OCCASIONAL NOTES

Yeast, Flies, Worms, and Fish in the Study of Human Disease

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The sequencing of the human genome has revealed an almost complete "parts list" for the study of the genetic basis of disease.[1,2] The Online Mendelian Inheritance in Man data base lists more than 1000 human genes that have been implicated in specific diseases.[3] It is likely that within a few years the causative lesion in most diseases that result from a mutation in a single gene will have been characterized, and geneticists are using sophisticated methods to track genes in polygenic diseases — that is, diseases caused by defects in more than a single gene.

Often, however, the rapid pace of the discovery of disease-causing genes is not matched by the pace of our understanding of how these genes cause the clinical manifestations of a disease. In the search for the genetic basis of a disease, it is not uncommon to discover an abnormal protein the normal function of which is not known. Some information about a novel protein may be gained by studying its distribution in normal tissues and their subcellular compartments and by examining the consequences of overexpression of the protein in cultured cells or inactivation of the corresponding gene in knockout mice. These investigative approaches are an important starting point, but they may not help in understanding the role of a novel gene in the functional context of known signaling pathways. They also are not easily adaptable to high-throughput analyses, in which tens of thousands of mutant organisms can be screened for alterations in specific biochemical pathways, or to testing large libraries of chemicals for their effects on abnormal phenotypes. Analyses with relatively simple organisms, however, which at first seem remote from humans, are likely to help reveal the function of genes implicated in human disease. These simple organisms include the yeast *Saccharomyces cerevisiae*, the fruitfly *Drosophila melanogaster*, the nematode *Caenorhabditis elegans* (hereafter referred to as yeast, fly, and worm, respectively), and the zebrafish *Danio rerio*.

YEAST, FLIES, WORMS, AND FISH

The use of simple model organisms in biologic research is certainly not new. Indeed, much of the knowledge of the fundamentals of gene regulation comes from studies that were conducted in bacteria,[4] reportedly leading Jacques Monod to remark, "What is true for [*Escherichia*] *coli* is also true for the elephant." Two premises underlie the use of simple organisms in medical research. First, most of the important biologic processes have remained essentially unchanged throughout evolution — that is, they are conserved in humans and simpler organisms. Second, these processes are easier to unravel in simple organisms than in humans. The short generation times of yeast, flies, worms, and zebrafish accelerate genetic studies in these organisms. Mutant strains can be generated efficiently, and the mutations responsible for specific phenotypes can be identified rapidly. Moreover, the effects of gene inactivation or overexpression, as well as interactions among different genes, can readily be identified. Like the human genome, the genomes of the yeast, fly, and worm have been almost fully sequenced,[5-7] and completion of the zebrafish genome is expected within a few years. The ability to compare evolutionarily conserved gene-family members (orthologues) among these species is one of the important benefits of the genome projects.

The genetic approaches used to study each of these organisms have been reviewed recently elsewhere.[8-11] In this article, we provide an overview of their usefulness in studying genes with direct relevance to human disease. Although some laboratories are now conducting large-scale mutagenesis screening in mice, the discussion here is restricted to nonmammalian organisms.

IDENTIFYING GENES CAUSING HUMAN DISEASE IN MODEL ORGANISMS

It is thought that 60 to 80 percent of disease-causing genes in humans have orthologues in the fly genome.[12,13] This estimate depends on the stringency of the criteria used to identify similarities in amino acid sequences, and the proportion may be even higher when functional studies are used to uncover related genes. Worms appear to have slightly fewer orthologues of human genes than do flies.[12] The vertebrate zebrafish is likely to have a counterpart for almost every disease-causing gene in humans. For this reason, these small, nearly transparent fish have been subjected to large-scale mutagenesis experiments, which produce numerous mutants with a wide variety of defects in organ development that can be readily detected under low magnification[14] (Fig. 1). Completion of the sequencing of the zebrafish genome will greatly facilitate the isolation of genes responsible for abnormalities in vertebrate organ development. In addition, the consequences of the abnormal expression of genes can be studied at the level of the whole organism in zebrafish. For example, the generation of c-*myc*–driven T-cell leukemia cells can be monitored in real time by means of a green fluorescent protein marker as they are disseminated from the thymus to other organs.[15]

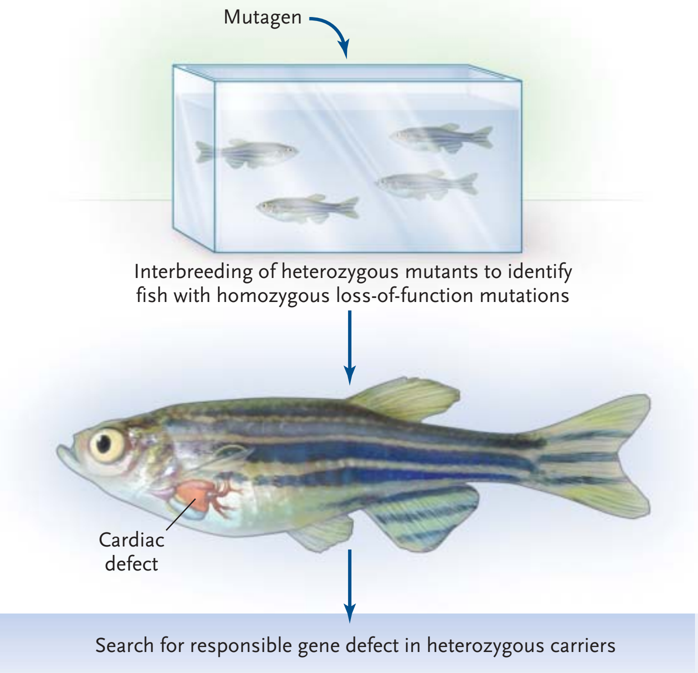

Figure 1. Abnormal Development of the Heart in Mutagenized Zebrafish.

The near-transparency of zebrafish makes it possible to screen for developmental defects in many different organs. Zebrafish can survive for days with gross abnormalities of the heart, kidney, or other organs. Exposure of zebrafish to mutagens has been used to generate extensive collections of mutants with specific abnormalities associated with both heterozygous and homozygous mutations. The identity of the mutated gene responsible for a specific phenotype can be determined with the use of positional cloning strategies.

Beyond the conservation of gene sequences across species, the usefulness of a model organism is dictated primarily by its suitability for the study of particular cellular pathways. For instance, the understanding of genes involved in regulating the cell cycle and of the checkpoints that monitor for damaged DNA began with studies in yeast cells, which are particularly well suited for investigations of the effects of mutations on cell division[16-18] (Fig. 2). Similarly, much of the understanding of genes that regulate the organization of tissues and the differentiation of cells in the human embryo is based on insights gained from studies of mutations that perturb segmentation in the fly embryo.[19] Genetic studies in the worm, in which it is feasible to account for the developmental fate of each of the animal's 1090 cells, improved the understanding of apoptosis (programmed cell death).[20] Research in flies, yeast, and worms led to Nobel prizes in 1995, 2001, and 2002, respectively.

DISCOVERING NEW GENES

Studies in model organisms often reveal the first clues to the identity of a genetic defect in a human disease. For instance, the instability of mononucleotide and dinucleotide repeat sequences (microsatellite DNA) in colorectal cancer cells from patients with hereditary nonpolyposis colon cancer (the Lynch syndrome) resembles the defects in DNA replication seen in yeast cells with defective mismatch repair genes. Indeed, inactivating mutations of the human orthologues of these yeast genes, the *MSH2* and *MLH1* genes, are the most frequent causes of familial colon cancer.[21] Similarly, gene-mapping studies of families affected by the basal-cell nevus syndrome (Gorlin's syndrome) narrowed the search to a chromosomal locus that carries the orthologue of the fly gene *patched*. In flies, mutations in this gene alter the differentiation of groups of cells in the embryonic epidermis. Remarkably, germ-line mutations in the human orthologue of *patched* occur in Gorlin's syndrome, and somatic mutations in

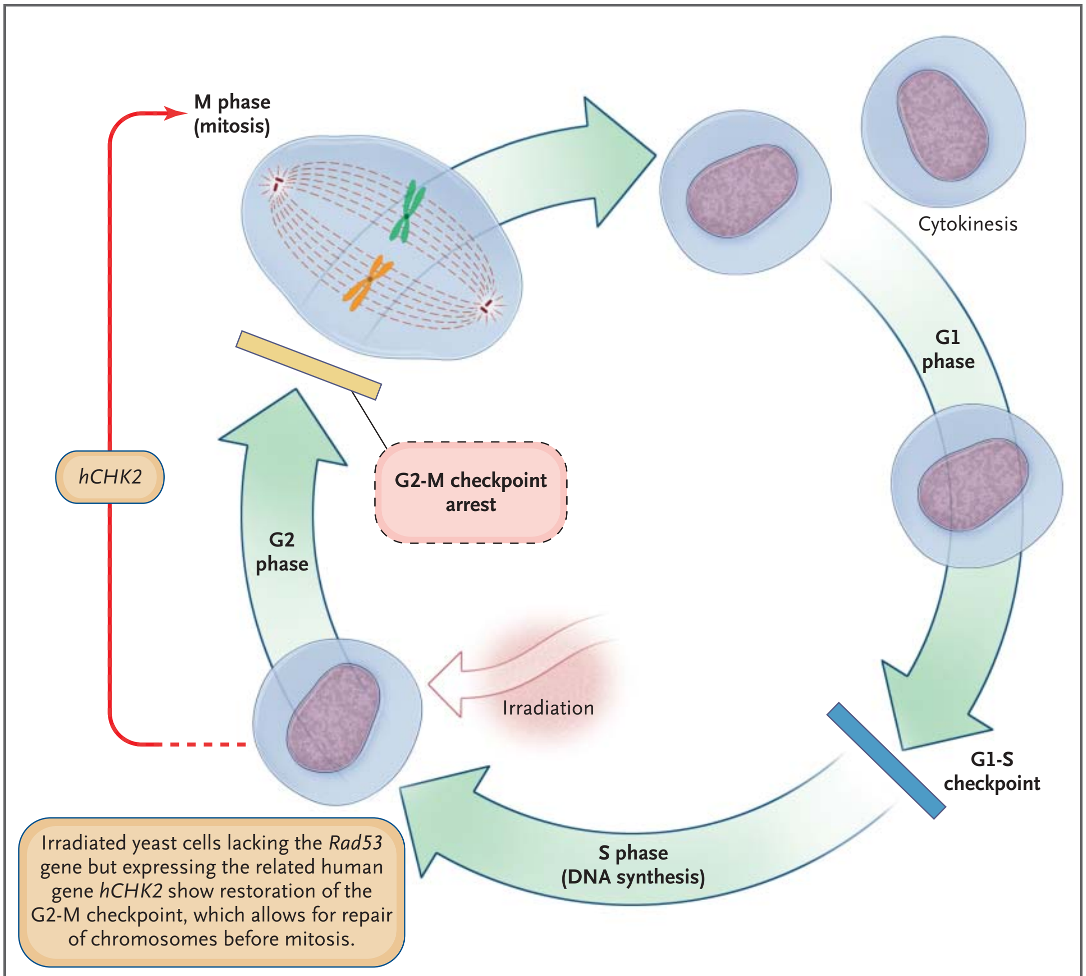

Figure 2. Correction of a Defect in a Gene Regulating the Cell Cycle in Yeast by Its Human Orthologue.

The cell cycle, in yeast and in humans, is defined by specific phases, including gap 1 (G1), DNA synthesis (S phase), gap 2 (G2), and mitosis (M). At checkpoints in the cell cycle there are mechanisms that monitor the integrity of DNA before new DNA is synthesized (G1-S checkpoint) and, similarly, that ensure chromosomal integrity before mitosis (G2-M checkpoint). Irradiated yeast cells that lack the G2-M checkpoint gene *Rad53* cannot proceed normally through this checkpoint, and their growth is arrested. The introduction of a normal *hCHK2* gene, the human orthologue of *Rad53,* is sufficient to restore partial checkpoint function and allow the yeast cells to proceed through mitosis. Germ-line mutations in *hCHK2,* which are present in 1 percent of the general population, appear to confer a moderate increase in the risk of breast cancer.

patched are evident in most cases of sporadic basal-cell carcinoma of the skin.[22,23]

Genetic screening in flies, worms, or yeast can also help to identify genes that regulate cell proliferation. Both the yeast *CDC4* gene and its fly orthologue *archipelago* (*ago*) are involved in a mechanism that suppresses cell proliferation by promoting the degradation of cyclin E, a protein required for entry into S phase — the phase of DNA synthesis — of the cell cycle.[24-26] The human orthologue *hCDC4/hAGO* has a similar function and is mutated in breast and ovarian cancer cell lines[24,25] and in up to 16 percent of endometrial carcinomas.[27] Another example is the protein ferroportin1, which in humans is hypothesized to export cellular iron into the circulation and which was first identified in anemic zebrafish.[28] The human orthologue of the *ferroportin1* gene was subsequently shown to be mutated in certain cases of hemochromatosis.[29,30] As these examples suggest, the relative ease with which newly identified genes can be linked to phenotypes in model organisms makes the use of such organisms a powerful approach for identifying their orthologues involved in human diseases.

DEFINING CELLULAR PATHWAYS

Model organisms provide researchers with a unique method of placing genes within a functional pathway — the so-called modifier screen.[9,10] This method involves inducing random mutations in an organism known to have a specific mutation in a gene of interest. The added mutations in other genes may modify the usual phenotype, thereby providing clues to the molecular pathway in which the gene of interest is important. Modifier genes, as those identified with this method are called, often function in the same cellular pathway as the gene of interest. For example, overexpression of a gene in the highly organized compound eye of the fly causes a defect that is easily measured. This defect may be exacerbated or suppressed by a mutation in another gene that functions in the same pathway. Inducing random mutations in the fly genome with the use of a chemical mutagen or irradiation allows tens of thousands of flies to be screened for the rare individual in which a mutation alters the phenotype of the initial mutant strain (Fig. 3).

Modifier screening has been used to gain insight into disease-causing genes implicated in human neurodegeneration.[31] Abnormal expansion of the stretches of glutamines in specific proteins underlies Huntington's disease, spinocerebellar atrophy, and other inherited neurologic diseases.[32] Remarkably, the expression of proteins engineered to contain polyglutamine stretches in flies or worms also triggers neurodegeneration, and, as in human neurodegeneration, the severity correlates with the length of the polyglutamine stretch.[33-35] Modifier screening in flies has shown that reduced activity of cellular heat-shock proteins, which facilitate the correct folding of proteins, hastens neurodegeneration, whereas overexpression of heat-shock proteins has a protective effect.[36-38] Similar experiments have also suggested that polyglutamine stretches interfere with histone acetyltransferases, which regulate gene expression by adding acetyl groups to the histones that encase chromatin. Drugs known to inhibit the opposing histone deacetylases, and hence to restore histone acetylation, slow neurodegeneration in the fly model,[39] an observation with implications for neurodegeneration in humans.

Similar genetic screening may prove to be of interest in the study of Parkinson's disease. In the fly, overexpression of the gene for α-synuclein, which has been implicated in the human disease, causes degenerative changes in dopaminergic neurons and abnormalities in movement.[40] A model of early-onset Alzheimer's disease has been established in the worm by mutation of its orthologue of the human gene *PRESENILIN*. The effects of the mutation are reversed by overexpression in the worm of the human transmembrane proteins APH-1 and PEN-2.[41] Further study of these proteins may yield insights into the function of presenilins and may thus suggest new therapeutic targets.

The discovery of RNA-mediated interference (RNAi), a scientific breakthrough first achieved in the worm and recently extended to the fly and mammalian cells, is likely to revolutionize the study of gene function.[42] The introduction into cells of double-stranded RNA molecules complementary to a particular gene triggers degradation of the endogenous messenger RNA through a specific nuclease pathway. RNAi provides the basis for a strategy for rapidly inactivating any gene of interest. In the worm, RNAi is so potent that when the adult organism is fed bacteria engineered to contain the appropriate double-stranded RNA, the endogenous messenger RNA will degrade in virtually all cells (Fig. 4). Worms with a mutant phenotype can be grown in thousands of wells, each containing bacteria expressing a different double-stranded RNA. The RNA that enhances or suppresses the mutant phenotype is thus identified. RNAi works well in cultured fly and human cells, and several methods have been developed that permit limited use in vivo. This approach readily lends itself to automation. When used in conjunction with the known sequences of all genes in both the model organism and humans, RNAi is likely to have a major role in the evolving field of "functional genomics," the high-throughput analysis of every gene product encoded by the genome. Indeed, in a technical tour de force, in two recent studies RNAi was used to examine the consequences of inactivation of 86 percent of the known genes in the worm.[43,44] In one of these investigations the worms were examined for changes in fat storage. Human orthologues of these genes may help to determine susceptibility to obesity or diabetes.

TOWARD THERAPEUTICS

In general, the development of useful therapeutic agents lags behind the understanding of the mechanisms of disease. There are currently no drugs in regular use that were developed as a result of studies in simple model organisms, but two studies have led to the identification of possible therapeutic agents. In the first study, the use of cyclopamine was found to be beneficial in the treatment of basal-cell carcinoma.[45] Cyclopamine, a compound in the corn lily, *Veratrum californicum*, causes fetal prosencephaly in sheep. There is a similar abnormality in mice and humans lacking a *hedgehog* gene, which encodes a signaling molecule essential for early tissue differentiation.[46] Genetic screening in the fly first clarified the signaling pathway whereby the *hedgehog* gene interacts with the transmembrane protein Patched and its partner Smoothened to initiate a signaling cascade that regulates cellular differentiation. Cyclopamine was found to inhibit Smoothened, thereby suppressing *hedgehog* signaling,[47,48] whereas Patched is now known to be the target of mutations in Gorlin's syndrome and basal-cell carcinoma, both diseases associated with increased *hedgehog* signaling. Together, these observations raise the possibility that topical cyclopamine could be used in the treatment of locally advanced skin cancers.

Another discovery concerns the possible use of sirolimus (rapamycin), an antibiotic with immunosuppressive properties, in the treatment of the congenital abnormality tuberous sclerosis. Rapamycin antagonizes the function of the TOR (target of rapamycin) kinase, a signaling molecule activated by several growth-promoting stimuli.[49] Studies in the fly indicated that the tuberous sclerosis genes TSC1 and TSC2 restrict cell growth and that they function in cellular pathways known to impinge on TOR activation.[50-52] The finding that in tuberous sclerosis mutations in TSC1 and TSC2 cause excessive TOR kinase activity[53] provides a rationale for exploring the possible use of rapamycin in severe cases of tuberous sclerosis.

Beyond these fortuitous examples lies the prospect of large-scale screening of drugs based on phenotypes defined in model organisms. Traditional screening for new drugs that modulate cellular pathways requires the use of large libraries of compounds to test for inhibition of enzymatic activity, in vitro binding to a purified protein, or more complex mammalian-cell–based assays. By contrast, abnormal signaling in the eye of the fly, aberrant apoptosis in the worm, or developmental defects in zebrafish might make possible novel approaches to the discovery of drugs that correct the malfunction

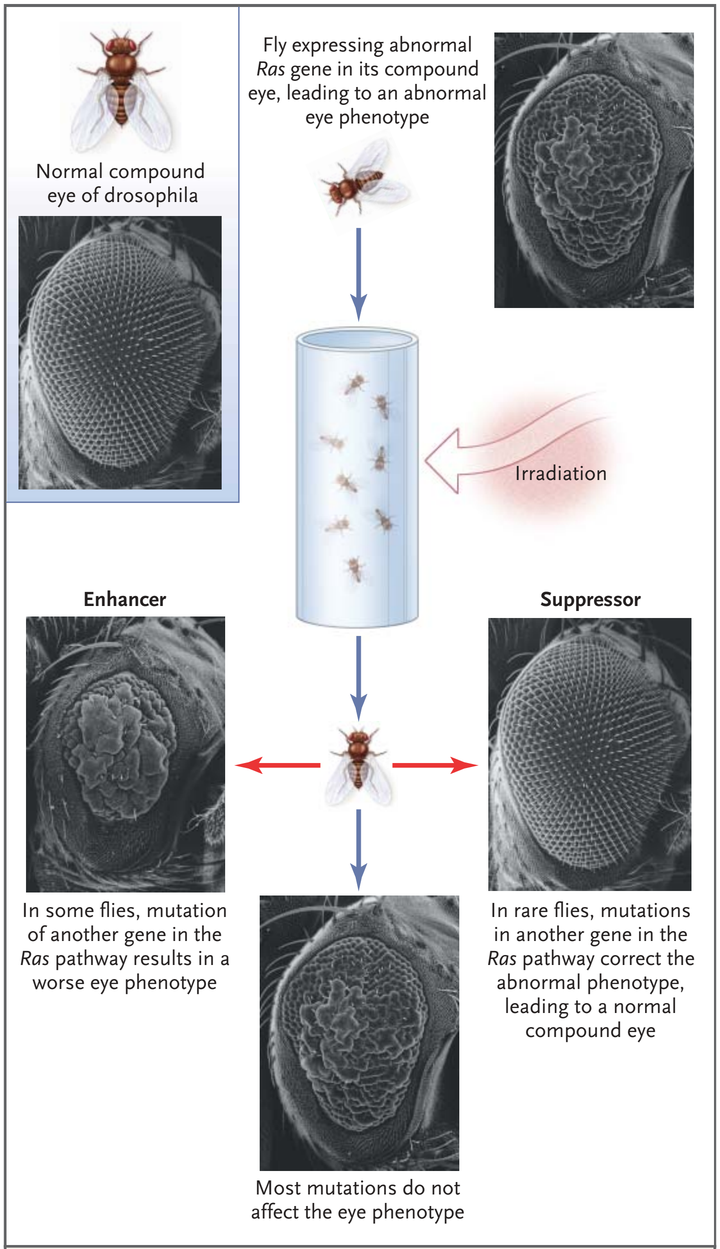

Figure 3. Modifier Screening in *Drosophila melanogaster.*

The elaborate structure of the compound eye of the fly is unnecessary for its feeding and breeding in the laboratory environment, but it makes possible the detection of abnormalities in the multiple cellular pathways required for cellular proliferation and differentiation. Expression of an abnormal *Ras* gene in fly retinal cells leads to an abnormal appearance of the fly's eye, a so-called rough-eye phenotype. Inducing random mutations in other genes (e.g., with the use of irradiation) may cause additional mutations in genes that function in the same pathway as *Ras*. Genes that undergo a mutation that causes a worsening of the phenotype are called enhancers, whereas genes that cause a correction of the phenotype are called suppressors. Genetic mapping is used to identify both these types of genes and to study how they interact with the *Ras* gene.

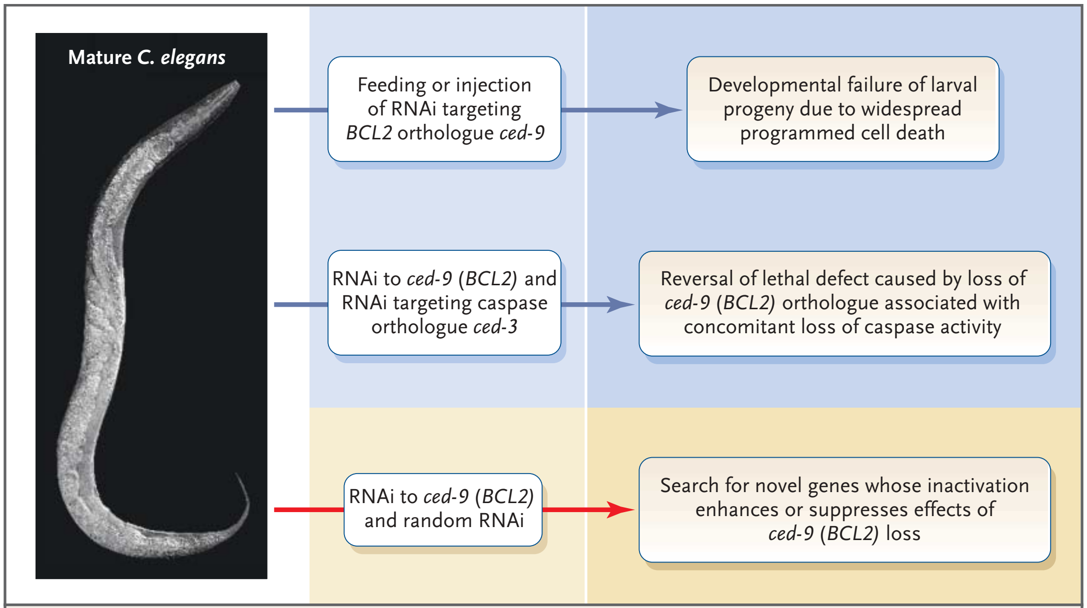

Figure 4. Inactivation of Apoptosis Genes in *Caenorhabditis elegans* by RNA-Mediated Interference.

With the fate of all 1090 cells in the worm *C. elegans* now traced, any additional cells resulting from suppression of programmed cell death (apoptosis) can be identified. Introduction of RNA-mediated interference (RNAi) into a mature worm to target the orthologue of *BCL2* (called *ced-9*) leads to developmental failure of larval progeny from widespread apoptosis. This result can be corrected by coinjection of RNAi against the caspase orthologue *ced-3,* the primary trigger of cell death. Screening for new genes that either enhance or suppress the cell-death phenotype can be performed by injecting worms with RNAi that targets *ced-9* together with RNAi that targets every known gene in the *C. elegans* genome.

of a specific cellular pathway in the context of a whole organism.

THE FUTURE

The complete annotation of the human genome and the sequencing of the genomes of an increasing number of model organisms will together provide an unparalleled opportunity to use comparative genomics to study gene function. There remains much to learn from the study of the conservation, divergence, and convergence of genes and their pathways during evolution, and with the use of model organisms, these advances can make important contributions to medical research. Early studies of the fruitfly yielded many pillars of human genetics, among them the chromosomal theory of inheritance and the mutagenic effects of x-rays. Along with a menagerie of worms, zebrafish, and other small creatures, the fly has now entered a new stage of discovery, in which the modeling of specific cellular pathways implicated in human diseases may contribute to the search for new treatments.

Jacques Monod's reported observation, mentioned earlier, that discoveries about gene regulation point to a relation between *E. coli* and the elephant may now be expanded to include yeast, the worm, the zebrafish, and also — as the 18th-century poet William Blake foresaw — the fly:

Am not I
A fly like thee?
Or art not thou
A man like me?[54]

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