the very first sign of cancer. But that still gives the patient a jump on the cancer, and analysis of survival data for 46 patients who have undergone surgery after first being alerted by the test indicates that 80% will live at least 5 years.

An even higher survival rate, of course, would follow from cancer prevention. With that end in mind, cancer epidemiologists are trying to pinpoint the risk factors associated with esophageal adenocarcinoma. Some are known already, such as smoking and diets low in fruits and vegetables and high in fats. But, says epidemiologist Thomas Vaughan of the Fred Hutchinson Cancer Center, "all the known risk factors account—roughly—for only half of the cases." Those risk factors also fail to explain the soaring incidence of esophageal adenocarcinoma over the past decade, he says.

Vaughan, in collaboration with epidemiologists from NCI, Yale University in New Haven, Connecticut, and Columbia University in New York City, started a 3-year-long study in 1992 intended to solve the riddle. He and his colleagues are scrutinizing the diets, degrees of obesity, and medical treatments of 700 patients with esophageal adenocarcinoma, 700 patients with other types of stomach and esophageal cancers, and 700 healthy people. For instance, the study will pay close attention to whether or not the adenocarcinoma patients ate abnormally large amounts of processed meats such as hot dogs, says Vaughan. These foods are high in chemicals called nitrosamines (and their precursors), which are known to cause cancer in lab animals.

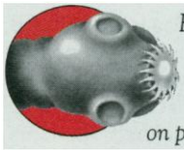
Other prime suspects are certain medicines that have been increasingly prescribed since the 1970s. According to Vaughan, the study consortium is particularly interested in two classes of drugs. One class includes the H_2-blockers that are used to treat stomach ulcers and—ironically—esophageal reflux. These drugs, which suppress production of stomach acid, may promote bacterial growth in the stomach, and certain stomach bacteria produce nitrosamines. The other class of drugs, which includes calcium blockers that are used to treat hypertension and asthma and certain anti-depressant drugs, triggers excessive esophageal reflux as a side effect.

Besides identifying and eliminating risk factors, cancer researchers have one more lead to follow. Occasionally, Barrett's esophagus—the precancerous condition—spontaneously reverts to normal. "It's the strangest thing we've ever seen," says Reid, "We would like to identify the cause and be able to do that on a regular basis." And that's a goal the current work might bring a step or two closer—an achievement that, if the incidence of cancer of the esophagus keeps growing at its current rate, could be very valuable indeed.

–Rachel Nowak

PARASITOLOGY

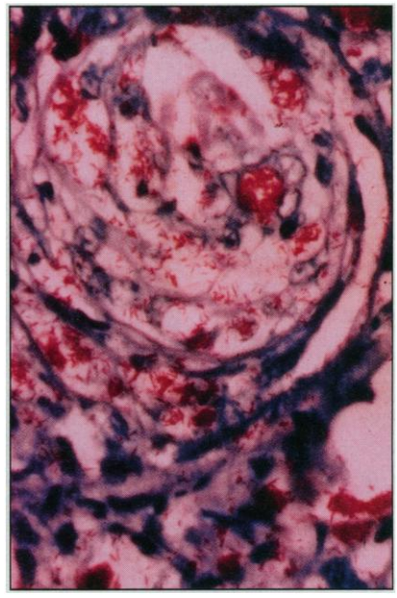
Genome Initiatives Tackle Developing World's Big Killers



For more News, Policy Forums, Perspectives, and Articles on parasitology, see special section starting on page 1857.

Despite the hundreds of millions of dollars that are now being poured into the Human Genome Project, our own genome will be far from the first to be sequenced in its entirety. In fact, the genomes of many simpler organisms will be sequenced long before our own genetic blueprint is deciphered—and included among their number will be a range of important human pathogens and parasites. Earlier this month, for instance, the genomics company Collaborative Research Inc. of Waltham, Massachusetts, announced that it had started a 6-month project to sequence the 1.8 million bases of DNA carried by *Helicobacter pylori*, a bacterium that is believed to induce stomach ulcers and cancer.

The same company is nearly halfway through sequencing the genome of *Mycobacterium leprae*, the leprosy bacterium—a project that should be completed within 2 years. And these bacterial projects are just the tip of the iceberg: A flurry of genome projects for human pathogens are now gearing up, including several targeting protozoan and worm parasites responsible for diseases that are major killers in tropical regions. Most of these projects are hampered by the lack of funding that seems to beset research on these diseases, but their proponents argue that they offer direct benefits to human health that are well worth additional investment.



VISUALS UNLIMITED

Leprosy bacterium. Half of *Mycobacterium leprae*'s genome has been sequenced.

For protozoa and worms, which possess genomes several times larger than those of bacteria, it's not yet practical to begin whole-genome sequencing. So, taking a cue from the Human Genome Project, researchers are trying to assemble genome maps and selectively sequence genes that are expressed in the organism. They hope to identify genes influencing metabolic pathways that could be drug targets or genes that encode antigens that could be built into vaccines.

Since so far only a few tens of genes have been identified in most parasites, many researchers believe the projects currently under way will revolutionize the field. "[We're] going to be able to generate information orders of magnitude faster," says molecular geneticist James Ajioka, who heads a Cambridge University lab that is starting to map the genomes of *Leishmania*, the African sleeping sickness agent *Trypanosoma brucei*, and *Toxoplasma gondii*, a protozoan that can cause fatal encephalitis in immunosuppressed people.

As ever with tropical parasitology, however, money is a problem. Although diseases such as malaria are responsible for more than 1 million deaths every year, they are not a high priority for funding agencies in the developed north (see p. 1857). Nevertheless, a few funders are starting to take an interest in parasite genomes. Last summer Britain's Wellcome Trust launched a 3-year, $1-million effort to produce a physical genome map of the malaria parasite, *Plasmodium falciparum*, involving labs in Australia, Britain, and the United States. And the leading international parasitology agency, the Geneva-based Special Program for Research and Training in Tropical Diseases (TDR)—which is run by three United Nations agencies—will soon start funding genome projects for five other major disease organisms: the protozoans that cause leishmaniasis, African sleeping sickness, and Chagas' disease; the nematode worms responsible for lymphatic filariasis; and the liver flukes that cause schistosomiasis.

Unlike the human genome effort, these projects will involve developing countries. TDR is actively encouraging the formation of lab networks involving developing-world researchers—a policy that will help bring these scientists into the molecular biology mainstream. "Having a genome effort...

gives you a common ground to talk to people," says geneticist Sérgio Pena of the Federal University of Minas Gerias in Belo Horizonte, Brazil, who recently launched a project to sequence expressed genes in the liver fluke *Schistosoma mansoni*.

Developing- and developed-world researchers alike, however, face a difficult task in analyzing pathogen genomes. One major problem is that, in most parasites, it's difficult—and in some cases impossible—to produce genetic linkage maps. Human geneticists typically hunt genes using both physical and genetic linkage maps. A physical map consists of a series of overlapping cloned fragments of DNA, lined up in the order they appear on the genome, while a linkage map shows the position of polymorphic marker sequences that vary among individuals. Researchers look for polymorphic markers that are inherited within families together with an identifiable trait. When they identify such a marker, they know that a gene influencing the trait must lie nearby; they can then search for it in the corresponding cloned DNA fragment from a physical map.

For parasite geneticists, however, this strategy is extremely difficult to follow. For a start, researchers aren't sure that some parasites, such as the various species of *Leishmania*, undergo sexual reproduction; if they don't, it will be impossible to follow the inheritance of polymorphic markers in sexual crosses. And while other parasites do reproduce sexually, obtaining crosses in the lab is very difficult, because they reproduce only inside their host or the insect or other vector that spreads the disease. For the malaria parasite, the procedure takes several years, as the parasites must be cultured both in a mosquito, where sexual reproduction occurs, and then in a chimpanzee, from which researchers extract the parasites and search for the small proportion of sexual crosses. "Nobody in their right mind would have chosen *Plasmodium falciparum*" for genome studies, notes Thomas Wellems of the National Institute of Allergy and Infectious Diseases in Bethesda, Maryland.

Despite these formidable obstacles, Wellems has managed to produce a rudimentary *P. falciparum* linkage map by analyzing one of the two crosses that have been performed in the malaria parasite. John Boothroyd of Stanford University and David Sibley of Washington University in St. Louis, meanwhile, have completed a similar map for *Toxoplasma gondii*, and in Ajioka's Cambridge University lab, Sara Melville is working to integrate into a *Trypanosoma brucei* physical map polymorphic markers that can be used in linkage studies.

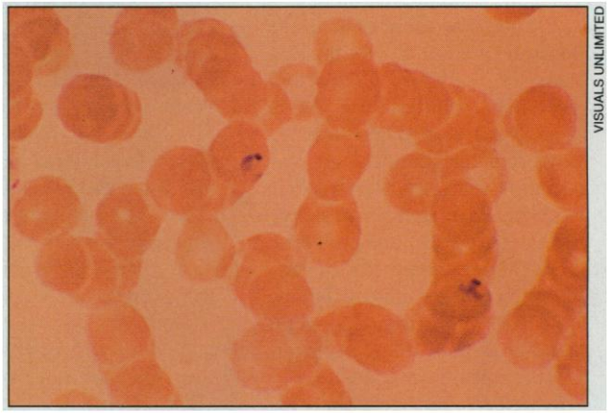
But even in the absence of a workable linkage map, parasite geneticists have a few tricks that can help them pinpoint important genes. In *Leishmania*, for example, Harvard University molecular geneticist Stephen Beverley is adopting a "mutation-recovery" strategy. The idea is to mutate *Leishmania* cells using a chemical and then select those defective in a particular trait. Beverley can then insert randomly selected fragments of *Leishmania* DNA into individual mutant parasites by cloning them into cosmids (loops of DNA derived from a virus that infects bacteria) until they find a fragment that restores the trait. "For every mutant we've tested, we're able to recover the gene," says Beverley, who has already identified the gene for an enzyme that generates a *Leishmania* cell surface carbohydrate that seems to play an important role in the parasite's virulence.

Other researchers hope to find a short cut to important genes by sequencing libraries of complementary DNA (cDNA). These libraries contain only expressed genes, because cDNA is produced by exposing messenger RNA to a reverse transcriptase enzyme, which produces short sections of DNA with a sequence matching that from which the RNA was transcribed. Indeed, researchers studying the nematode worm *Brugia malayi*, which causes filariasis, have opted to dispense with mapping and focus on a "genes-first" cDNA approach. Their advantage is that a closely related species, *Caenorhabditis elegans*, is already one of the leading model organisms for genome analysis, which should make it easier to determine the function of new cDNA sequences. "We're hoping by comparison with the *C. elegans* database to be able to identify genes very quickly," says molecular geneticist Stephen Williams of Smith College in Northampton, Massachusetts.

In the malaria parasite, meanwhile, John Dame and colleagues at the University of Florida, Gainesville, are employing a variant of the cDNA approach. In addition to sequencing cDNA from the main human blood form of *P. falciparum*, Dame's group is working on chromosomal DNA cut up by the enzyme mung bean nuclease, which digests chromosomes to produce a DNA library extremely rich in genes. This means the Florida team is sequencing genes expressed at every stage in the parasite's life cycle, rather than just those expressed by the blood form. The combination of cDNA and mung bean nuclease library sequencing, claims Dame, could cut the cost of cloning a *P. falciparum* gene from tens of thousands of dollars down to a mere $250.

The proponents of parasite genome analysis hope arguments like Dame's will convince funding agencies that parasite genome-sequencing projects could give good value for the money. But the current financial picture remains bleak: TDR's first deadline for parasite genome proposals is next month, but the $600,000 that TDR can afford to set aside this year is only a fraction of the sum required to support the full range of projects being planned.

The best hope of breaking this impasse is finding money for pathogen genome analysis within the richly funded Human Genome Project. One sign that the players in that project might be receptive is that several powerhouse human genome labs are already joining the effort. The Institute for Genomic Research (TIGR), the private-sector human cDNA sequencing outfit in Gaithersburg, Maryland headed by Craig Venter, has provided technical assistance for the *Schistosoma mansoni* project; the Centre d'Etude du Polymorphisme Humain (CEPH) in Paris is hosting a team of Latin American and Spanish researchers preparing DNA libraries to begin physical mapping of *Trypanosoma cruzi*, the agent of Chagas' disease; and the Sanger Center in Hinxton, near Cambridge, United Kingdom, is helping Ajioka with his mapping projects.



Malaria parasite. A multi-lab effort to map the genome of *Plasmodium falciparum* is under way.

But what's really required is for major genome funding agencies to take a direct interest. If the ultimate goal of the Human Genome Project is to improve human health, argues molecular geneticist Stewart Cole of the Pasteur Institute in Paris, who assembled the DNA clones for the leprosy sequencing project, pathogen projects deserve support.

The first *M. leprae* DNA fragment to be sequenced, he says, contained a gene that confers resistance to the drug rifampin—a discovery that led to a rapid diagnostic test to detect patients suffering from drug-resistant leprosy. Few of the established model genome organisms, Cole asserts, are yielding results so directly relevant to human health. "If you can sequence a model organism that also has a huge impact on public health," he argues, "then that will be a much more worthwhile investment."

–Peter Aldhous