Stalking the Start of Colon Cancer

Research teams have now linked hereditary colon cancer to damage in two genes. Both are part of a DNA repair process that, when disrupted, may set the stage for the disease

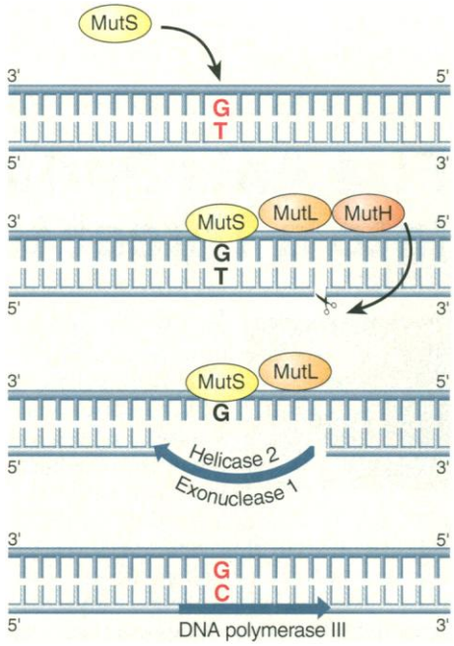
The study of colon cancer genetics is red hot: For the second time in 3 months, two teams of researchers have raced to uncover a gene involved in a hereditary form of colon cancer. And for the second time the race ended in a dead heat, although the two groups took very different routes to the finish line—one of which exploited private DNA databases that are controversial but cut the time needed to get the gene.

The two teams, one led by Bert Vogelstein and Kenneth Kinzler of the Johns Hopkins School of Medicine, the other by Richard Kolodner of Harvard's Dana-Farber Cancer Institute, isolated a gene known as *hMLH1*, which may play a role in up to 30% of cases of hereditary non-polyposis colon cancer (HNPCC). The discovery follows a similar tie in December, when the same groups announced isolation of a gene called *hMSH2* (*Science*, 10 December 1993, p. 1645). Defects in that gene, they estimated, accounted for as many as 60% of the cases of HNPCC; combined, defects in the two genes can account for the vast majority of these cancer cases. Kolodner's group reported their results in the 17 March *Nature*, and the Hopkins team describes their find on p. 1625 of this issue of *Science*.

The two genes are part of a DNA repair pathway that may provide one of the fundamental roads to cancer when it is disrupted by mutations. "This is an extremely important development," says Lee Hartwell, a geneticist and cancer researcher at the University of Washington in Seattle. "In the last decade, most of the focus [in cancer research] has been on genes that change in tumor cells: oncogenes and tumor suppressor genes. Now we are going back to view what is probably the primary cause of cancer, which is how a cell develops the capacity to evolve quickly from a normal to cancerous cell."

Genetic housekeepers. Mutations in the two recently uncovered genes apparently have a central role in a process known as DNA "mismatch repair." The building blocks of DNA—nucleotides—are normally paired in a specific pattern between the two complementary DNA strands. Mistmatched nucleotides signal a genetic error. In their normal roles, *hMLH1* and *hMSH2*, like genetic proofreaders, seem able to spot mismatches and orchestrate the enzymes that effect repairs. "These genes are the mothers of all housekeeping genes," says Vogelstein.

ILLUSTRATION: K. SUTLIFF



Repair crew. These enzymes (MutS, MutL, MutH) fix DNA mismatches in *E. coli*. Damage to genes coding for their human counterparts could trigger hereditary colon cancer.

If these housekeepers don't function, errors accumulate in the course of many generations of cell division—and eventually a cancerous mutation occurs.

The Hopkins and Harvard teams have been focusing on the role of DNA repair genes in colon cancer; in December both groups pinned *hMSH2* to chromosome 2. However, the teams knew other genes were involved, since *hMSH2* accounted for only 60% of HNPCC cases. By tracking genetic markers in families with a high incidence of the disease, other research teams, led by Albert de la Chapelle at the University of Helsinki and Magnus Nordenskjöld at Karolinska Hospital in Stockholm, determined that one other contributing gene probably lay on the short arm of chromosome 3.

Since *hMSH2* was a mismatch repair gene, researchers were fairly certain "that the gene on chromosome 3 was probably involved in the same mechanism," says Kolodner. And earlier work on mismatch repair systems in bacteria and yeast highlighted a gene, called *MutL*, as another critical player. This gene appeared to be highly conserved—very similar in nucleotide sequence from organism to organism—so the researchers set out to find a human gene on chromosome 3 with a similar sequence, hoping it would play a similar role.

Both groups knew they could use conventional techniques to fish out a human version of *MutL*. Those conventional methods rely on DNA "probes," based on the genetic sequence of the bacterial and yeast genes, which bind to similar human genetic sequences. Yet the nonhuman sequences have just enough differences from the human ones so that such probes might have a hard time sticking to their target. Fearing that finding a match in this manner could take months, both teams opted for short cuts.

Two roads to discovery. Kolodner and collaborator Richard Fishel of the University of Vermont College of Medicine went to someone who, they thought, already had pieces of the human gene. Michael Liskay at Oregon Health Sciences University in Portland had been studying the role of the equivalent of *MutL* in mice. In 1990 he had used probes from bacterial *MutL* to fish out the mouse gene. During the next few years, out of curiosity, he used those probes to look for the human gene, and he came up with two short, incomplete fragments. Kolodner realized Liskay's fragments could be ready-made probes for the full gene. Liskay agreed. Within a few weeks the group had retrieved a full copy of the human gene and mapped it to region 21 of chromosome 3.

The Hopkins group, meanwhile, took a relatively untested road, traveling via The Institute for Genomic Research (TIGR) in Gaithersburg, Maryland. Researchers at TIGR, along with counterparts at Human Genome Sciences (HGS), the commercial arm of the institute, have been mapping genes backward. They start by capturing messenger RNAs (mRNA) in cells, which cart instructions from genes to the protein-manufacturing ribosomes. The scientists make mirror-image copies of the mRNAs, known as complementary DNAs (cDNAs), which should—in theory—be equivalent to the original gene. TIGR and HGS have copied more than 100,000 fragments of these cDNA instructions, and they store the sequences on a database in a supercomputer. The approach means the scientists often know the structure of these gene stand-ins—but not the function.

Vogelstein and Kinzler gambled that since housekeeping genes are widely expressed in cells, TIGR and HGS already had

a human cDNA sequence in their database similar to bacterial *MutL*. In December, they called J. Craig Venter, TIGR's director, and asked him if he had identified any such candidates. "Within a few minutes of checking," says Kinzler, "they told us that they had three such genes." The cDNA fragment tagged by the computer as the closest match to *MutL*, as it happened, mapped to chromosome 3, position 21. Further analysis revealed the complete gene: *hMLH1*.

The paths of the two groups again converged for the final task: proving that families afflicted with HNPCC consistently had mutations in *hMLH1*. To do so, both teams turned to genetic samples from families previously identified as likely to harbor an HNPCC gene at chomosome 3. And both teams found mutations in *hMLH1*.

The precise functions of these genes on the human repair pathway haven't been worked out, but they do show similarities to their bacterial analogs (see diagram p. 1559). The human gene discovered in December, *hMSH2*, works much like its bacterial equivalent *MutS*—by initially spotting mismatched nucleotides, says Kolodner. If that's the case, it seems logical that *hMLH1* would act like *MutL*, which grabs on to *MutS* and orchestrates the other repair proteins. But no work with the human system yet supports this inference. Nor have scientists charted the entire pathway, for 10% of HNPCC cases are not accounted for by the two known genes—suggesting that at least a third gene and its product are at work. (Indeed, both teams have identified at least one gene as a likely candidate.)

Clearer than the specific mechanism at this point is the speed with which cDNA sequencing produced results. "[The Hopkins-TIGR-HGS study] provides a wake-up call to the future value of the databases that will hold cDNA sequences," says Jefferey Trent, chief of the Laboratory for Cancer Genetics at the National Center for Human Genome Research. "The reason is very simple," adds Vogelstein. "If TIGR or HGS has the gene already, it takes essentially 5 minutes to get this gene, whereas it could take months with the traditional approach." Though Kolodner notes that traditional gene hunters are keeping pace for now, he confesses he's considered the cDNA approach for finding additional human mismatch repair enzymes. "The potential of those databases wasn't lost on us," he says.

Still, many researchers are concerned about work done with the cDNA database because of its effect on research collaborations. The databases belong to private organizations, worries Francis Collins, the director of the National Center for Human Genome Research at the National Institutes of Health, which could keep much of the information out of the public domain. "I'd be a little more relieved if all this work was going to be made available to the scientific community," says Collins (*Science*, 11 October 1991, p. 184). Venter insists that TIGR's work on a large number of sequences will be made public by October. HGS, however, only allows academic researchers access to their libraries if they sign an agreement to give HGS first crack at commercializing any meaningful results; Vogelstein has just signed such an agreement.

One other issue raised by the elucidation of this housekeeping pathway is whether scientists now know enough about it to develop a screening test for HNPCC. Accurate tests require the ability to spot all the different mutations to these genes that cause the disease. At this point, "we have to know a lot more about the spectrum of mutations in these genes," says Collins. But with the repair pathway nearly characterized, the next race—to identify that mutation spectrum—is already well under way.

–Robert F. Service

CONSERVATION BIOLOGY

Fire Ants Parlay Their Queens Into a Threat to Biodiversity

SANFORD D. PORTER

In 1942, an entomologically minded teenager named Edward Wilson spotted some strange ants in a vacant lot near his home in the port city of Mobile, Alabama. They were tiny red things (like the one above) swarming about a conical mound almost a foot high; when threatened, they attacked en masse, stinging repeatedly and painfully. Wilson, now a distinguished entomologist at Harvard, still thinks back to that day with regret. "I am always wishing," he says, "that I had rushed to City Hall in my Boy Scout uniform and told them to wipe out the ants on that lot."

Wilson had made the first recorded observation of the red imported Argentine fire ant, *Solenopsis invicta*—an accidentally introduced species that has become famous as a catastrophic example of what can happen when organisms from one ecosystem are released in another. Within two decades, the ants on that lot spread through the South, hitching rides on sod and nursery shipments. Because this furiously active predator can make outdoor life difficult—even killing small pets—the federal government launched a massive eradication program in 1957. It failed. Today *S. invicta* has invaded territory from southern Virginia to central Texas, and many entomologists believe its appearance in California is only a matter of time.

Southerners long since concluded they have to live with a permanent nuisance, but that may change. Fire ants are spreading in a new, more virulent form that lives in such remarkably high densities it could inflict untold harm on entire ecosystems. Meanwhile, the long-dormant federal fire ant program has begun to gear up for a new round of combat, replacing old chemical weapons with modern armaments of biological control—natural pathogens, parasites, and predators. "Nobody has ever tried biological control on a social insect before," says Walter R. Tschinkel, an entomologist at Florida State University. "If it works, it would be really exciting. More than that, it better work, because the [new form of fire] ants may really be something to worry about."

One of about 20 fire ant species in the New World, *S. invicta* spreads like a weed. Mature queens are 70% ovary and can lay 5000 eggs a day. Their offspring, the workers, forage through a network of tunnels that may extend 50 feet from the central mound. Most ant species have a few hundred workers in a colony; *S. invicta* can have up to half a million—which is why they seem to be everywhere in affected areas.

The ants Wilson saw were territorial and "monogyne"—that is, each colony had a single egg-laying queen. In the 1980s, however, fire ants increasingly appeared in a nonterritorial, "polygyne" form, which creates interconnected "super-colonies" that may have scores of egg-laying queens. "There are places now around Austin [a polygyne center] where you can almost hop from one mound to another like a frog on a lily pond," says Sanford D. Porter, an entomologist at the Insects Affecting Man and Animals Research Laboratory, an Agriculture Department facility in Gainesville, Florida. "You'd better hop lightly, of course."

Genetically, the two forms seem indistinguishable, and nobody knows why the transition occurred. Small polygyne colonies appeared in Mississippi in the late 1970s, later others popped up in Louisiana, Florida, Georgia, and Alabama. Today they dominate Texas and may be ready to spread throughout the South. As a result, entomologists believe, the fire ant is transforming itself from an annoyance into a serious ecological problem. The reason is that polygyne super-colonies increase fire ant densities, already extremely high, by up to a fac-