by the Harris and Ozturk groups can provide valuable information about carcinogen action, the mutation they found can't, for some very practical reasons, be used directly as a means of screening for exposure to the carcinogen. For one thing, the mutation is present only in tumor cell DNA, not in normal liver, so it wouldn't provide an early warning of cancer risk. For another, it's not feasible to use carcinogen target tissue, such as liver or lung, for screening purposes. As Steven Tannenbaum, a molecular epidemiologist at the Massachusetts Institute of Technology points out, "You just can't go up to people and say, 'give me a piece of lung.'"

But according to Gerald Wogan of MIT, John Groopman of Johns Hopkins University School of Medicine, and their colleagues, it is possible to screen for aflatoxin exposures with much more readily obtainable blood and urine samples. Instead of looking for the actual carcinogenic mutation itself, these researchers take a tack used by many other molecular epidemiologists and look for "surrogates"—indicators that can show aflatoxin has been wreaking its mischief in an individual, but don't require sampling of target tissues.

Like most other carcinogens, aflatoxin has to be activated by enzymes in the body to unleash its cancer-causing power. Only the activated form of aflatoxin can attack the DNA, which it does by forming chemically bound "adducts" that can lead to carcinogenic mutations if they are not repaired. The reactive aflatoxin molecules can also form adducts with proteins, such as blood albumin. In their study, Wogan and his colleagues have compared the aflatoxin adduct concentrations in blood and urine samples from people living in Guangxi Province in China and The Gambia, West Africa, with the aflatoxin concentrations in the people's diets. The result? "In both populations the study is working very well," Wogan says. "We see a relation between the adducts and exposures."

> ***"What I find exciting is that as we put these markers into play we may have the opportunity to intercede."***
>
> —I. Bernard Weinstein

Adduct measurement is also proving to be an effective indicator of exposures to other carcinogens that require activation. For example, Perera, Regina Santella, who is also at Columbia University School of Public Health, and their colleagues have shown that adduct formation by the DNA of white blood cells reflects the occupational exposures of iron foundry workers in Finland and coke oven workers in Poland to the powerful carcinogens known as the polycyclic aromatic hydrocarbons (PAHs). What's more, the researchers have also found significant increases in the PAH-DNA adducts in people living in a highly polluted region of Poland, the first time such a link has been found to environmental pollution.

And the Tannenbaum group found that adduct formation between the blood pigment hemoglobin and 4-aminobiphenyl, a chemical in cigarette smoke that has been linked to bladder cancer, is highest in people who smoke black tobacco, next highest in people who smoke blond tobacco, and lowest in nonsmokers. That exactly parallels the carcinogen exposures of the three groups—and their risks of getting cancer. "We feel we've really closed the loop," Tannenbaum

Testing for Cancer Risk: Tough Questions Ahead

With the recent discoveries of a few genes involved in rare inherited cancers, screening healthy individuals for cancer susceptibility has at last become possible. Such genetic tests have been eagerly awaited, as they open up the possibility of early detection, intervention, and perhaps even prevention. While expectations are still high, the pioneers in this field are finding that the tests raise some thorny questions as well.

The problem, in a nutshell, is that cancer often involves changes in several genes, unlike the "classic" genetic diseases. In Huntington's, for instance, inheritance of the faulty gene means an individual will get the disease. But inheriting a cancer susceptibility gene does not necessarily lead to cancer, though the risk is high—sometimes extraordinarily so. Given that uncertainty, do the benefits of knowing about cancer susceptibility outweigh the risks of anxiety, depression, and potential job or insurance discrimination? Should patients undergo preventive treatments, and if so, how radical?

If early experience is any indication, the answers to such questions will vary widely with each new susceptibility gene discovered. At one end of the spectrum is the retinoblastoma gene. Although it was the first inherited cancer gene to be identified (in 1986), the benefits of screening were clear and screening began almost immediately. Explains geneticist Louise Strong of the M.D. Anderson Cancer Center at the University of Texas, Houston: "With the retinoblastoma gene, the outcome is predictable [about 95% of those who carry the gene will develop eye cancer by age 5], and there is something specific you can do. You can examine the infant from the time it is born every 4 to 6 weeks. And if you detect lesions, they can be treated very successfully without doing anything invasive, without losing the eye. And there is almost 100% survival."

But then there's the gene involved in the Li-Fraumeni cancer syndrome, discovered just last November. Extremely rare—so far only 100 families worldwide are known to be affected, though the number could be far higher—the syndrome is nevertheless a good test case because it raises some of the toughest issues of any cancer susceptibility gene that is likely to be discovered over the coming decades.

The syndrome was first identified in 1969 by Fred Li and Joseph Fraumeni of the National Cancer Institute, who described individuals extremely susceptible to not just one type of cancer but to at least six: breast cancer, soft tissue sarcomas, brain tumors, bone cancer, leukemia, and adrenocortical carcinoma. Li-Fraumeni patients tend to develop cancer as children or young adults, and those who survive their first cancer sometimes go on to develop a second, especially if they've been given radiation therapy.

Last November, Stephen Friend of the Massachusetts General Hospital Cancer Center, along with Li, Fraumeni, and Strong, found that at least some of these patients have a germline mutation in a tumor suppressor gene, p53, that has been implicated in several types of cancer. Finding the gene meant a new DNA test was possible to detect those who carry the Li-Fraumeni defect—but should such a test be offered? The difficulty is that once the mutation has been found, the physician doesn't know what, precisely, that finding means, other than that the individual has a

says. "We can say unquestionably that there's a set of compounds in cigarette smoke that causes bladder cancer."

Other researchers are assaying for carcinogen exposures by looking for the increased mutation rates they cause, although again the mutations are usually in surrogate genes. Richard Albertini and his colleagues at the University of Vermont in Burlington developed one widely used assay of this type. It involves determining what percentage of T cells in the blood have undergone mutations that have caused them to lose an enzyme known as HPRT (for hypoxanthine-guanine phosphoribosyltransferase), an enzyme the researchers chose mainly because there is an easy way of determining whether cells have it. In addition, William Bigbee, Ronald Jensen, and their colleagues at Lawrence Livermore National Laboratory in Livermore, California, have developed a similar assay that looks for mutations that have caused red blood cells to lose certain surface proteins, called glycophorins.

The adduct and mutational assays have somewhat different strengths and weaknesses. The adduct assays, for example, aren't much use in detecting exposures that occurred many years in the past, because the adducts will be lost within days to weeks after the exposures cease. The mutations, however, may last a long time—even a lifetime. Both the HPRT and glycophorin assays have detected increased mutations in survivors of the Hiroshima and Nagasaki nuclear bomb blasts more than 40 years after the radiation exposures.

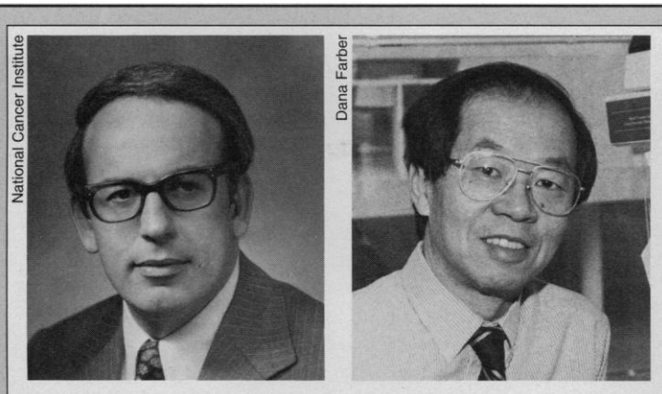
The very shortness of the adducts' lifetimes may give them an advantage in another regard, however. They might be used to monitor the effectiveness of intervention trials aimed at preventing cancer development. Groopman and his colleagues are already planning such a trial in the areas of China and Africa that have high liver cancer incidences. Their motivation to undertake it was, Groopman says, heightened by the Harris and Ozturk groups' results linking aflatoxin to the p53 gene mutation.

The plan is to treat the people with the schistosomiasis drug Oltipraz, which has been shown to block aflatoxin-induced liver cancer in rats. "There's virtually no way we are going to prevent these people from getting aflatoxin in the diet," Groopman says. "The economic resources aren't there." And since Oltipraz apparently blocks aflatoxin's carcinogenic effects by preventing its metabolic activation, it should be possible to get an idea of whether the drug is having an effect in humans by measuring their aflatoxin adduct concentrations. As mentioned previously, only the activated carcinogen can form the adducts.

But while molecular epidemiologists are confident that the biomarkers they are studying provide accurate indicators of exposures to environmental carcinogens, even they have to concede that they there is a key step still to come: proving that the markers will actually predict individual cancer risks. "I'd like to tell you that we know that," Albertini says, "but the relationship to health is dangling and this has to be nailed down."

Still, there is some rationale for the "cautious optimism" Perera described in discussing these markers. "What's been really intriguing," she notes, "is that we all see a large inter-individual variability." Even the control groups, who were not supposed to have any unusual carcinogen exposures, have their "outliers"—people with much higher biomarker concentrations than others. The individual variability suggests that the biomarkers can be used to find the people who are most sensitive to carcinogen action and therefore most likely to get cancer.

The next step is to do the prospective studies needed to find out whether that is



Syndrome discoverers: *Joseph Fraumeni* (left), *Fred Li.*

50% chance of developing cancer by age 30.

"We don't know what to recommend if someone has an asymptomatic young child," says Strong. "That child may develop cancers anywhere. And it is very difficult to screen for soft tissue sarcoma, bone cancer, and brain cancer. There is nothing you can recommend, other than common-sense things like being alert to lumps and bumps that don't go away."

And even if these cancers can be detected early, investigators are uncertain about whether that is always an advantage. "For some diseases, like breast cancer, early detection is a way to cure it," says Li, now at the Dana Farber Cancer Center. "But what is the benefit of early diagnosis of leukemia? That is a question mark. So does early diagnosis benefit these patients or are you just giving them the bad news earlier and prolonging stress and anxiety?"

These questions are complex and stubborn enough that Li convened a workshop earlier this summer at the National Institutes of Health (NIH) to examine them. At the workshop, participants agreed that the first task is to untangle the relations among Li-Fraumeni syndrome, the p53 germline mutation, and other cancers. So far, Friend's group and others have found the germline mutation in dozens of Li-Fraumeni families; they are in the process of evaluating more. At this early stage, it is still unclear whether an individual can have the mutation but not the syndrome, or vice versa.

To complicate the picture further, Friend and others suspect that the same germline p53 mutation may be more widely involved in human cancer—in people without a history of Li-Fraumeni syndrome—which raises questions about screening other cancer patients or perhaps the general population. To find out, dozens of researchers are scouring tissue banks, looking for the mutation in a variety of cancers. At Massachusetts General, Friend has been evaluating women with early-onset breast cancer as well as children with multiple primary malignancies.

For now, participants at the NIH workshop agreed, testing for the germline p53 mutation should be offered only to Li-Fraumeni families and other high-risk groups, perhaps including women with early-onset breast cancer or individuals with radiogenic tumors. The group will meet again in November to take a second stab at crafting guidelines for screening, looking at who to test, what sorts of education and counseling are needed, and how best to monitor and care for those who test positive.

One reason that investigators would like to sort out such issues quickly is that discoveries of other susceptibility genes are expected to follow closely on the heels of Li-Fraumeni. With any luck, those new genes will prove to be more like retinoblastoma than Li-Fraumeni—but no one is banking on it.

■ LESLIE ROBERTS