient. It was the first inherited gene defect shown to be associated with cancer. By March of the following year, Kolodner and another colleague had identified a second human MMR gene that causes Lynch syndrome, known as MLH1.

As Kolodner characterized his new genes over the next few years, he developed



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diagnostic tests that were used for years to diagnose Lynch syndrome. He and his colleagues also showed how MMR genes could play a key role in the genesis of spontaneous colon cancers, which account for the vast majority of such cancers. "We showed," says Kolodner, "the gene for MLH1 is methylated and silenced—so it is not expressed—in non-inherited colon cancers." The methods used to detect such silencing, which were also developed in Kolodner's lab, remain in use today.

A NEW MUTATOR

In 1974, researchers put forward a "mutator phenotype hypothesis," which held that cancer genomes must have high rates of mutation to account for all the changes to tumor suppressor genes and oncogenes that drive the progression of cancer. "Our detection of defects in MMR genes," says Kolodner, "was among the first elements of proof for that idea because you had a gene defect that caused high

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The approach Kolodner took to find MSH2 is characteristic of his research. In searching for an answer to any new question, his lab tends to begin with yeast, performing genetic experiments on them that are technically cumbersome or impossible in mammalian cells. How things work in yeast often reflects what goes on in human cells, especially in the genetics and biochemistry fundamental to cellular life.

“MMR defects cause a particular class of mutations,” says Kolodner. “Mostly single base changes, and insertions and deletions of one or a few DNA bases. But in cancer there are more drastic genetic alterations that for all practical purposes are also mutations.”

These include events in which entire chunks of chromosomes are rearranged,

deleted or chopped off and glued to other chromosomes, causing massive genetic disruption. More advanced and drug resistant cancers, in particular, are prone to such aberrations.

“It was my view that genome rearrangements in cancers might also reflect a mutator phenotype, which is to say they are caused by defects in genes unknown,” says Kolodner. He got a chance to test this idea when experiments done in his lab revealed mutations in yeast that appeared to cause large genome rearrangements of the kind seen in cancer.

MUTATORS IN SCADS

These, Kolodner figured, might be the genome instability suppressing genes (GIS) that, when mutated, cause gross chromosomal rearrangements, known in the shorthand as GCRs. Kolodner’s team—including Ludwig San Diego associate investigator Christopher Putnam, postdoc

Anjana Srivatsan and Sandro de Souza, who received Ludwig funding for the work—devised an ambitious approach to finding the GIS genes. They combined methods from yeast genetics and bioinformatics to identify GIS genes—finding them first in yeast and then applying what they learned to human cancers.

The researchers first used assays and technologies from Kolodner’s lab to screen thousands of mutant yeast strains for genes that suppress GCRs. They identified 182 GIS genes, 98 of which had not been noted before. They also uncovered more than 400 previously unknown cooperating genome instability suppressing genes (cGIS), which only affect genome stability when combined with other mutations. Only a few dozen such genes had been described before.

To see if these genes had counterparts implicated in human cancers, the team searched The Cancer Genome Atlas (TCGA)—which contains genomic data from thousands of patients—for genes similar to those they had found in yeast. They also added to this list human genes that are not found in yeast but that participate in the same biochemical pathways and protein complexes as the analogous yeast GIS genes. They then looked for defects in these genes in the TCGA in each of three cancers: ovarian cancer, colorectal cancer and acute myeloid leukemia (AML).

The leukemia served as a control because it is a cancer that has a relatively stable genome. The other two, however, have extremely unstable genomes. The researchers found that 93% of ovarian cancers and 66% of colorectal cancers had genetic defects affecting one or more of the predicted GIS genes. The AML, as they expected, had no such defective genes.

Kolodner now has an embarrassment of

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genetic riches to mine as he moves forward. “The problem is this is a huge project,” he says. “We simply couldn’t do all the interesting experiments that can be done, so we’re focusing on a few things.”

That includes tumor suppressor genes they’ve identified in their analysis. They’re also expanding their analysis to uncover GIS defects in other types of cancer, and mutating the GIS genes they’ve identified to see if they can’t induce genome instability in human cells. Kolodner is now working with the Ludwig’s Small Molecule Discovery Program to see if their discoveries might be exploited to develop new cancer diagnostics and therapies. ■