tochrome c). In this model, deciding between necrosis and apoptosis depends on two events: the cellular stores of adenosine triphosphate at the time of PT pore opening, and competition between the rate of bioenergetic collapse and activation of the apoptosis cascade. Clearly, a principal goal is to understand the mechanisms that tip photoreceptor cells in favor of either necrosis or apoptosis in response to cellular stress. Diverse *Drosophila* mutants with a variety of visual system defects promoting either necrosis or apoptosis in response to light activation are important reagents for the systematic dissection of this decision process. The Acharya *et al*. work points the way by identifying ceramide as the potential integrator of the different pathways leading to photoreceptor cell death.

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CANCER

Developing Molecular Biomarkers for Cancer

David F. Ransohoff

The development of a noninvasive test for cancer has been the Holy Grail of cancer detection research for three decades. In 2003, there is reason to be cautiously optimistic that such a test can actually be developed. On page 1753 of this week's issue, Cui *et al*. (*1*) report a step forward in this endeavor. They have developed a DNA-based blood test that may predict the risk of developing colorectal cancer (CRC). Their work raises interesting issues about cancer biology, the use of biomarkers to test for cancer, and the process of biomarker discovery.

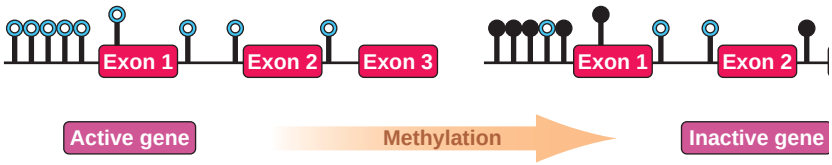
Cui *et al*. examined colon tissue biopsies and blood samples from 172 colonoscopy patients for loss of imprinting (LOI) in the insulin-like growth factor II (*IGF2*) gene. LOI is an epigenetic phenomenon in which certain genes are generally silenced during embryonic development through the addition of methyl groups (methylation) (see the figure). Gene methylation also may take place later in life. Cui and colleagues show that LOI of the *IGF2* gene is associated with a family history of developing CRC and with a personal history of colon adenomas and CRC (*1*). The association of LOI with familial CRC is potentially important because 30 to 50% of sporadic CRC is associated with familial risk, yet the genetic basis for this common association is largely unknown. In contrast, genetic mechanisms for the dramatic but very uncommon inherited CRC syndromes—familial adenomatous polyposis coli and hereditary nonpolyposis colorectal cancer—are well established.

These investigators demonstrated that LOI was present in 28% of persons with a family history of CRC and—although the number of patients was small—in 56% of those with a personal history of CRC. In contrast, LOI was present in only about 10% of healthy individuals. If LOI turns out to "mediate" risk for common sporadic CRC, this relationship could provide not only the basis for a blood-based diagnostic biomarker but also insights into the biology of sporadic CRC.

LOI may work through the epigenetic phenomenon of methylation. Both DNA methylation and DNA mutations disrupt the transfer of information from DNA to RNA to the functional protein. Methylation of cytosines in the DNA is often associated with changes in gene expression. In many cases, methylation results in repression of transcription (*2*) (see the figure). The regulation of methylation is complicated and interesting because, unlike mutation of the DNA, methylation can be chemically reversed, and it can be either acquired or inherited. The product of the *IGF2* gene is an important tumor growth factor, and the DNA sequence that regulates expression of *IGF2* is normally switched off through methylation. However, with LOI the methylation status of this DNA sequence is reversed. In the subjects studied by Cui *et al*., LOI appears to have been inherited or acquired early in life because it turned up in multiple biopsies of colon tissue, and sometimes in white blood cells as well. In contrast, LOI would be expected to be patchy in colon tissue if it occurred later in life from clonal expansion of cells at a single focus.

The work of Cui *et al*. is a step toward the goal of developing a noninvasive test for detecting cancer, but whether it will be useful clinically requires consideration of several factors. First, LOI does not assess directly the presence of a CRC, but rather the "tendency" of colon tissue to become cancerous. A marker as indirect as one that assesses "tendency" might nevertheless be clinically useful if it, either alone or with a panel of other markers, were sufficiently sensitive that a negative test strongly predicted a low lifetime risk of CRC, making conventional screening unnecessary (*3*). Identification of a large subgroup of persons for whom conventional screening including colonoscopy is unnecessary would constitute a major advance in the development of cancer biomarkers.

Other types of molecular marker research may be aimed not at measuring the lifetime "tendency" of developing adenoma or CRC but rather at direct detection of



Switching genes off. Methylation of cytosine bases in DNA prevents the DNA from being read, resulting in silencing of the gene. Methylation can be both inherited or acquired and is potentially reversible.

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the tumor tissue. Targets include the DNA mutations that appear as normal colon tissue progresses toward adenoma and then CRC (*4*). Stool (*5*, *6*) or blood samples from the patient can be screened for DNA mutations derived from tumor tissue. Proteomics-based assays presumably detect protein-based tumor products, allowing discrimination between persons with and without cancer (*7*).

Two dramatically different approaches to biomarker discovery are currently being pursued, and it is not yet clear whether either (or both) will result in practical clinical tests. Cui *et al*. use a hypothesis-driven approach in which a molecule like IGF2, thought to be involved in the biology of cancer, provides the target for which a marker is developed. Hypothesis-driven research assesses plausibly important candidate genes or proteins, one by one. Successful discovery or development is likely to involve assembling a panel of multiple markers when individual markers cannot provide the sensitivity and specificity needed (*6*). Development of any individual marker may require that substantial technical challenges are overcome (*5*). In concept, the step by step approach, building on what is known about cancer biology, is very logical and appealing.

A very different approach termed "discovery-based research" (*8*) has become possible and popular, because of the availability of high-throughput techniques that allow simultaneous assessment of tens of thousands of genes or proteins. With this approach, there is no need to identify targets a priori. Huge portions of the genome may be screened simultaneously, for example, with RNA expression microarrays that can be used to predict a prognosis of cancer (*9*). Or the entire proteome of serum may be scanned by mass spectroscopy to discriminate among persons with and without cancer (*7*). Products of discovery-based research include complicated patterns of gene expression and mass spectroscopy peaks. These patterns may be used to identify new candidate genes or proteins, which then can be assessed individually with conventional techniques (*10*). Alternatively, in a new approach to biomarker discovery, the patterns themselves may be used as the test—for example, "expression signatures" (*9*) or algorithm-interpreted proteomics patterns (*7*)—without needing to understand precisely which genes or proteins account for the pattern. This kind of "black box" research may eventually provide robust and useful results, but the products of this approach must be validated in a manner that is absolutely fastidious, in order to avoid problems of overfitting, confounding, and bias (*11*).

The field of biomarker research is much more promising today than it was 30 years ago because current knowledge about the molecular biology of cancer provides so many potential targets. Further, powerful tools such as the polymerase chain reaction, microarrays, and mass spectroscopy can screen multiple targets simultaneously. However, the complexity of data generated by these methods is as frustrating and fascinating as the examination of fractals, which look similarly complex at high and low resolution. Simply because we can look at larger numbers of smaller phenomena does not mean that insights will automatically arise. The journey to discover useful biomarkers will require imaginative exploration, fastidious validation, and some good luck (*12*).

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EVOLUTION

Little Else But Parasites

J. K. M. Brown

Infectious diseases are a major driving force in host-pathogen coevolution. In host populations, the constant barrage of attack by different parasite species, each with a range of genotypes, drives the genetic diversity of the host. The reason is that genetic variation (polymorphism) in the host that confers resistance against parasites reduces the probability that a parasite can infect the next susceptible host encountered. The maintenance of variation in parasite populations has been more difficult to explain. Most theoretical models assume that virulence does not become fixed because it incurs a cost in terms of fitness, yet the existence, nature, and size of such costs and their relationship to the population structure of the host remain controversial.

On page 1735 of this issue, Thrall and Burdon (*1*) report variation in populations of wild flax (*Linum marginale*). They show that increased virulence of a parasite of wild flax, the rust fungus (*Melampsora lini*), is associated with reduced spore production, a clear fitness cost. Virulent isolates of rust fungus were found more frequently growing in highly resistant populations of wild flax, and, intriguingly, showed reduced reproductive fitness. Meanwhile, avirulent forms of the parasite dominated susceptible wild flax populations and often exhibited greater reproductive fitness. (When applied to plant pathogens, "virulence" means their ability to cause disease in a host plant of a specific genotype. "Aggressiveness" would be the term equivalent to "virulence" as used in medical and veterinary pathology to describe quantitative variation in pathogenicity.) The trade-off between parasite virulence and reproduction may help to maintain a diverse array of resistance genes in the host plant population. Thrall and Burdon present the clearest evidence to date for a trade-off between virulence and fitness in wild plant populations.

The gene-for-gene relationship, which operates in many host plant–parasite interactions (*2*), is an attractive model for explaining the coevolution of the host plant and parasite. In the gene-for-gene model, each parasite virulence gene is matched by a host plant gene for resistance to the parasite. In many cases, the differences between resistant and susceptible responses of plants to parasites are very distinct and highly specific to the genotypes of host and parasite. In contrast, most disease resistance genes detected in humans have relatively small quantitative effects that reduce but do not eliminate the risk of a person becoming infected; no matching gene pairs have yet been found in human hosts and any of our many parasites (*3*).

A major challenge for theoretical biologists has been to account for maintenance of polymorphism in gene-for-gene systems. Until recently, most theories postulated allele-for-allele models in which a series of alleles at one locus in the host match a similar multi-allelic locus in the parasite. Here, there is an obvious trade-off because the presence of one resistance allele excludes the possibility of any other allele at the same locus on the same chromosome. However, this system is not biologically re-

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