Human DNA Polymerase ι: Wrong for the Right Reasons

In a recent collaborative study, Thomas A. Kunkel, director of the NIEHS Environmental Biology Program, NIEHS deputy director Samuel H. Wilson, and Roger Woodgate, an investigator at the National Institute of Child Health and Human Development, report novel findings about DNA repair and genome stability in humans. Woodgate's laboratory recently discovered and purified polymerase iota (pol ι), one of a superfamily of DNA polymerases that synthesize short stretches of DNA and are relatively inaccurate—that is, they may allow the A, C, G, or T base moieties to be matched to an incorrect mate (for instance, matching G with T or A, rather than with its proper mate, C). The scientists speculate that these enzymes' importance therefore lies in DNA repair and damage-induced mutagenesis, not in DNA replication.

The highly accurate DNA polymerases that carry out DNA replication are blocked by DNA damage. In contrast, some of the less accurate polymerases, also called translesion synthesis (TLS) polymerases, bypass damage in the DNA, fall off after inserting a few nucleotides, and then allow an accurate replicative polymerase to continue. Thus, TLS polymerases may allow damaged cells to survive at the cost of a slightly higher mutation rate in regions of DNA damage.

The results of the collaboration between the Kunkel, Wilson, and Woodgate laboratories, published in the 16 March 2001 issue of *Science*, indicate that pol ι has a novel enzymatic activity called 5′-dRPase, which removes damage from the 5′ end of a broken DNA strand. This activity was previously demonstrated in polymerase beta, which is active in a repair pathway called base excision repair (BER). dRPase activity is required during many BER reactions, suggesting that pol ι could participate in BER.

In addition, Kunkel and colleagues show that the sequence of DNA in question greatly influences whether pol ι forms a correct base pair (namely AT, TA, GC, or CG) or an incorrect base pair (for example, GT). Pol ι forms an AT base pair inserting T opposite A 40- to 100-fold more efficiently than it forms the other correct base pairs, a property that had not been reported previously. However, says Kunkel, "The most unusual thing about pol ι is its preference to insert G opposite T rather than A opposite T." The rate at which pol ι makes this error during DNA synthesis is higher than observed for any base pair. Based on their results, Kunkel and colleagues suggest two novel roles for pol ι in BER.

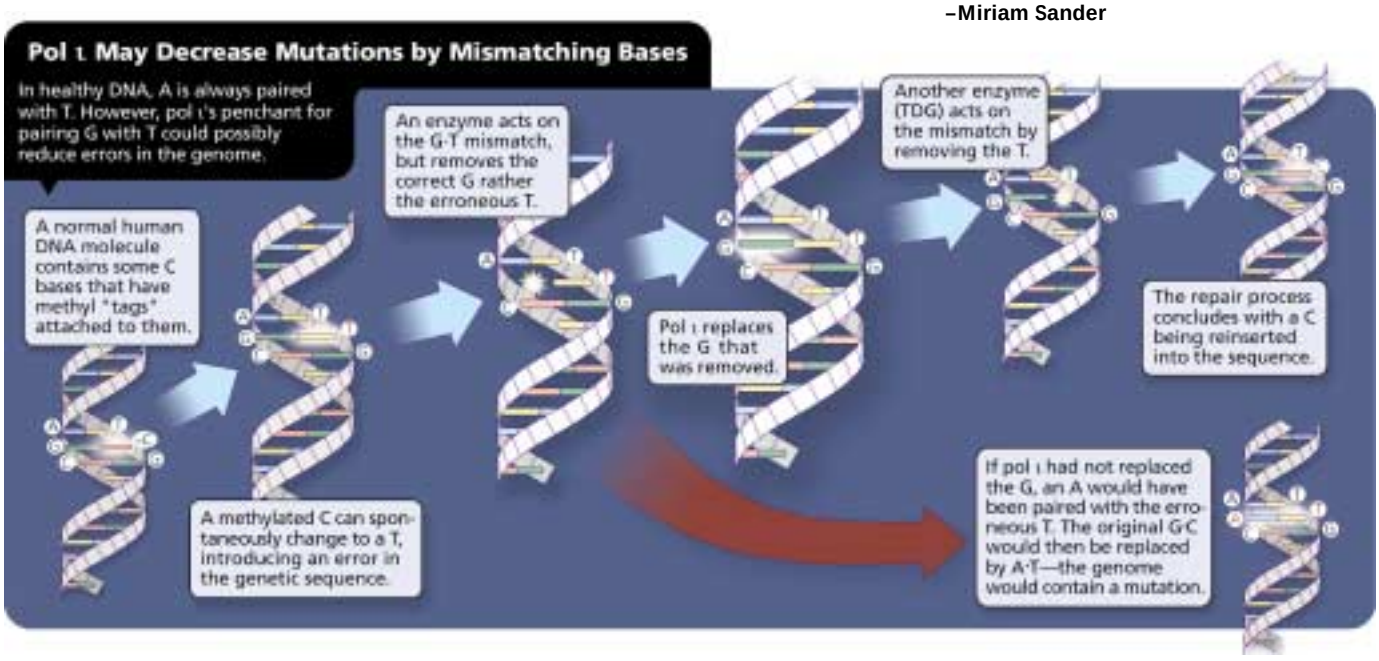
In the first possible role, pol ι may play a role repairing uracil (U) in DNA. U is a normal, correct base in RNA that is not found in normal undamaged DNA. It forms in DNA spontaneously and enzymatically when a C base undergoes a normal damage process known as deamination. It can also be misinserted during normal DNA replication. In this role, U is removed enzymatically from an incorrect AU base pair, and then pol ι replaces it with T, a reaction that calls on the dRPase activity of pol ι and that is highly accurate.

In the second role, pol ι could also prevent misrepair of GT mismatches. In this scenario, a GT base pair forms spontaneously when C undergoes deamination. When this occurs, G is the correct base and T is the incorrect base in the mispair. The incorrect T base should be removed and replaced with a C base, but if the correct G is removed instead, a mutation could result. In this case, Kunkel and colleagues suggest that pol ι might reinsert G opposite T as a "correct" insertion event that stabilizes the genome, allowing the normal sequence to be restored.

This is a surprising suggestion, because it is thought that TLS polymerases are inaccurate and that they form mispairs that increase the mutation rate. Kunkel's idea is that pol ι forms a mispair in a specific context where it actually decreases the mutation rate. Errol Friedberg, a professor of pathology at the University of Texas Southwestern Medical Center at Dallas, finds this "a very interesting idea" that suggests that these polymerases "may have very specialized functions that allow them to be error-free."

Friedberg points out that "the biochemical evidence needs to be backed up by genetic evidence" in order to establish the full extent of the role or roles of pol ι *in vivo*. It is clear that pol ι creates errors with high frequency in certain sequence contexts; thus, this enzyme, like other TLS polymerases, must be tightly regulated *in vivo*. More research is needed to clarify the biologic roles and the regulation of pol ι and other TLS polymerases.

–Miriam Sander



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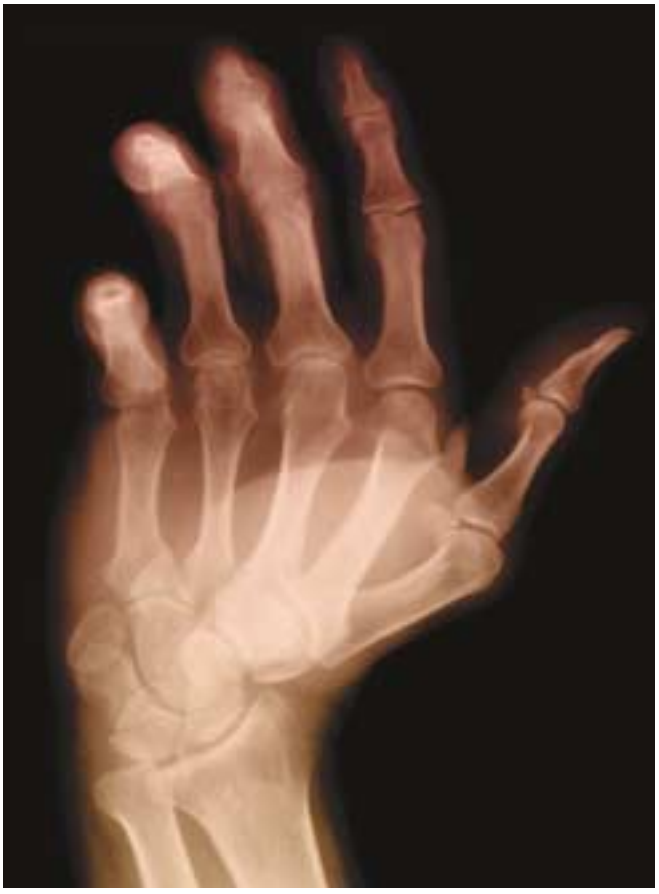
New Research Group to Target Autoimmune Disorders

A recently formed clinical research group will soon begin studying how the complex interplay between genetics and the environment may influence the development of autoimmune disorders. Working out of the NIH's Warren Grant Magnuson Clinical Center in Bethesda, Maryland, the Environmental Autoimmunity Group first will investigate myositis, a little-known disorder that causes muscle inflammation and weakness throughout the human body and can affect breathing, digestion, and function of the heart muscle. Down the road, the team will broaden its focus to include other autoimmune disorders with suspected environmental triggers, including rheumatoid arthritis, lupus, type 1 diabetes mellitus, multiple sclerosis, scleroderma (a thickening of the skin), and autoimmune thyroid disease.

"Members of the group will work in collaboration with their colleagues at the NIEHS campus in North Carolina and those in other institutes at the Clinical Center at NIH," says Perry Blackshear, NIEHS director of clinical research, who will work with the group. Frederick W. Miller, a specialist in immunology, internal medicine, and rheumatology, will lead the group in close association with senior clinical investigator Lisa G. Rider, who specializes in immunology, pediatrics, and pediatric rheumatology. Biologist Terrance P. O'Hanlon and postdoctoral researcher Ejaz A. Shamim will join them.

According to Miller, scientists have long suspected that certain genetic and environmental risk factors can work together to lead to disease. Myositis—a chronic, incurable, potentially fatal disease that afflicts at least 30,000 children and adults in the United States—may be triggered by infections or exposure to certain drugs or sunlight in people who are genetically predisposed. The group's initial goal will be to understand the disorder's possible genetic and environmental risk factors, which they plan to accomplish through several stages of studies.

"Some studies will have to be hypothesis-generating investigations," explains Miller. After that will come cohort–control studies to compare significant differences between normal individuals and patients, followed by further studies to confirm those results. "To better understand genetic risk factors, we're also considering a number of case–control studies using a targeted gene approach," Miller says. He adds, "In order for us to know how to treat the disease, we have to know how to measure the disease." This will require validation studies of disease assessment tools. "This is necessary before we can do a treatment study," says Miller.



Getting a handle on autoimmune disorders. A new NIEHS research group seeks to understand the gene–environment link in autoimmune diseases such as rheumatoid arthritis.

Myositis, like many autoimmune disorders, is often underdiagnosed for two chief reasons. First, patients exhibit nonspecific symptoms common to many diseases and disorders (such as muscle or joint pain and fatigue), or there is no clinical manifestation of the illness until long into the disease phase. Second, the disorders themselves are rare, and physicians don't diagnose disorders they know little or nothing about. Armed with more knowledge of the disease, physicians will eventually stand better equipped to diagnose and treat it. But because myositis is one of the rarest autoimmune disorders, Miller says, one initial challenge will be finding enough patients—100 or so for each study. For that reason, the research group will collaborate with doctors in other locations.

The research team will also explore the possibility that certain autoimmune disorders may actually be collections of a number of so-called elemental disorders, each with its own pathogenesis and trigger. "This may be a major confounder of current studies," Miller explains. There is increasing evidence, he says, that myositis, rheumatoid arthritis, lupus, and scleroderma as they are defined today are actually aggregate conditions composed of different elemental disorders.

The new team is unique in that it will study both adults and children. Rider says the team's focus on the environment marks a significant departure from much of past research on childhood rheumatic diseases, which has focused more on genetics. Among other things, the team will study the roles that infectious agents and sunlight play in the development of a form of myositis called dermatomyositis. "We'll also be looking at a phenomenon known as microchimerism, which is the result of migration of cells between mothers and fetuses," Rider says. In microchimerism, a small number of cells circulating inside an individual's body actually come from another individual's body. The most common form of microchimerism occurs during pregnancy, when cells from the fetus's and mother's bodies can interchange. Microchimerism is suspected to play a role in both myositis and scleroderma. "We suspect that [microchimerism] has a role in juvenile dermatomyositis and now need to understand whether it has a role in pathogenesis," Rider says.

Rider suspects a number of environmental agents, including silica, mercury, estrogenic compounds, pesticides, and others still unidentified may influence disease. More than likely, multiple environmental triggers combine to influence a single autoimmune disorder. "As these diseases are polygenetic, they are also going to be polyenvironmental," she predicts.

–Jennifer Medlin

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