Garland Publishing Inc.
717 Fifth Avenue
New York, NY 10022-8101
Telephone (212) 751-7447
Fax (212) 308-9399

October 1, 1996

Ethel Barnard
Information Systems Specialist
ACRS/ACNW Office
(301) 415-5589

Dear Ms. Barnard,

Thank you for your recent correspondence. We are able to grant permission for you to use chapter 21 from the second edition of Alberts, et al, Molecular Biology of the Cell. These pages are to be used as background information for a meeting minutes of the USNRC'c Advisory Committee on Nuclear Waste and the Advisory Committee on Reactor Safeguards. These pages will not be sold or used in any publication for sale.

Permission is granted for this one-time use only and is free of charge. All citations and credits in the text must be reproduced as they appear in the text. Proper credit for the entire selection must be given to the authors and to Garland Publishing. Include in your citation the title, date and page numbers that apply.

Thank you for pursuing your request. Please contact me with any further questions.

Sincerely,

Michael R. Galligan
Assistant Editor

Cancer

21

Chapter 21 Michael Bishop (University of California, San Francisco), John Cairns (Harvard School of Public Health), Ruth Ellman (Institute of Cancer Research, Sutton, U.K.), Hartmut Land (Imperial Cancer Research Fund, London), Bruce Ponder (Institute of Cancer Research, Sutton, U.K.).

Roughly one person in five, in the prosperous countries of the world, will die of cancer; but that is not the reason for devoting a chapter of this book to the subject. Heart disease causes more deaths, and many other illnesses result in just as much distress; in the world as a whole, other health problems, such as malnutrition and parasitic infections, are more serious. In the context of cell biology, however, cancer has a unique importance, for the family of diseases grouped under this heading reflect disturbances of the most fundamental rules of behavior of the cells in a multicellular organism. To understand cancer and to devise rational ways to treat it, we have to understand both the inner workings of cells and their social interactions in the tissues of the body. Thus basic cancer research has been the source of many advances in our knowledge of normal cells. By-products of cancer research have become crucial tools in the current revolution of cell biology—tools such as reverse transcriptase from RNA tumor viruses, used to make cDNA (see p. 183), and myeloma cell lines derived from cancerous B lymphocytes, used to make monoclonal antibodies. One may debate how much the massive resources devoted to laboratory research on cancer have so far contributed directly toward improvements in cancer treatment; but there can be no doubt that, by contributing to progress in cell biology, the cancer research effort has profoundly benefited a much wider area of medical knowledge than that of cancer alone.

We have already discussed how cancer research has begun to reveal the molecular mechanisms underlying the normal controls of cell growth and division (see pp. 752–761). In this concluding chapter we examine the disease itself. In the first section we shall consider the nature of cancer and the natural history of the disease from a cellular standpoint; in the second section we focus on its molecular basis.

Cancer as a Microevolutionary Process[1]

The body of an animal can be viewed as a society or ecosystem whose individual members are cells, reproducing by cell division and organized into collaborative assemblies or tissues. In our earlier discussion of the maintenance of tissues (in Chapter 17), our concerns were similar to those of the ecologist: cell births, deaths, habitats, territorial limitations, the maintenance of population sizes, and the like. The one ecological topic conspicuously absent was that of natural selection: we said nothing of competition or mutation among somatic cells. The reason is that a healthy body is in this respect a very peculiar society, where self-sacrifice, rather

than competition, is the rule for every class of cells except one: all somatic cell lineages are committed to die, leaving no progeny but dedicating their existence to support of the germ cells, which alone have a chance of survival. There is no mystery in this, for the body is a clone, and the genome of the somatic cells is the same as the genome of the germ cells; thus, by their self-sacrifice for the sake of the germ cells, the somatic cells help to propagate copies of their own genes.

In contrast, therefore, with free-living cells such as bacteria, which compete to survive, the cells of a multicellular organism are committed to collaboration. Any mutation that gives rise to nonaltruistic behavior by individual members of the cooperative will jeopardize the future of the whole enterprise. Thus mutation, competition, and natural selection operating *within* the population of somatic cells are ingredients for a disaster. It is, in essence, just this type of disaster that occurs in cancer: cancer is a disease in which individual cells begin by prospering selfishly at the expense of their neighbors but in the end destroy the whole cellular society and die.

In this section we discuss the development of cancer as a microevolutionary process, occurring on a time scale of months or years in a population of cells in the body, but dependent on the same principles of mutation and natural selection that govern the long-term evolution of all living organisms.

Cancers Differ According to the Cell Type from Which They Derive[2]

Cancer cells are defined by two heritable properties: they and their progeny (1) reproduce in defiance of the normal restraints and (2) invade and colonize territories normally reserved for other cells. It is the combination of these features that makes cancers peculiarly dangerous. An isolated abnormal cell that does not proliferate more than its normal neighbors does no significant damage, no matter what other disagreeable properties it may have; but if its proliferation is out of control, it will give rise to a tumor or **neoplasm**—a relentlessly growing mass of abnormal cells. So long as the neoplastic cells remain clustered together in a single mass, however, the tumor is said to be **benign,** and a complete cure can usually be achieved by removing the mass surgically. A tumor is counted as a cancer only if it is **malignant,** that is, only if its cells have the ability to invade surrounding tissue. Invasiveness usually implies an ability to break loose, enter the bloodstream or lymphatic vessels, and form secondary tumors or **metastases** at other sites in the body (Figure 21–1). The more widely a cancer metastasizes, the harder it becomes to eradicate.

Cancers are classified according to the tissue and cell type from which they arise. Cancers arising from epithelial cells are termed **carcinomas;** those arising from connective tissue or muscle cells are termed **sarcomas.** Cancers that do not fit in either of these two broad categories include the various **leukemias,** derived from hemopoietic cells, and cancers derived from cells of the nervous system. Table 21–1 lists the types of cancers that are common in the United States, together with their incidence and the death rate from them. Each of the broad categories has many subdivisions according to the specific cell type, the location in the body, and the structure of the tumor; many of the names used are fixed by tradition and have no modern rational basis. In parallel with the set of names for malignant tumors, there is a related set of names for benign tumors: an *adenoma,* for example, is a benign epithelial tumor with a glandular organization, the corresponding type of malignant tumor being an *adenocarcinoma* (Figure 21–2); a *chondroma* and a *chondrosarcoma* are, respectively, benign and malignant tumors of cartilage. About 90% of human cancers are carcinomas, perhaps because most of the cell proliferation in the body occurs in epithelia, or perhaps because epithelial tissues are most frequently exposed to the various forms of physical and chemical damage that favor the development of cancer.

Each cancer has characteristics that reflect its origin. Thus, for example, the cells of an epidermal *basal-cell carcinoma,* derived from a keratinocyte stem cell in the skin, will generally continue to synthesize cytokeratin intermediate filaments, whereas the cells of a *melanoma,* derived from a pigment cell in the skin, will often (but not always) continue to make pigment granules. Cancers originating

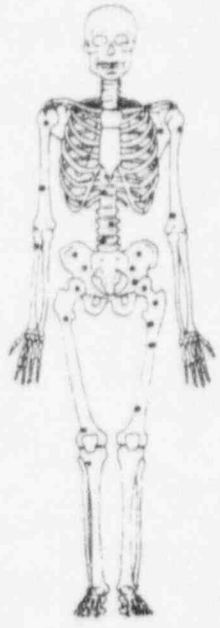


Figure 21–1 Malignant tumors typically give rise to metastases, making the cancer hard to eradicate. The drawing shows common sites in the bone marrow for metastases from carcinoma of the prostate gland. (From Union Internationale Contre le Cancer, TNM Atlas: Illustrated Guide to the Classification of Malignant Tumors, 2nd ed. Berlin: Springer, 1986.)

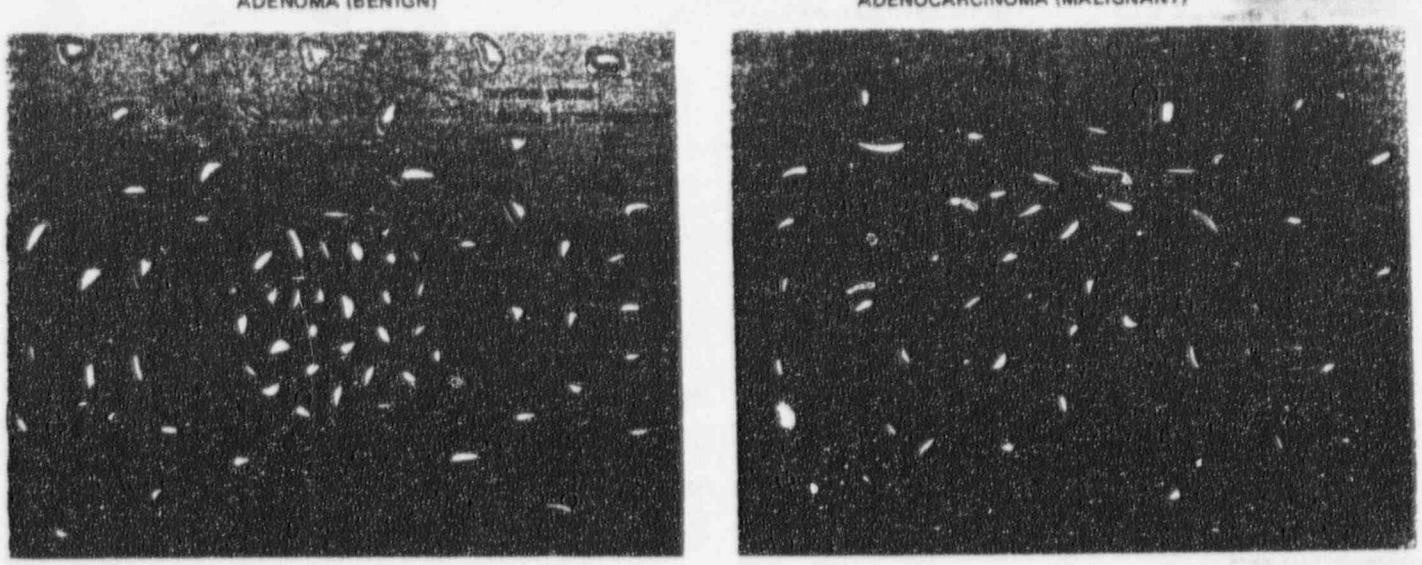
Table 21–1 Cancer Incidence and Cancer Mortality in the United States, 1986

Type of Cancer	New Cases per Year		Deaths per Year	
Total cancers	930,000		472,000	
Cancers of epithelia: carcinomas	789,000	(85%)	381,400	(81%)
Oral cavity and pharynx	29,500	(3%)	9,400	(2%)
Digestive organs (total)	217,800	(23%)	119,700	(25%)
Colon and rectum	130,000	(14%)	60,000	(13%)
Pancreas	25,500	(3%)	24,000	(5%)
Stomach	24,700	(3%)	14,300	(3%)
Liver and biliary system	13,600	(1%)	10,600	(2%)
Respiratory system (total)	164,500	(18%)	135,400	(29%)
Lung	149,000	(16%)	130,100	(28%)
Breast	123,900	(13%)	40,200	(9%)
Skin (total)	(>400,000)*		7,500	(2%)
Malignant melanoma	23,000	(2%)	5,600	(1%)
Reproductive tract (total)	169,800	(18%)	49,400	(10%)
Prostate gland	90,000	(10%)	26,100	(6%)
Ovary	19,000	(2%)	11,600	(2%)
Uterine cervix	14,000	(2%)	6,800	(1%)
Uterus (endometrium)	36,000	(4%)	2,900	(1%)
Urinary organs (total)	60,500	(7%)	19,800	(4%)
Bladder	40,500	(4%)	10,600	(2%)
Cancers of hemopoietic and immune system: leukemias and lymphomas	70,100	(8%)	41,100	(9%)
Cancers of central nervous system and eye: gliomas, retinoblastoma, etc.	15,600	(2%)	10,600	(2%)
Cancers of connective tissues, muscles and vasculature: sarcomas	7,100	(1%)	4,200	(1%)
All other cancers + unspecified sites	48,200	(5%)	34,800	(7%)

*Nonmelanoma skin cancers are not included in total of all cancers, since almost all are cured easily and many go unrecorded.

In the world as a whole, the five most common cancers are those of lung, stomach, breast, colon/rectum, and uterine cervix, and the total number of new cancer cases per year is just over 6 million. Note that only about half the number of people who develop cancer die of it. (Data for USA from American Cancer Society, Cancer Facts and Figures, 1986.)

Figure 21–2 An adenoma (a benign glandular tumor) and an adenocarcinoma (a malignant glandular tumor) contrasted. There are many forms that such tumors may take; this diagram illustrates schematically types that might be found in the breast.



from different cell types are, in general, very different diseases. The basal-cell carcinoma, for example, is only locally invasive and rarely forms metastases, whereas the melanoma is much more malignant and rapidly gives rise to many metastases (behavior that recalls the migratory tendencies of the normal pigment-cell precursors during development—see p. 944). The basal-cell carcinoma is usually easy to remove by surgery, leading to complete cure; but the malignant melanoma, once it has metastasized, is often impossible to extirpate and consequently fatal.

Most Cancers Derive from a Single Abnormal Cell[3]

The origins of most cancers can be traced to a single isolated **primary tumor;** this suggests that they are derived by cell division from a single cell that has undergone some heritable change that enables it to outgrow its neighbors. By the time it is first detected, however, a typical tumor already contains about a billion cells or more (Figure 21–3), often including many normal cells—fibroblasts, for example, in the supporting connective tissue that is associated with a carcinoma. It is not easy to prove that the cancer cells are a clone descended from a single abnormal cell; but where evidence is available, it usually confirms that the cancer has a monoclonal origin. In almost all patients with *chronic myelogenous leukemia,* for example, the leukemic white blood cells are distinguished from the normal cells by a specific chromosomal abnormality (the so-called Philadelphia chromosome, created by a translocation between the long arms of chromosomes 22 and 9, as shown in Figure 21–4). It is unlikely that the genetic accident responsible for this abnormality would have occurred in several cells at once in the same individual; it is much more likely that all the leukemic cells are descendants of the same single mutant cell. Indeed, when the DNA at the site of translocation is cloned and sequenced, it is found that the site of breakage and rejoining of the translocated fragments is identical in all the leukemic cells in any given patient, but differs slightly (by a few hundred or thousand base pairs) from one patient to another, as expected if each case of the leukemia arises from a unique accident occurring in a single cell.

Another way to show that a cancer has a monoclonal origin is by exploiting the phenomenon of X-chromosome inactivation (see p. 577). A normal woman is a random mixture, or mosaic, of two classes of cells—those in which the paternal X chromosome is inactivated and those in which the maternal X chromosome is inactivated. The inactivation of one X chromosome in each cell occurs early in embryonic development, and thereafter the daughters of a dividing somatic cell always have the same X chromosome inactivated as the parent cell. Consequently, the state of X-chromosome inactivation—maternal or paternal—can be used as a heritable marker to trace the lineage of cells in the body. In the great majority of tumors that have been analyzed—both benign and malignant—all the tumor cells have been found to have the same X chromosome inactivated, strongly suggesting that they are derived from a single deranged cell (Figure 21–5).

Most Cancers Are Probably Initiated by a Change in the Cell's DNA Sequence[4]

If a single abnormal cell is to give rise to a tumor, it must pass on its abnormality to its progeny: the aberration has to be heritable. A first problem in understanding a cancer is to discover whether the heritable aberration is due to a genetic change—that is, an alteration in the the cell's DNA sequence—or to an *epigenetic* change—that is, a change in the pattern of gene expression without a change in the DNA sequence. Heritable epigenetic changes, reflecting cell memory (see p. 570 and p. 898), are a familiar feature of normal development, as manifest in the stability of the differentiated state (see p. 952) and in such phenomena as X chromosome inactivation (see p. 577); and there is no obvious a priori reason why they should not be involved in cancer. For one rare and extraordinary type of cancer—the teratocarcinoma (see p. 897)—there is indeed evidence in favor of an epigenetic origin. There are, however, good reasons to think that most cancers are initiated by genetic change (although epigenetic changes may play a part in the subsequent

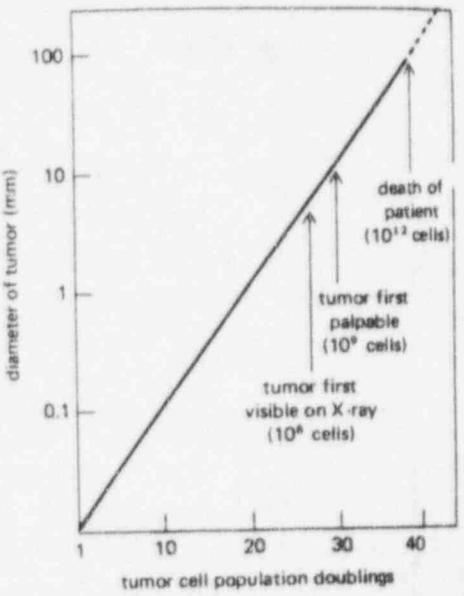


Figure 21–3 The growth of a typical human tumor, with the diameter of the tumor plotted on a logarithmic scale. Years may elapse before the tumor becomes noticeable.

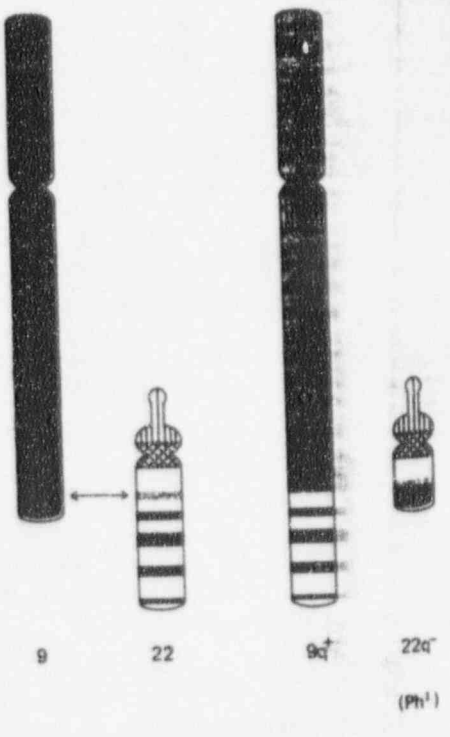


Figure 21–4 The translocation between chromosomes 9 and 22 responsible for chronic myelogenous leukemia. The smaller of the two resulting abnormal chromosomes is called the Philadelphia chromosome, after the city where the abnormality was first recorded.

development of the disease). Thus cells of a given cancer can often be shown to have a shared abnormality in their DNA sequence, as we have just seen for chronic myelogenous leukemia; many other examples will be discussed in the second half of this chapter. But this does not prove that genetic change is an essential *first* step in the causation of cancer. A more cogent argument is that most of the agents known to cause cancer cause genetic change; and, conversely, agents that cause genetic change cause cancer. This correlation between **carcinogenesis** (the generation of cancer) and *mutagenesis* is clear for three classes of agents: chemical carcinogens (which typically cause simple local changes in the nucleotide sequence), ionizing radiation such as x-rays (which typically cause chromosome breaks and translocations), and viruses (which introduce foreign DNA into the cell). The role of viruses in cancer will be discussed later; we pause here to discuss **chemical carcinogens.**

In general, a given cancer cannot be blamed entirely on a single event or a single cause: as we shall see, cancers as a rule result from the chance occurrence in one cell of several independent accidents, with cumulative effects. The cell's environment influences the frequency of these accidents in a variety of ways, and most cancers should be viewed as the outcome of a random process that is made more probable by a mixture of contributory environmental factors (see p. 1195). There are, however, some unusually carcinogenic agents that increase the likelihood of the critical events to the point where it becomes virtually certain, given a high enough dosage, that at least one cell in the body will turn cancerous. The compound 2-naphthylamine, used in the chemical industry in the early part of this century, is one notorious example: in one British factory, all of the men who had been employed in distilling it (and were thereby subjected to prolonged exposure) eventually developed bladder cancer.

Many quite disparate chemicals have been shown to be likewise carcinogenic when they are fed to experimental animals or painted repeatedly on their skin. Some of these carcinogens act directly on the target cells; many others take effect only after they have been changed to a more reactive form by metabolic processes—notably by a set of intracellular enzymes known as the cytochrome P-450 oxidases, which normally help to convert ingested toxins and foreign lipid-soluble materials into harmless and easily excreted compounds but which fail in this task with certain substances, converting them instead into direct carcinogens (Figure 21–6). Although the known chemical carcinogens are very diverse, most of them have at least one property in common: they cause mutations. The mutagenicity can be demonstrated by various methods, one of the most convenient being the *Ames test*, in which the carcinogen is mixed with an activating extract prepared from rat liver cells and added to a culture of specially designed test bacteria; the resulting mutation rate of the bacteria is then measured (Figure 21–7). Most of the compounds scored as mutagenic by this bacterial assay also cause mutations and/or chromosome aberrations when tested on mammalian cells, and they have chemical structures that can be seen to imply an ability to react with DNA. When mutagenicity data from these various sources are combined and compared with carcinogenicity data from studies of cancer induction *in vivo*, it is found that the majority of known carcinogens are mutagenic and, conversely, that the majority of mutagens are carcinogenic.

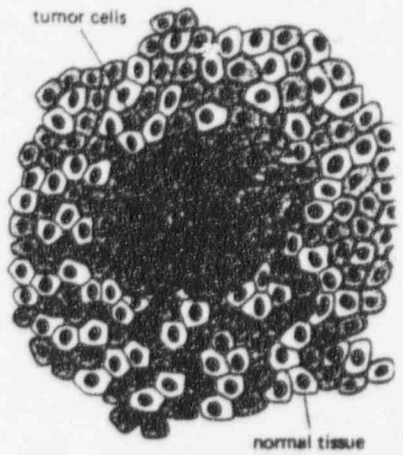
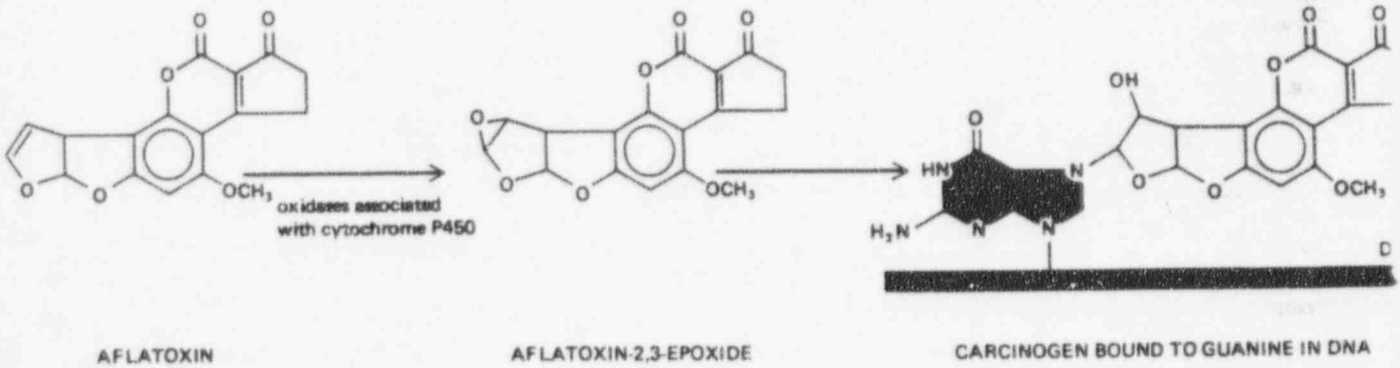


Figure 21–5 Evidence from the analysis of X-inactivation mosaics demonstrating the monoclonal origin cancers. As a result of a random process that occurs in the early embry practically every normal tissue in a woman's body is a mixture of cells wit different X chromosomes heritably inactivated (indicated here by the mixture of colored cells and black cell in the normal tissue). When the cells a cancer are tested for their expressio of an X-linked marker gene, however, they are usually all found to have the same X chromosome inactivated. This implies that they are all derived from single cancerous founder cell.

Figure 21–6 Many chemical carcinogens have to be activated by a metabolic transformation before they will cause mutations by reacting with DNA. The compound illustrated here *aflatoxin B1*, a toxin from a mold (*Aspergillus flavus oryzae*) that grows grain and peanuts when they are stor under humid tropical conditions. It is thought to be a contributory cause of liver cancer in the tropics.



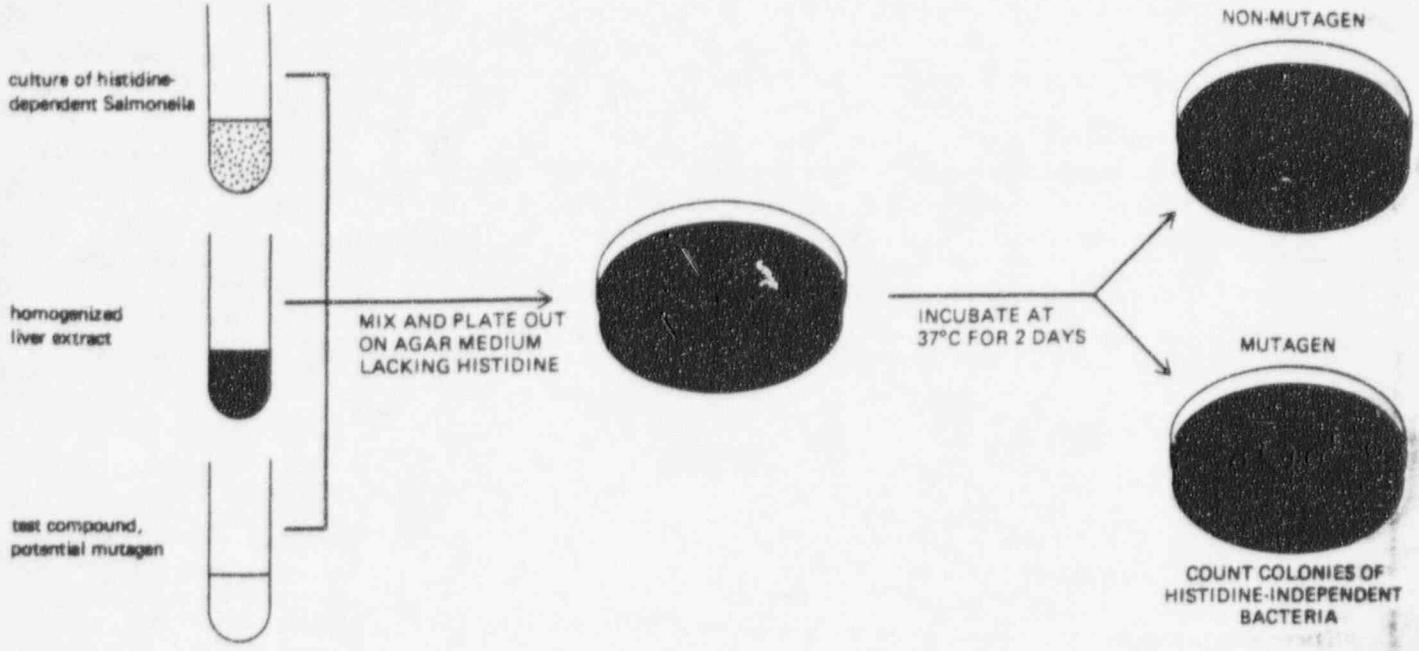


Figure 21–7 The Ames test for mutagenicity. The test uses a strain of *Salmonella* bacteria that require histidine in the medium because of a defect in a gene necessary for histidine synthesis. Mutagens can cause a further change in this gene that reverses the defect, creating revertant bacteria that do not require histidine. To increase the sensitivity of the test, the bacteria also have a defect in their DNA repair machinery that makes them especially susceptible to agents that damage DNA. A majority of compounds that are mutagenic by tests such as this are also carcinogenic, and vice versa.

There is, nevertheless, a significant minority of carcinogens that do not appear to be mutagenic. We shall discuss below (p. 1196) how non-mutagenic substances may promote the development of cancer by affecting the behavior of pre-existing mutant cells. But first we must consider how frequently such mutant cells are likely to arise in the normal course of events.

A Single Mutation Is Not Enough to Cause Cancer[1,5]

Something on the order of 10^{16} cell divisions take place in a human body in the course of a lifetime; in a mouse, with its smaller number of cells and its shorter lifespan, the number is about 10^{12}. Even in an environment that is free of mutagens, mutations will occur spontaneously, at an estimated rate of about 10^{-6} mutations per gene per cell division—a value set by fundamental limitations on the accuracy of DNA replication and repair (see p. 229). Thus, in a lifetime, every single gene is likely to have undergone mutation on about 10^{10} separate occasions in any individual human being, or about 10^{6} occasions in a mouse. Among the resulting mutant cells one might expect that there would be many that have disturbances in genes involved in the regulation of cell division and that consequently disobey the normal restrictions on cell proliferation. From this point of view, the problem of cancer seems to be not why it occurs, but why it occurs so infrequently.

Evidently, the survival of mammals must depend on some form of double—or more than double—insurance in the mechanisms that protect us from being overrun by mutant clones of cells that have a selective advantage over our healthy normal cells: if a single mutation in some particular gene were enough to convert a typical healthy cell into a cancer cell, we would not be viable organisms. Many lines of evidence indicate that the genesis of a cancer does indeed require that several independent rare accidents occur together in one cell. One such indication comes from epidemiological studies of the incidence of cancer as a function of age. If a single mutation were responsible, occurring with a fixed probability per year, the chance of developing cancer in any given year should be independent of age. In fact, for most types of cancer the chance goes up very steeply with age—typically as the third, fourth, or fifth power (Figure 21–8). From such statistics it has been estimated that somewhere between three and seven independent random events, each of low probability, are typically required to turn a normal cell into a cancer cell; the smaller numbers apply to leukemias, the larger to carcinomas.

Now that specific mutations responsible for the development of cancer have been identified, it has become possible to test the effects of the mutant genes in transgenic mice (see p. 267); as we shall see later (p. 1212), the results give addi-

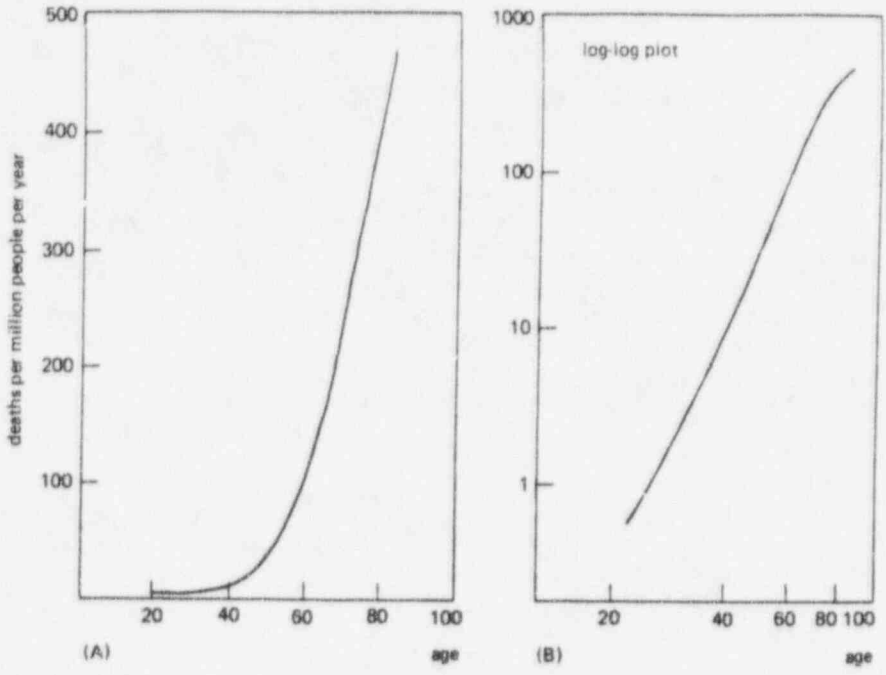


Figure 21–8 Number of deaths from cancer of the large intestine in the United States in one year, plotted as a function of age at death: the same data are shown on an ordinary linear scale in (A) and on a logarithmic scale in (B). The incidence of cancer rises steeply as a function of age—roughly as the 5th power, in this example (that is, the slope of the log-log plot in (B) is about 5). This suggests that a cell must accumulate the disruptive effects of several independent accidents before it will give rise to a cancer. (From U.S. Department of Health, Education and Welfare: Vital Statistics of the United States, Vol. II: Mortality. Washington, D.C.: U.S. Government Printing Office, 1968.)

tional and more direct evidence for the hypothesis that a single mutation is insufficient to cause cancer. The hypothesis is also supported by many older studies of the phenomenon of **tumor progression**, whereby an initial mild disorder of cell behavior evolves gradually into a full-blown cancer. These observations of how tumors develop, moreover, provide insight into the nature of the multiple changes that must occur for a normal cell to become a cancer cell and into the factors that control their occurrence.

Cancers Develop in Slow Stages from Mildly Aberrant Cells[1,5,6]

For those cancers that have a discernable external cause, there is almost always a long delay between the causal event(s) and the onset of the disease: the incidence of lung cancer does not begin to rise steeply until after 10 or 20 years of heavy smoking; the incidence of leukemias in Hiroshima and Nagasaki did not show a marked rise until about 5 years after the explosion of the atomic bombs, and it did not reach its peak until 8 years had elapsed; industrial workers exposed for a limited period to chemical carcinogens do not usually develop the cancers characteristic of their occupation until 10, 20, or even more years after the exposure (Figure 21–9); and so on. During this long incubation period, the prospective cancer cells undergo a succession of changes. Chronic myelogenous leukemia, mentioned earlier, provides a clear and simple example. This disease begins as a disorder characterized by a nonlethal overproduction of white blood cells and

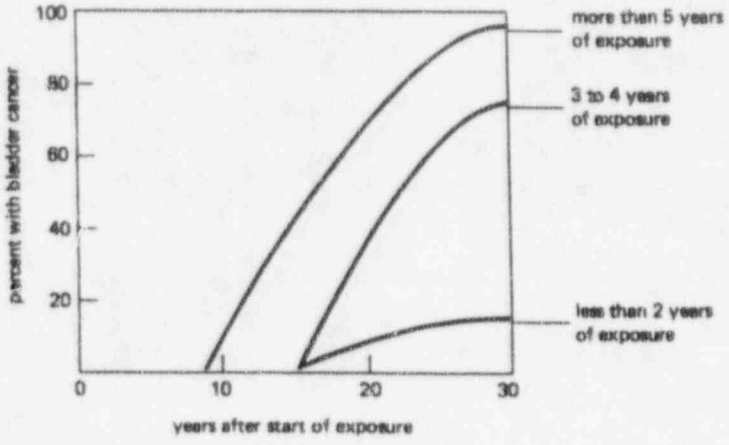
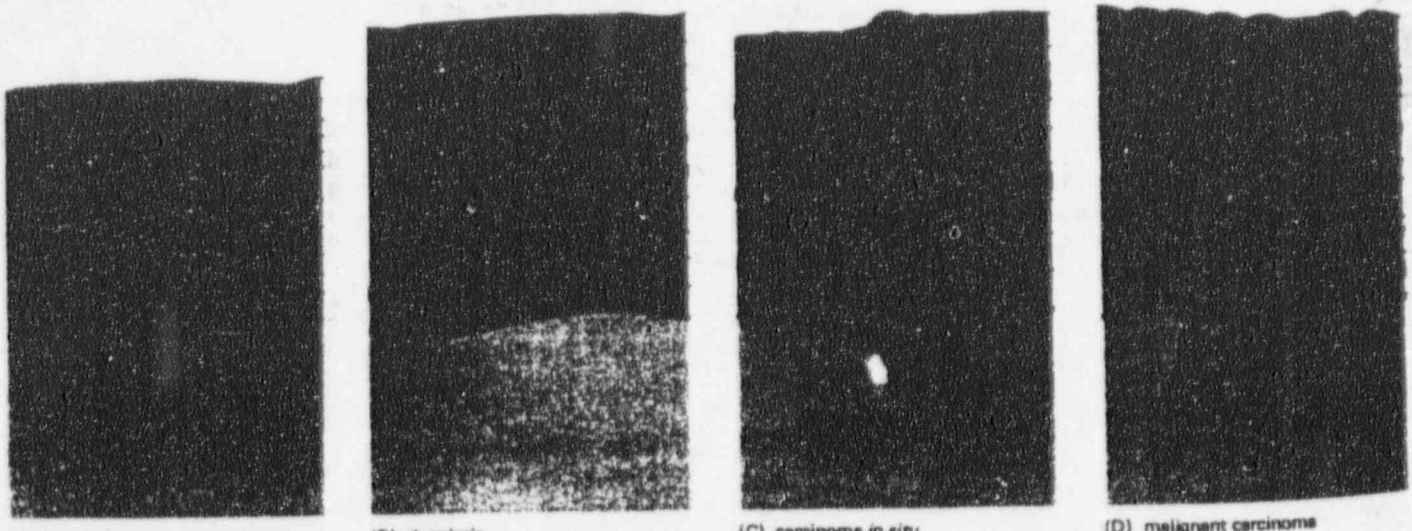


Figure 21–9 The length of the delay before onset of bladder cancer in a set of 78 men who had been exposed to the carcinogen 2-naphthylamine, grouped according to the duration of their exposure. (Modified from J. Cairns, Cancer: Science and Society. San Francisco: Freeman, 1978. After M.H.C. Williams, in Cancer, Vol. III (R.W. Raven, ed.). London: Butterfield, 1958.)

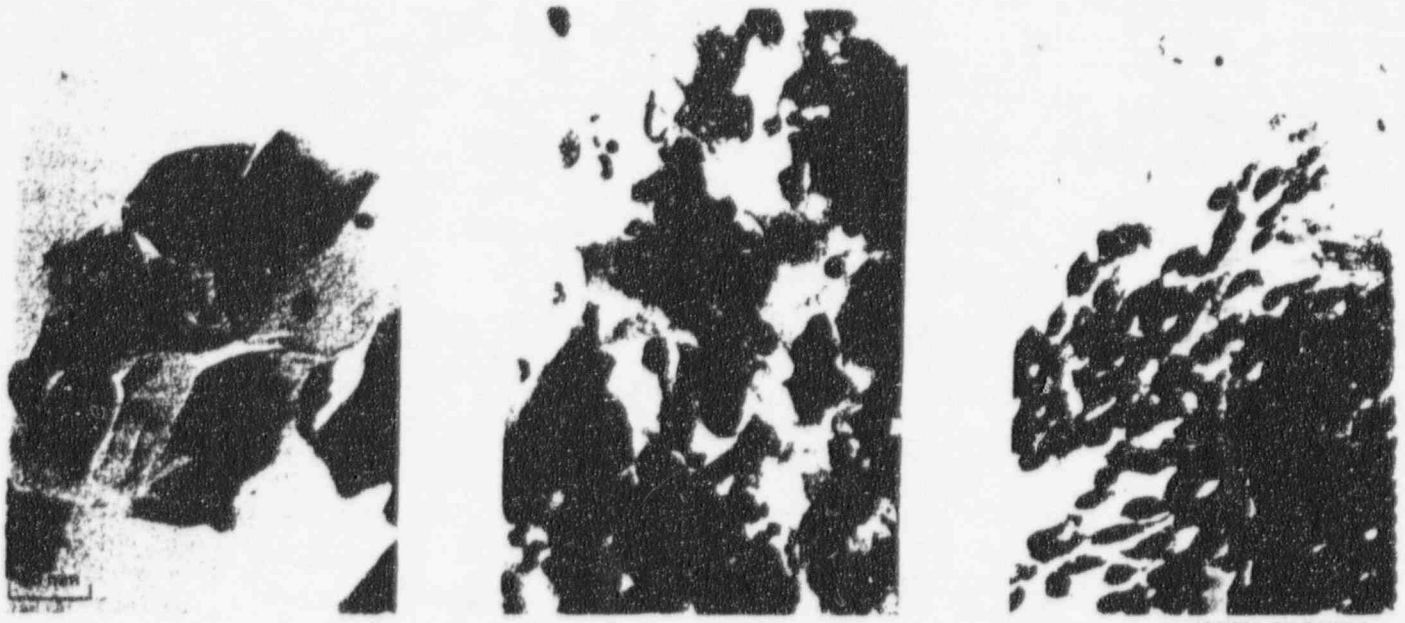
continues as such for several years before changing into a much more rapidly progressing illness that usually ends in death within a few months. In the chronic early phase the leukemic cells in the body are distinguished simply by their possession of the chromosomal translocation mentioned previously (see p. 1190). In the subsequent acute phase of the illness the hemopoietic system is overrun by cells that show not only this chromosomal abnormality but also several others. It appears as though members of the initial mutant clone have undergone further mutations that make them proliferate more rapidly (or divide more times before they terminally differentiate), so that they come to outnumber both the normal hemopoietic cells and their cousins that have only the primary disorder.

Carcinomas and other solid tumors are thought to evolve in a similar way. Although most such cancers in humans are not diagnosed until a relatively late stage, in a few cases it is possible to observe the early steps in the development of the disease. Cancers of the *uterine cervix* (the neck of the womb) provide a typical example. These cancers derive from the multilayered cervical epithelium, which has an organization similar to that of the epidermis of the skin (see p. 968). Normally, proliferation occurs only in the basal layer, generating cells that then move outward toward the surface, differentiating into flattened, keratin-rich, nondividing cells as they go, and finally being sloughed off from the surface (Figure 21–10A). When many specimens of this epithelium from different women are examined, however, it is not unusual to find patches of **dysplasia**, where dividing cells are no longer confined to the basal layer and there is some disorder in the process of differentiation (Figure 21–10B). Cells are sloughed from the surface in abnormally early stages of differentiation, and the presence of the dysplasia can be detected by scraping a sample of cells from the surface and viewing it under the microscope (the "Pap smear" technique—Figure 21–11). Left alone, the dysplastic patches will often remain harmless or even regress spontaneously; more rarely, however, they may progress, over a period of several years, to give rise to patches of so-called **carcinoma *in situ*** (Figure 21–10C). In these more serious lesions (somewhat misleadingly named, since they are not yet fully malignant), the usual pattern of cell division and differentiation is much more severely disrupted, and all the layers of the epithelium consist of undifferentiated proliferating cells, which are often highly variable in size and karyotype; the abnormal cells are still confined, however, to the epithelial side of the basal lamina. At this stage it is still easy to achieve a complete cure by destroying or removing the abnormal tissue surgically. Without such treatment the abnormal patch may still remain harmless or regress; but in an estimated 20–30% of cases it will develop, again over a period of several years, to give rise to a truly malignant cervical carcinoma (Figure 21–10D), whose cells break out of the epithelium by crossing the basal lamina and begin to invade the underlying connective tissue. Surgical cure becomes progressively more difficult as the invasive growth spreads.

Figure 21–10 The stages of progression in the development of cancer of the epithelium of the uterine cervix. In dysplasia, the most superficial cells still show some signs of differentiation; but this is incomplete, and proliferating cells are seen abnormally far above the basal layer. In carcinoma *in situ*, the cells in all the layers are proliferating and apparently undifferentiated. True malignancy begins when the cells cross the basal lamina and begin to invade the underlying connective tissue. Several years may elapse from the first signs of dysplasia to the onset of full-blown malignant cancer.



(A) normal (B) dysplasia (C) carcinoma *in situ* (D) malignant carcinoma



(A) (B) (C)

Figure 21–11 Photographs of cells collected by scraping the surface of the uterine cervix (the Papanicolaou or "Pap smear" technique). (A) Normal; the cells are large and well differentiated, with highly condensed nuclei. (B) Dysplasia; the cells are in a variety of stages of differentiation, some quite immature. (C) Invasive carcinoma; the cells all appear undifferentiated, with scanty cytoplasm and a relatively large nucleus; debris in the background includes blood cells that have leaked out at the site of the ulcer created by the carcinoma. (Courtesy of Edward Miller.)

Tumor Progression Involves Successive Rounds of Mutation and Natural Selection[6,7]

As illustrated by the two very different examples just discussed, cancers in general seem to arise by a process in which an initial population of slightly abnormal cells, descendants of a single mutant ancestor, evolves from bad to worse through successive cycles of mutation and natural selection. This evolution involves a large element of chance and usually takes many years; most of us die of other ailments before cancer has had time to develop. To understand the causation of cancer it is essential to understand the factors that may speed up the process.

In general, the rate of evolution, whether in a population of cells exploiting the opportunities for cancerous behavior in the body or in a population of organisms adapting to a new environment on the surface of the Earth, would be expected to depend on four main parameters: (1) the *mutation rate*, that is, the probability per gene per unit time that any given member of the population will undergo genetic change; (2) the *number of individuals in the population;* (3) the *rate of reproduction*, that is, the average number of generations of progeny produced per unit time; and (4) the *selective advantage* enjoyed by successful mutant individuals, that is, the ratio of the number of surviving fertile progeny they produce per unit time to the number of surviving fertile progeny produced by nonmutant individuals. The selective advantage depends both on the nature of the mutation and on environmental conditions, and further complications arise if heritable epigenetic changes occur, either randomly or in reaction to specific cues.

Experimental studies on the induction of cancer in animals illustrate these evolutionary principles. In the light of such studies, one can begin to make sense of the confusing variety of factors that affect the incidence of human cancers—factors ranging from cigarette smoke (for cancer of the lung) to the age at which a woman has her first baby (for cancer of the breast). The mutation rate per cell is not the only significant variable in the development of cancer.

The Development of a Cancer Can Be Promoted by Factors That Do Not Alter the Cells' DNA Sequence[6,8]

The stages by which an initial mild lesion progresses to become a cancer can be most easily observed in the skin. Skin cancers can be elicited in mice, for example, by repeatedly painting the skin with a mutagenic chemical carcinogen such as benzo(a)pyrene (a constituent of coal tar and tobacco smoke) or the related com-

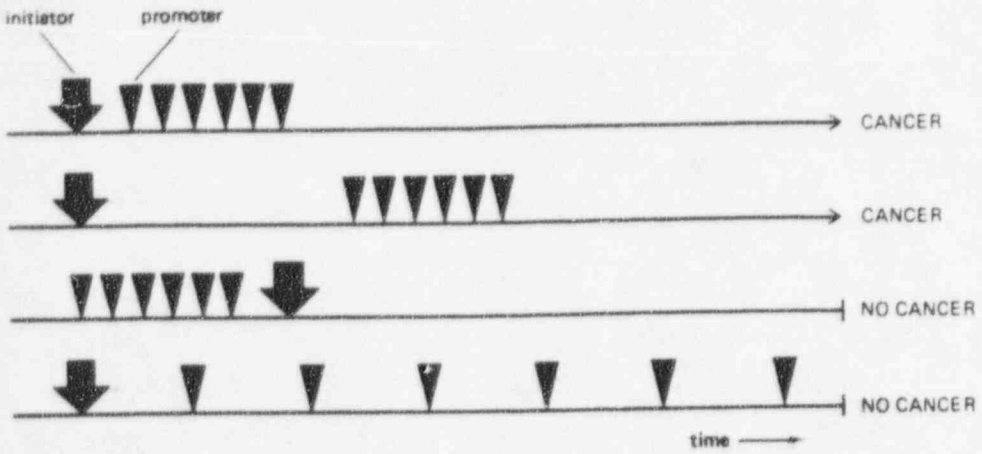


Figure 21–12 Some possible schedules of exposure to a tumor initiator (mutagenic) and a tumor promoter (nonmutagenic), and their outcomes. Cancer ensues only if the exposure to the promoter follows exposure to the initiator, and only if the intensity of exposure to the promoter exceeds a certain threshold. Cancer can also occur as a result of repeated exposure to the initiator alone.

pound dimethylbenz(a)anthracene (DMBA). A single application of the carcinogen, however, usually does not by itself give rise to a tumor or any other obvious lasting abnormality. Yet it does cause latent genetic damage, and this can be detected through a greatly increased incidence of cancer when the cells are exposed either to further treatments with the same substance or to certain other, quite different, insults. A carcinogen that sows the seeds of cancer in this way is said to act as a **tumor initiator.** Simply wounding skin that has been exposed once to such an initiator can cause cancers to develop from some of the cells at the edge of the wound. Alternatively, repeated exposure over a period of months to certain substances known as **tumor promoters,** which are not themselves mutagenic, can cause cancer selectively in skin previously exposed to a tumor initiator. The most widely studied tumor promoters are *phorbol esters,* such as tetradecanoylphorbol acetate (TPA), which we have already encountered in another context as artificial activators of protein kinase C (and hence as agents that activate part of the phosphatidylinositol intracellular signaling pathway—see p. 704). These substances cause cancers at high frequency only if they are applied *after* a treatment with a mutagenic initiator (Figure 21–12).

As one might expect for genetic damage, the hidden changes caused by a tumor initiator are irreversible: thus they can be uncovered by treatment with a tumor promoter even after a long delay. The immediate effect of the promoter is apparently to stimulate cell division (or to cause cells that would normally undergo terminal differentiation to continue dividing instead); and in the region that had previously been exposed to the initiator this results in the growth of many small, benign, wartlike tumors, called *papillomas.* The greater the prior dose of initiator, the larger the number of papillomas induced; it is thought that each papilloma (at least for low doses of the initiator) consists of a single clone of cells descended from a mutant cell that the initiator has engendered. Both wounding and the application of the promoter probably act by inducing the expression of some of the "social control" genes that directly or indirectly affect cell proliferation (see p. 752). Such genes may remain quiescent in the resting epithelium, so that any mutations they have undergone in response to the initiator go undetected; by

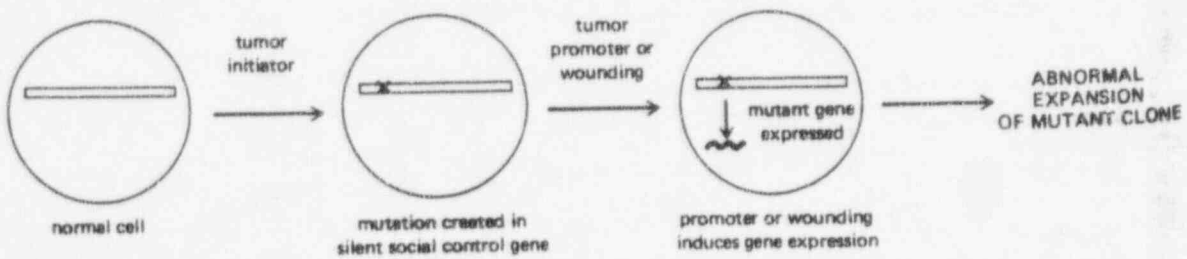


Figure 21–13 One hypothesis proposed to explain the observed effect of tumor promoters on the development of tumors. Alternatively, the mutant gene might be constitutively expressed but have no effect until the promoter activates other genes required for cell proliferation.

inducing gene expression, the promoter or the stimulus of wounding may uncover the mutations and enable them to begin influencing cell proliferation (Figure 21–13).

A typical papilloma might contain about 10^5 cells—more than a thousand times as many as there are in a normal epidermal proliferative unit (see p. 969). If exposure to the tumor promoter is stopped, almost all the papillomas regress, and the skin regains a largely normal appearance—as expected from the hypothesis illustrated in Figure 21–13. In a few of the papillomas, however, further changes occur that enable growth to continue in an uncontrolled way, even after the promoter has been withdrawn. These changes seem to originate in occasional single papilloma cells, at about the frequency expected for spontaneous mutations. In this way a small proportion of the papillomas progress to become cancers. Thus the tumor promoter apparently favors the development of cancer, in this system at least, by expanding the population of cells that carry an initial mutation: the more such cells there are, the greater the chance that at least one of them will undergo another mutation carrying it one more step along the road to malignancy. Although naturally occurring cancers do not necessarily arise through the specific sequence of distinct initiation and promotion steps just described, their evolution must be governed by similar principles. They too will evolve at a rate that depends both on the frequency of mutations and on influences affecting the survival, proliferation, and spread of certain types of mutant cells once they have been created.

Most Cancers Result from Avoidable Combinations of Environmental Causes[9]

The development of a cancer generally involves many steps, each governed by multiple factors, some dependent on the genetic constitution of the individual, others dependent on his or her environment and way of life. By changing our surroundings or our habits, therefore, we should, in principle, be able to reduce drastically our chance of developing almost any given type of cancer. This is demonstrated most clearly by a comparison of cancer incidence in different countries: for almost every cancer that is common in one country, there is another country where the incidence is several times lower (Table 21–2); and migrant populations tend to take on the pattern of cancer incidence typical of the host country, implying that the differences are due to environmental, not genetic, factors. From such data it is estimated that 80–90% of cancers should be avoidable. Unfortunately, different cancers have different environmental risk factors, and a country that happens to escape one such danger is no more likely than other countries to escape the rest; thus the incidence of all cancers combined (among individuals of a given age) is similar from country to country. There are, however, some subgroups whose abstinent way of life does seem to reduce the total cancer death rate: the incidence of cancer among strict Mormons in Utah, for example, is only about half that among Americans in general.

While such epidemiological observations indicate that cancer can be avoided, it remains difficult to identify the specific environmental risk factors or to establish how they act. Some certainly operate as mutagenic tumor initiators, directly provoking genetic change; others presumably serve as tumor promoters that help to enlarge the population of cells liable to progress, through further mutation, to full-blown cancer. The carcinogens in tobacco smoke, like the aflatoxin on tropical peanuts (see Figure 21–6), probably belong mostly in the first category, while the reproductive hormones that circulate in a woman's body at different stages of her life may belong in the second category (Figure 21–14). It is possible that some factors act in still other ways—for example, by causing heritable epigenetic changes. Of course, it is not necessary to understand how cancer-causing agents act in order to identify them and show how to avoid them. In this task, cancer epidemiology has had some notable successes and promises more to come; simply by revealing the role of smoking, it has shown a way to reduce the total cancer death rate in North America and Europe by as much as 30%. The prevention of cancer is not only better than cure but seems also, given our present state of knowledge, to be much more readily attainable.

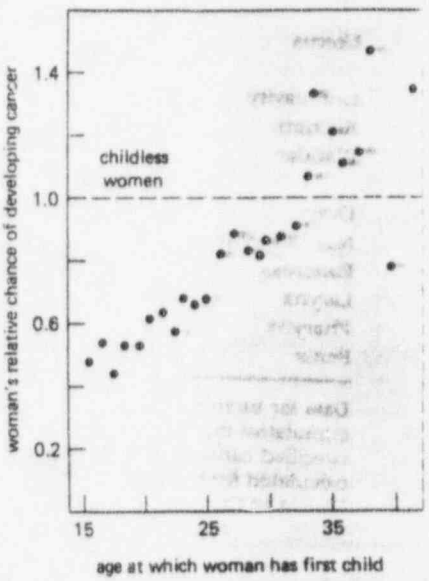


Figure 21–14 The relative probability of breast cancer developing at some time in a woman's life plotted as a function of the age at which she gives birth to her first child. The graph shows the value of the probability relative to that for a childless woman. The longer the delay before bearing the first child, the higher the probability of breast cancer, suggesting that exposure to certain combinations of reproductive hormones may promote development o the cancer. There is some evidence from laboratory studies that the first full-term pregnancy may result in a permanent epigenetic change in the cells of the breast, altering their subsequent responses to hormones. Many other factors—for example, the amount of fat in the diet—are also highly correlated with breast cancer. (From J. Cairns, Cancer: Science and Society. San Francisco: Freeman, 1978. After B. MacMahon, P. Cole, and J. Brown, *J. Natl. Cancer Inst.* 50:21–42, 1973.)

Table 21–2 Variation Between Countries in the Incidence of Some Common Cancers

Site of Origin of Cancer	High–Incidence Area	Cumulative Incidence (%) in High–Incidence Area	Low–Incidence Area	Ratio of Rates in High– and Low–Incidence Areas
Skin	Australia (Queensland)	20	India (Bombay)	>200
Esophagus	Iran	20	Nigeria	300
Lung	England	11	Nigeria	35
Stomach	Japan	11	Uganda	25
Uterine cervix	Columbia	10	Israel (Jewish)	15
Prostate	United States (blacks)	9	Japan	40
Liver	Mozambique	8	England	100
Breast	Canada	7	Israel (non-Jewish)	7
Colon	United States (Connecticut)	3	Nigeria	10
Uterus	United States (California)	3	Japan	30
Oral cavity	India (Bombay)	2	Denmark	25
Rectum	Denmark	2	Nigeria	20
Bladder	United States (Connecticut)	2	Japan	6
Ovary	Denmark	2	Japan	6
Nasopharynx	Singapore (Chinese)	2	England	40
Pancreas	New Zealand (Maori)	2	India (Bombay)	8
Larynx	Brazil (São Paulo)	2	Japan	10
Pharynx	India (Bombay)	2	Denmark	20
Penis	Parts of Uganda	1	Israel (Jewish)	300

Data for uterine cervix, breast, uterus, and ovary are for women; others are for men. The cumulative incidence is defined as the percentage of the population that would develop the specified cancer by the age of 75, in the absence of other causes of death; the ratio of rates is calculated for the 35- to 64-year age group. (Slightly modified from R. Doll and R. Peto. The Causes of Cancer. New York: Oxford University Press, 1981.)

The Search for Cancer Cures Is Hard but Not Hopeless[10]

The difficulty of curing a cancer is like the difficulty of getting rid of weeds. Cancer cells can be removed surgically or destroyed with toxic chemicals or radiation; but it is hard to eradicate every single one of them. Surgery can rarely ferret out every metastasis, and treatments that kill cancer cells are generally toxic to normal cells as well. If even a few cancerous cells remain, they can proliferate to produce a resurgence of the disease; and unlike the normal cells, they may evolve resistance to the poisons used against them. Yet the outlook is not hopeless. In spite of the difficulties, effective cures using anticancer drugs (alone or in combination with other treatments) have been devised for some formerly highly lethal cancers (notably Hodgkin's lymphoma, testicular cancer, choriocarcinoma, and some leukemias and other cancers of childhood). For several of the more common cancers, moreover, appropriate surgery or local radiotherapy enables a large proportion of patients to recover if the illness is diagnosed at a reasonably early stage; and even where a cure at present seems beyond our reach, there are treatments that will prolong life or at least relieve distress.

A great deal of clinical cancer research centers on the problem of how to kill cancer cells selectively. For the most part, current methods exploit relatively subtle differences between normal and neoplastic cells with respect to proliferation rate, metabolism, and radiosensitivity, and they have unpleasant toxic side effects. A few types of cancer cells are especially vulnerable to selective attack because they depend on specific hormones or because their surfaces have unusual chemical features that can be recognized by antibodies. In general, however, progress with

the vexing problem of anticancer selectivity has been slow—a matter of trial and error and guesswork as much as rational calculation.

In the search for better ways of curbing the survival, proliferation, and spread of cancer cells, it is important to examine more closely the strategies by which they thrive and multiply.

Cancerous Growth Often Depends on Derangements of Cell Differentiation[11]

We have so far emphasized that cancer cells defy the normal controls on cell division: this is their central property. But many tissues are organized in such a way that even an uncontrolled increase in the frequency of cell division will not by itself produce a steadily growing tumor. The example of the uterine cervix, discussed above on page 1194, illustrates this point. Like the epidermis of the skin and many other epithelia, the epithelium of the uterine cervix normally renews itself continually by shedding terminally differentiated cells from its outer surface and generating replacements from stem cells in the basal layer (see p. 970). On average, each normal stem cell division generates one daughter stem cell and one cell that is condemned to terminal differentiation and a cessation of cell division. If the stem cell simply divides more rapidly, terminally differentiated cells will be produced and shed more rapidly, and a balance of genesis and destruction will still be maintained. Thus if a transformed stem cell is to generate a steadily growing clone of progeny, the basic rules must be upset: either more than 50% of the daughter cells must remain as stem cells, or the process of differentiation must be deranged so that daughter cells embarked on this route retain an ability to carry on dividing indefinitely and avoid being discarded at the end of the production line (Figure 21–15).

Presumably, the development of such properties underlies the progression from a mild dysplasia of the uterine cervix to carcinoma *in situ* and malignant cancer (see Figure 21–10). Similar considerations apply to the development of cancer in other tissues that rely on stem cells, such as the skin, the lining of the gut, and the hemopoietic system. Several forms of leukemia, for example, seem to arise from a disruption of the normal program of differentiation, such that a committed progenitor of a particular type of blood cell continues to divide indefinitely, instead of differentiating terminally in the normal way after a strictly limited num-

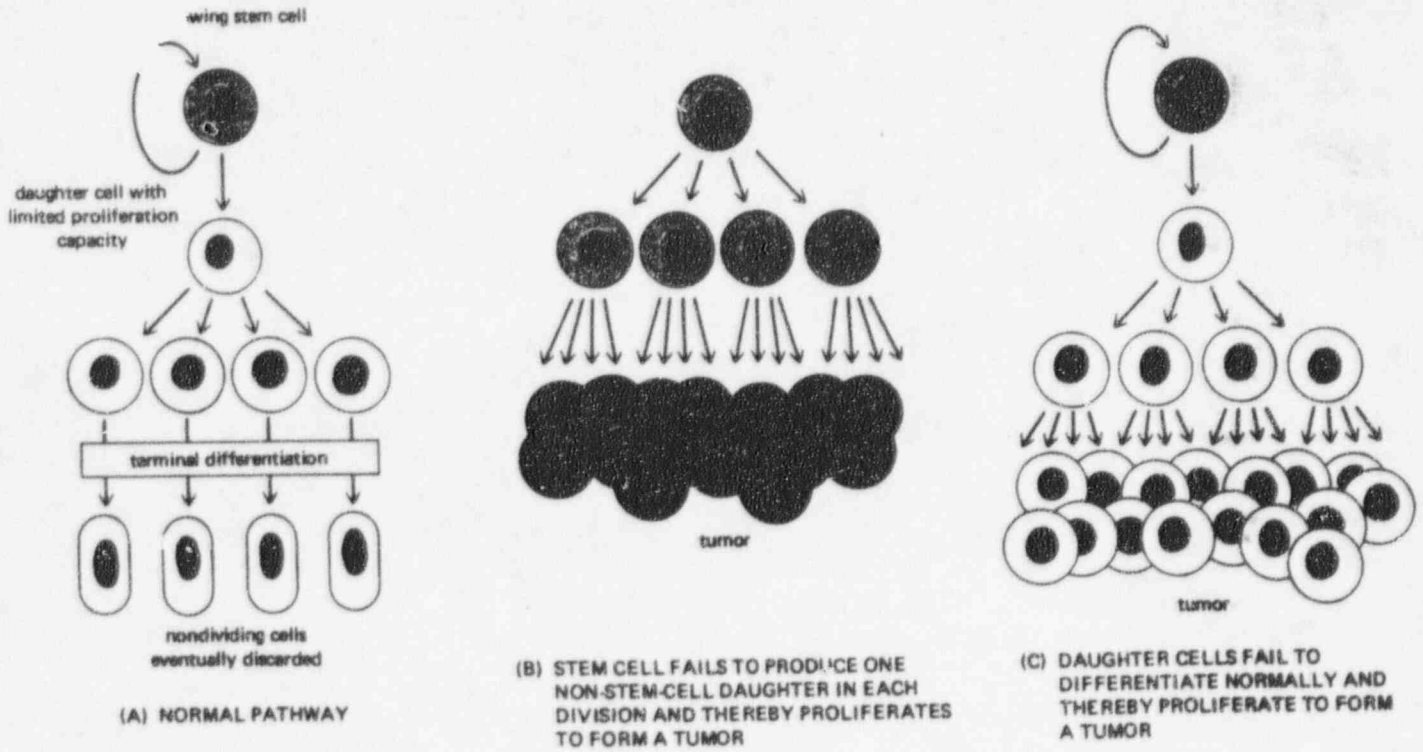


Figure 21–15 The stem-cell strategy for producing new differentiated cells, and two types of derangement that can give rise to the unbridled proliferation characteristic of cancer. Note that an excessive cell-division rate for the stem cells will not by itself have this effect.

ber of division cycles (see p. 979). In general, mutations or epigenetic changes that block the normal maturation of cells toward a nondividing, terminally differentiated state must play an essential part in many cancers. In the treatment of cancer, therefore, there is some prospect that drugs that promote cell differentiation may turn out to be a useful alternative or supplement to drugs that simply kill dividing cells.

To Metastasize, Cancer Cells Must Be Able to Cross Basal Laminae[12]

It is the ability to metastasize that makes cancers hard to eradicate surgically or by localized irradiation. To disseminate widely in the body, the cells of a typical solid tumor must be able to loosen their adhesion to their original neighbors, escape from the tissue of origin, burrow through other tissues until they reach a blood vessel or a lymphatic vessel, cross the basal lamina and endothelial lining of the vessel so as to enter the circulation, make an exit from the circulation elsewhere in the body, and survive and proliferate in the new environment in which they find themselves (Figure 21–16). The final steps are probably the most difficult: many tumors release quite large numbers of cells into the circulation, but only a minute proportion of these cells succeed in founding metastatic colonies.

A few types of normal cells—notably white blood cells—already have some or all of the properties needed to disseminate through the body, but for most cancers the ability to metastasize probably requires additional mutations or epigenetic changes. Such transformations, like the others involved in the development of cancer, are thought to occur at random in the initial tumor population: only

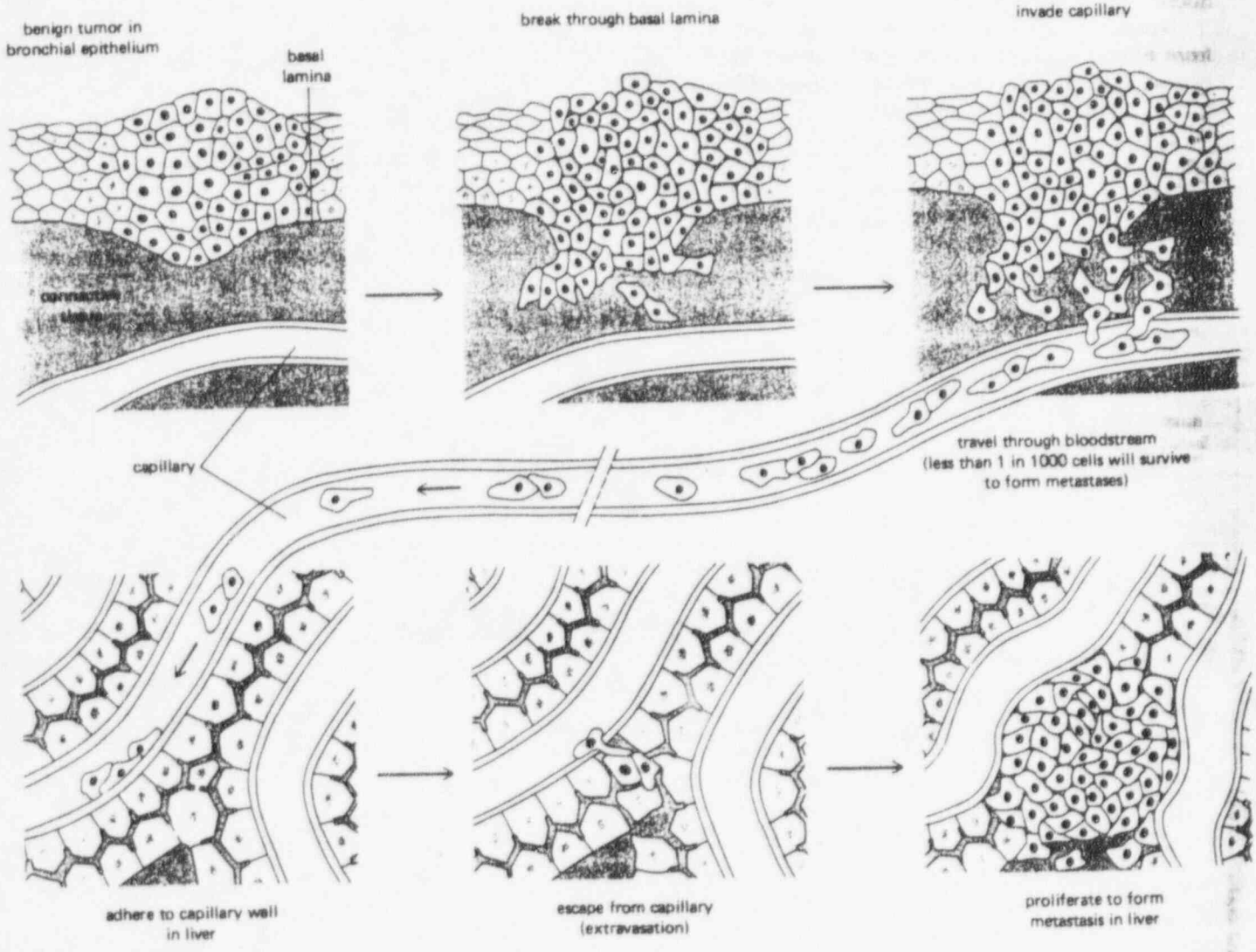


Figure 21–16 Steps in the process of metastasis. This example illustrates the spread of a tumor from the lung to the liver. Tumor cells may enter the bloodstream directly by crossing the wall of a blood vessel, as diagrammed here, or, more commonly perhaps, by crossing the wall of a lymphatic vessel. The lymphatic vessels ultimately discharge their contents (lymph) into the bloodstream, but tumor cells that have entered a lymphatic vessel often become trapped in lymph nodes along the way, giving rise to lymph-node metastases. Studies in animals show that typically less than one in every thousand malignant tumor cells that enter the bloodstream will survive to produce a tumor at a new site. The likelihood of metastasis depends on the characteristics of the host tissue as well as on those of the cancer cell.

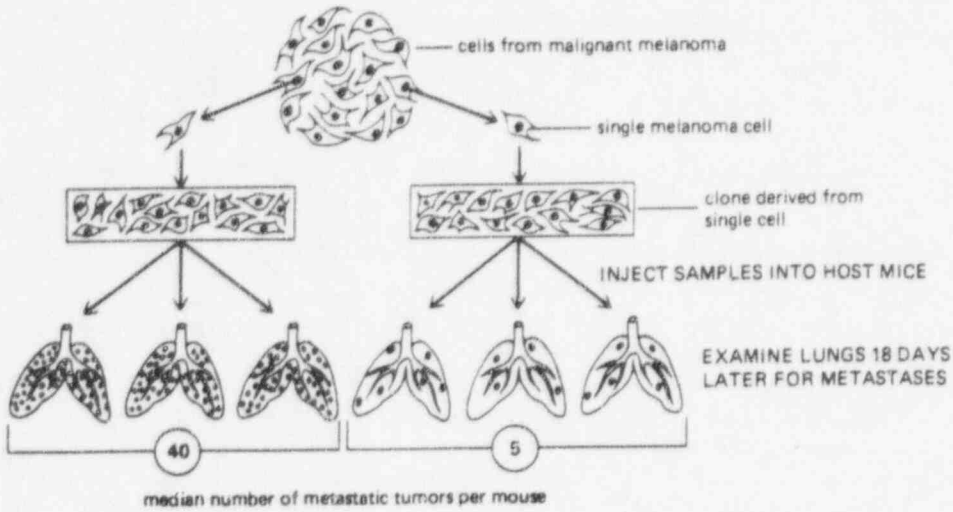


Figure 21–17 Experiment showing that there are clonally heritable differences between the cells of a single tumor with respect to the ability to metastasize. Cells derived from a single cancer cell line are subcloned, and standard aliquots of each subclone are tested by injection into the bloodstream of host mice. The subclones differ markedly in the number of resulting metastases per mouse.

those few cells that acquire the properties needed for metastasis and that happen to land in a suitable environment will be able to found secondary tumors. In accordance with this concept of evolution through random variation and natural selection, it is found that the cells of a single tumor are heterogeneous in metastatic capacity (Figure 21–17).

An understanding of the molecular mechanisms of metastasis should eventually allow the design of treatments to block it. Some progress is being made along these lines. It has been shown, for example, that for tumor cells to cross a basal lamina they must have laminin receptors (see p. 822), which enable the cells to adhere to the lamina, and they must secrete type IV collagenase, which helps them digest the lamina (Figure 21–18). Antibodies or other reagents that block either laminin attachment or the activity of type IV collagenase have been found to block metastasis in experimental animals. It remains to be seen whether human cancer patients can be helped by treatments that halt metastatic spread in this way.

Defects of DNA Repair, Replication, and Recombination Accelerate the Development of Cancer[1,13]

As we have emphasized, the incidence of tumors and their rate of progression from benign to malignant depends on the frequency of mutations. The mutation rate may be high because of mutagens in the environment or because of intracellular defects in the machinery governing replication, recombination, and repair of DNA. People with the rare genetic disorder *xeroderma pigmentosum*, for example, have a defect in the system of enzymes required to repair the type of damage done to DNA by ultraviolet irradiation (see p. 225); as a result, the slightest exposure of the skin to sunlight is liable to provoke skin cancers. A more general predisposition to cancer is seen in *Bloom's syndrome*, where there is a defect in the enzyme *DNA ligase I*, required for DNA replication and repair, and in *Fanconi's anemia* and *ataxia-telangiectasia*, where there are less well characterized defects in the same functions. In these rare genetic disorders, the abnormality is inherited through the germ line and is therefore present in all the cells of the body. Similar genetic defects in DNA metabolism can also arise, however, through mutations originating in somatic cells, and there is evidence to suggest that such aberrations are a common and important factor in the development of many cancers.

Cancer cells often display an abnormal variability in the size and shape of their nuclei (Figure 21–19) and in the number and structure of their chromosomes; indeed, abnormal nuclear morphology is one of the key features used by pathologists to diagnose cancer. When cancer cells are grown in culture, they are often found to have an extraordinarily unstable karyotype: genes become amplified or deleted and chromosomes become lost, duplicated, or translocated with a far higher frequency than in normal cells in culture. Such chromosomal variability might simply be the consequence of hasty cell-division cycles occurring in a dif-

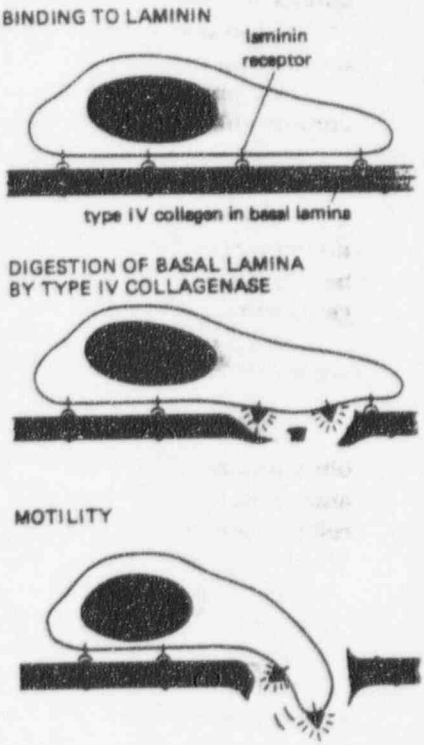


Figure 21–18 Three steps in crossing a basal lamina—a task that invasive tumor cells must be able to perform.

ferentiated cell that is poorly adapted for rapid proliferation. Alternatively, it might reflect a heritable deficiency in the machinery or control of DNA replication, repair, or recombination, arising by somatic mutation in any one of the many genes involved in these complex processes. Such a mutation would be liable to increase the likelihood of subsequent mutations in other classes of genes. For this reason one might expect it to be a common feature of cells that undergo the multiple mutations required to become cancerous. Suppose, for example, that three mutations in genes governing the social behavior of cells are required to convert a normal cell into a cancer cell and that each of these mutations normally occurs at a rate of 10^{-4} per cell per human lifetime. Then the probability for a single cell with the normal level of mutability to accumulate these three mutations in the course of a lifetime would be $10^{-4} \times 10^{-4} \times 10^{-4} = 10^{-12}$ per cell. But now let us suppose that each of these mutations occurs at a rate of 10^{-2} per cell per lifetime if there has been a prior mutation in some particular enzyme involved in DNA replication or repair. If this latter mutation itself has a probability of 10^{-4}, cancer cells will arise most frequently by the route that begins with the mutation that increases mutability: this route (given the simplest possible assumptions) involves a combination of four events whose joint probability is on the order of $10^{-4} \times 10^{-2} \times 10^{-2} \times 10^{-2} = 10^{-10}$ per cell per lifetime; it is thus 100 times more likely than the route involving only the minimal three mutations.

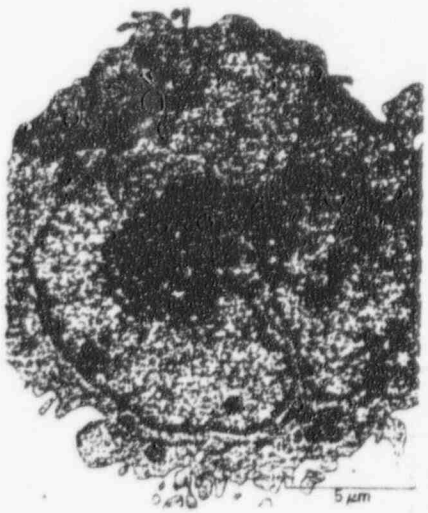


Figure 21–19 Typical abnormalities in the appearance of the nucleus of a cancer cell—in this example, an erythroleukemia cell. The cancer cell nucleus is large in relation to the amount of cytoplasm, with an irregularly indented envelope and a nucleolus that is also abnormally large and complex in its structure. (Courtesy of Daniel Friend.)

The Enhanced Mutability of Cancer Cells Helps Them Evade Destruction by Anticancer Drugs[10,14]

Whatever the origins of the abnormally high mutability of cancer cells, most malignant tumor cell populations are heterogeneous in many respects and capable of evolving at an alarming rate when subjected to new selection pressures. This aggravates the difficulties of cancer therapy. Repeated treatments with drugs that are selectively toxic to dividing cells can be used to kill the majority of neoplastic cells in a cancer patient, but it is rarely possible to kill them all: usually some small proportion are drug-resistant, and the effect of the treatment is to favor the spread and evolution of cells with this trait. To make matters worse, cells that are exposed to one drug often develop a resistance not only to that drug, but also to other drugs to which they have never been exposed.

This phenomenon of **multidrug resistance** is frequently correlated with a curious change in the karyotype: the cell is seen to contain additional pairs of miniature chromosomes—so-called *double minute chromosomes*—or to have a *homogeneously staining region* interpolated in the normal banding pattern of one of its regular chromosomes. Both these aberrations consist of massively amplified numbers of copies of a small segment of the genome (see Figures 21–26 and 21–31, below). Cloning of this amplified DNA has revealed that it often contains a specific gene, known as the *multidrug resistance* (*mdr*1) gene, which codes for a plasma-membrane-bound transport ATPase that is thought to prevent the intracellular accumulation of certain classes of lipophilic drugs by pumping them out of the cell. The amplification of other types of genes can also give the cancer cell a selective advantage: thus the gene for the enzyme dihydrofolate reductase (DHFR) often becomes amplified in response to cancer chemotherapy with the folic-acid antagonist methotrexate; and we shall see that some proto-oncogenes involved in cell-division control are similarly amplified in some cancers (see p. 1214).

While defects in DNA replication, recombination, or repair may help cancer cells to evolve by increasing their mutability, they may also make the cells more vulnerable to certain types of attack. This may explain the observation—exploited in therapy—that the cells of many tumors are killed more easily than normal cells by irradiation or by exposure to specific drugs that interfere with DNA metabolism. As we learn more about the molecular mechanisms of DNA replication, recombination, and repair, it should become possible to devise tests to pinpoint defects in these functions in individual cases of cancer. Using such information we may be better able to kill the delinquent cells by designing drugs that exploit their particular weaknesses.

Summary

Cancer cells, by definition, proliferate in defiance of normal controls (that is, they are neoplastic) and are able to invade and colonize surrounding tissues (that is, they are malignant). By giving rise to secondary tumors, or metastases, they become hard to eradicate surgically. Cancer cells usually retain many features of the specific cell type from which they are derived. Most cancers are thought to originate from a single cell that has undergone a somatic mutation, but the progeny of this cell must undergo further changes, probably requiring several additional mutations, before they become cancerous. This phenomenon of tumor progression, which usually takes many years, reflects the operation of evolution by mutation and natural selection among somatic cells; the rate of the process is accelerated both by mutagenic agents (tumor initiators) and by certain nonmutagenic agents (tumor promoters) that affect gene expression, stimulate cell proliferation, and alter the ecological balance of mutant and nonmutant cells. Thus many factors contribute to the development of a given cancer, and since some of these factors are avoidable features of the environment, a large proportion of cancers are in principle preventable.

Much effort in cancer research has been devoted to the search for ways to cure the disease by exterminating cancer cells while sparing their normal neighbors. A rational approach to this problem requires an understanding of the special properties of cancer cells that enable them to evolve, multiply, and spread. Thus neoplastic cell proliferation often seems to be associated with a block in differentiation, whereby the progeny of a stem cell are enabled to continue dividing instead of entering a terminal nondividing state; in principle, the proliferation could be curbed by promoting cell differentiation. To become malignant, tumor cells must be able to cross basal laminae; antibodies can be designed that interfere with this ability, thereby hindering metastasis. Cancer cells are often found to be abnormally mutable; this hastens evolution of the complex set of properties required for neoplasia and malignancy and helps the cancer cells develop resistance to anticancer drugs. At the same time, however, defects of DNA metabolism underlying such mutability may make the cancer cells uniquely vulnerable to a suitably designed therapeutic attack.

The Molecular Genetics of Cancer[15]

Because cancer is the outcome of a series of random genetic accidents subject to natural selection, no two cases even of the same variety of the disease are likely to be genetically identical. Nevertheless, all cancers can be expected to involve a disruption of the normal restraints on cell proliferation, and for each cell type there is a finite number of ways in which such disruption can occur. Moreover, some parts of the machinery for regulating cell proliferation are likely to be the same in many or all cell types, and similarly vulnerable. In fact, changes in a relatively small set of genes appear to be responsible for much of the deregulation of cell division in cancer. The identification and characterization of many of these genes has been one of the great triumphs of molecular biology in the past decade.

Cell proliferation can be regulated directly, through the mechanism that determines whether a cell passes the restriction point or "Start" of the cell-division cycle (see p. 745), or indirectly—for example, through regulation of the commitment to terminal differentiation (see p. 967). In either case the normal regulatory genes can be loosely classified into those whose products help stimulate cell proliferation and those whose products help inhibit it. Correspondingly, there are two mutational routes toward the uncontrolled cell proliferation that is characteristic of cancer. The first is to make a stimulatory gene hyperactive: this type of mutation has a dominant effect—only one of the cell's two gene copies need undergo the change—and the altered gene is called an **oncogene** (the normal allele being a **proto-oncogene**). The second is to make an inhibitory gene inactive: this type of mutation has a recessive effect—both the cell's gene copies must be

inactivated or deleted to free the cell of the inhibition—and the lost gene is sometimes called, for want of a better term, a **tumor suppressor gene.**

In addition to ordinary mutations, there is another type of genetic change that can lead to cancer: the cell-division control system can be subverted by foreign DNA introduced into the cell by a virus. In fact, insight into the molecular genetics of cancer came first from the study of such **tumor viruses,** which paved the way for an explosion of discoveries of oncogenes and proto-oncogenes. More recently, progress has been made in the more difficult task of identifying and cloning tumor suppressor genes, as we shall see at the end of this section.

Both DNA Viruses and RNA Viruses Can Cause Tumors[15,16]

Both DNA viruses and RNA viruses—retroviruses in particular—can play a part in transforming healthy cells into cancer cells. This can be demonstrated experimentally both in laboratory animals, where certain viruses cause cancers, and in cell culture, where the same viruses transform the behavior of infected cells, enabling them to divide in circumstances where untransformed cells will not (see p. 753 for an account of the properties of neoplastically transformed cells in culture). Two intensively analyzed examples are the *SV40 virus*, a DNA virus isolated from monkey cells, and the *Rous sarcoma virus*, a retrovirus isolated from chickens. It is harder, however, to incriminate viruses in human cancer: they do not stand out among the multiple causative factors involved in almost every variety of the disease, and it is thought likely that in most of the common human cancers they play no part. This may be because the immune system, by destroying virus-infected cells, protects us from many virus-induced cancers that might otherwise arise. Nonetheless, there is now good evidence that viruses do play a causative role in several types of human cancers (Table 21–3)—in some cases probably through

Table 21–3 Viruses Associated with Human Cancers

Virus	Associated Tumors	Areas of High Incidence	Other Suspected Risk Factors
DNA viruses			
Papovavirus family			
Papillomavirus (many distinct strains)	warts (benign)	worldwide	—
	carcinoma of uterine cervix	worldwide	smoking
Hepadnavirus family			
Hepatitis-B virus	liver cancer (hepatocellular carcinoma)	Southeast Asia; tropical Africa	aflatoxin from fungal contamination of food; alcoholism; smoking; other viruses
Herpesvirus family			
Epstein-Barr virus	Burkitt's lymphoma (cancer of B lymphocytes)	West Africa; Papua New Guinea	malaria
	nasopharyngeal carcinoma	southern China; Greenland (Inuit)	histocompatibility genotype (?); salted fish in infancy (?)
RNA viruses			
Retrovirus family			
Human T-cell leukemia virus type I (HTLV-I)	adult T-cell leukemia/ lymphoma	Japan (Kyushu); West Indies	—
Human immunodeficiency virus (HIV-1, the AIDS virus)	Kaposi's sarcoma (cancer of endothelial cells of blood vessels (?))	Central Africa	immune deficiency or suppression; infection with other virus (?)

For all the above viruses, the number of people infected is much larger than the number who develop cancer: the viruses must act in conjunction with other factors. Moreover, some of the viruses probably contribute to cancer only indirectly; for example, HIV-1, by obliterating cell-mediated immune defenses, may allow endothelial cells transformed by some other agent to thrive as a tumor instead of being destroyed by the immune system.

indirect promoting actions, in other cases by helping directly to cause neoplastic transformation of the cells they infect.

If a cell is to be stably transformed by a virus, a stable parasitic association must be established: the virus must not kill the cell, and the cell must retain the viral genes from one cell generation to the next—usually by integrating those genes into one or more of its own chromosomes, occasionally by retaining them as an extrachromosomal plasmid that replicates in step with the chromosomes. SV40 and the retroviruses, for example, are integrated into the chromosomes of the cells they transform; the *papillomaviruses*—a class of DNA viruses responsible for human warts and implicated in carcinomas of the uterine cervix (see Table 21–3)—are maintained in some conditions as plasmids, in other conditions as integrated elements. In either case the effect is to endow the cell with a heritably altered genome. DNA viruses and retroviruses differ fundamentally, however, in the nature of the viral genes that cause neoplastic transformation and in the relationship between cell transformation and the virus's usual life cycle.

DNA Tumor Viruses Subvert Cell Division Controls as Part of Their Strategy for Survival[15,17]

As explained in Chapter 5 (p. 250), a **DNA tumor virus** such as SV40 normally propagates in the wild by a process that does not depend on the production of cancer. An SV40 virus, having entered a host cell, typically does not become stably incorporated into the host cell genome. Instead, a protein (or set of proteins) encoded by a viral gene rapidly activates the host cell's machinery for DNA replication, and the virus then uses this subverted equipment to replicate its own genome, which in turn directs the synthesis of other viral components at the expense of the host cell until the host is killed, releasing a horde of new infectious virus particles. Much more rarely, the virus may enter a host cell of a type that is *nonpermissive* for viral proliferation and may persist there as a result of a genetic accident through which the virus becomes stably incorporated into one or more of the host cell's chromosomes. In these circumstances the viral gene that activates the host's machinery for DNA replication may still be transcribed, thereby driving the host cell into S phase and forcing it to undergo repeated division cycles: the viral gene acts as an oncogene, causing a cancerous transformation. It is, however, unlike the other classes of oncogenes to be discussed below in that it has no counterpart in the normal host cell genome; it has evolved as an essential part of the virus's equipment for its usual mode of propagation, and not by mutation from a cellular proto-oncogene.

DNA viruses are a diverse group, but the general principles just described apply, with some variations, to most of those that are implicated in cancer. One important variant is illustrated by the papillomaviruses, for which persistent association with the host cell is a normal part of the life cycle. These viruses belong to the *papovavirus* family that includes SV40, but they can apparently switch between a nonproductive mode of infection, in which they replicate in step with the host cell without harming it, and a productive mode, in which they reproduce rapidly and kill the host cell, so that large numbers of new virus particles are released to infect other cells. Like SV40, these viruses have to be able to subvert the host cell's DNA synthesis machinery, and the viral genes that have this function can act as oncogenes. Studies of cancer of the uterine cervix have suggested that carcinogenesis by papillomaviruses in humans requires the integration of a specific viral replication gene into a host chromosome, as illustrated in Figure 21–20.

Retroviruses Pick Up Oncogenes by Accident[15,18]

By contrast with DNA viruses, most **retroviruses** (see p. 254 and p. 754) are more or less harmless to the host cell, from which they are released continuously by budding from the plasma membrane, without causing neoplastic transformation. Occasionally, however, a retrovirus accidentally acquires from its host a cellular control gene—or a corrupted copy or fragment of such a gene—that has no useful

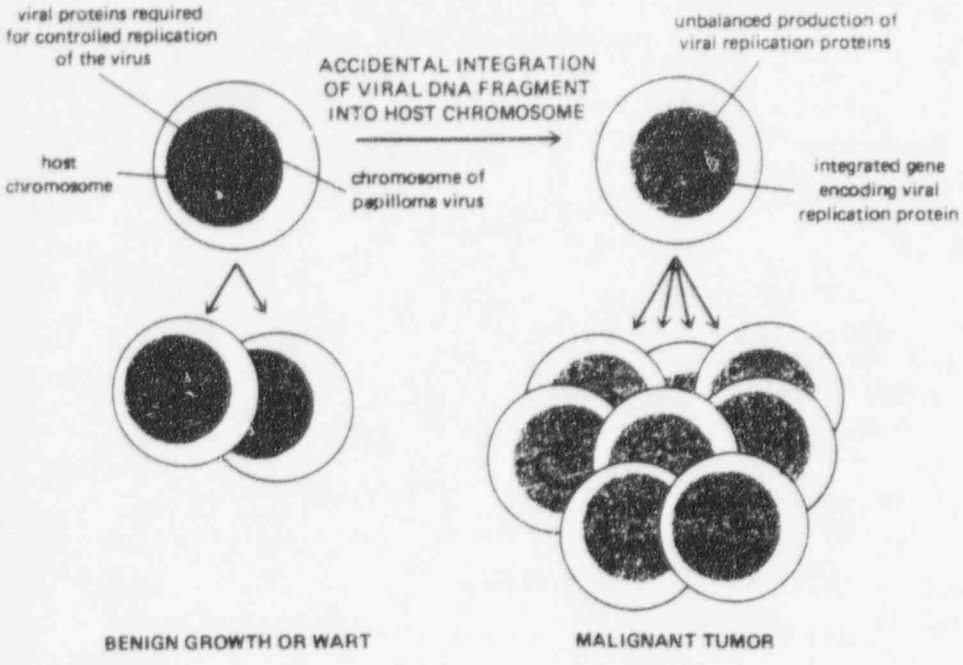


Figure 21–20 How certain papillomaviruses are thought to give rise to cancer of the uterine cervix. Papillomaviruses have double-stranded circular DNA chromosomes of about 8000 nucleotide pairs. In a wart or other benign infection, these chromosomes are stably maintained in the basal cells of the epithelium as plasmids whose replication is regulated so as to keep step with the chromosomes of the host (*left*). Rare accidents can cause the integration of a fragment of such a plasmid into a chromosome of the host, altering the environment of the viral genes and disrupting the control of their expression. The consequent unregulated production of a viral replication protein tends to drive the host cell into S phase, thereby helping to generate a cancer (*right*).

function in the viral life cycle but may drastically influence subsequent host cells. In particular, as we saw in Chapter 13 (see p. 754), a retrovirus such as the Rous sarcoma virus that has picked up a host-derived oncogene (Figure 21–21) will be easily detected because of its dominant transforming effect on infected host cells, which are driven to proliferate excessively. The oncogene can be identified by genetic dissection of the retrovirus; DNA probes can then be prepared from it to fish for homologous genes in normal cells. More than 20 oncogenes have been discovered by this approach (Table 21–4), each corresponding to its own closely similar counterpart—a proto-oncogene—present in the normal vertebrate cell genome (see p. 754). As discussed on page 756, these oncogenes fall into several

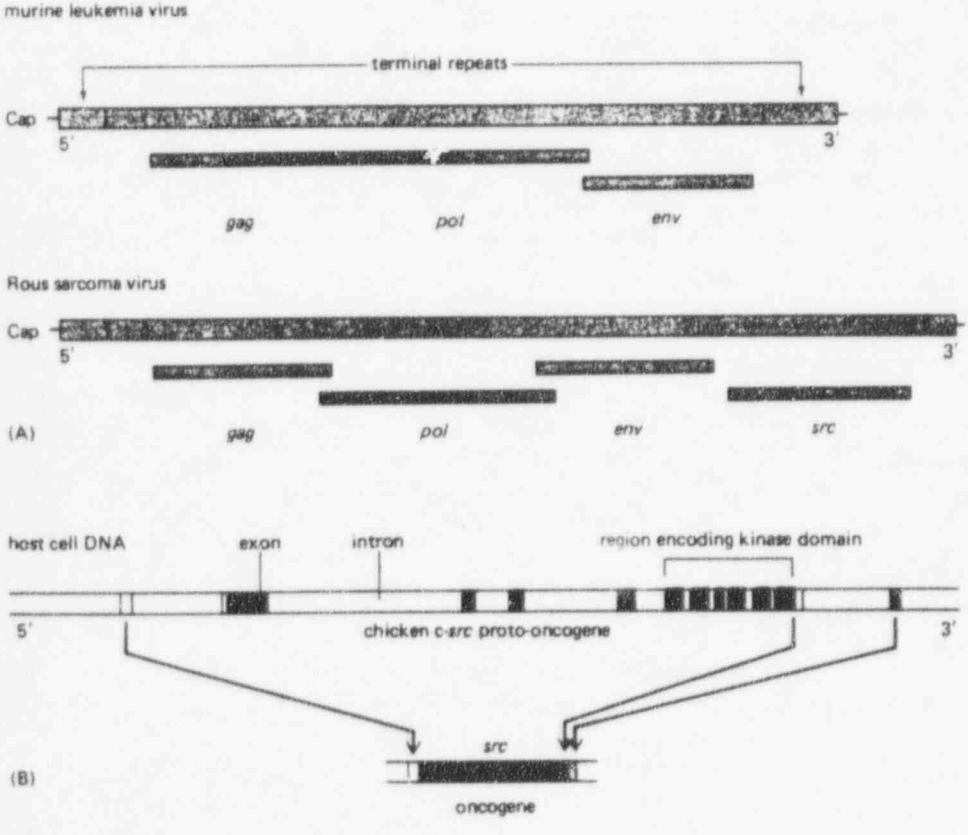


Figure 21–21 The structure of the Rous sarcoma virus. (A) The organization of the viral genome as compared with that of a more typical retrovirus (murine leukemia virus). Rous sarcoma virus is unusual among the retroviruses that carry oncogenes in that it has retained all the three viral genes required for the ordinary viral life cycle: *gag* (which produces a polyprotein that is cleaved to generate the capsid proteins), *pol* (which produces reverse transcriptase and an enzyme involved in integrating the viral chromosome into the host genome), and *env* (which produces the envelope glycoprotein). In other oncogenic retroviruses, one or more of these viral genes are wholly or partly lost in exchange for the acquisition of the transforming oncogene (see Figure 21–22), and therefore infectious particles of the transforming virus can be generated only in a cell that is simultaneously infected with a nondefective, nontransforming *helper virus*, which supplies the missing functions. (B) The relationship between the v-*src* oncogene and the cellular c-*src* proto-oncogene from which it has been derived. The introns present in c-*src* have been spliced out of v-*src*; in addition, v-*src* contains mutations that alter the amino acid sequence of the v-src protein, making it hyperactive and unregulated as a tyrosine-specific protein kinase (see p. 757). Rous sarcoma virus has been highly selected (by cancer research workers) for its ability to transform cells to neoplasia, and it does this with unusual speed and efficiency.

Table 21–4 Oncogenes Originally Identified Through Their Presence in Transforming Retroviruses

Oncogene	Proto-oncogene Function (where known)	Source of Virus	Virus-induced Tumor
abl	protein kinase (tyrosine)	mouse	pre-B-cell leukemia
		cat	sarcoma
akt	?	mouse	T-cell lymphoma
crk	activator of tyrosine-specific protein kinase(s)	chicken	sarcoma
erb-A	thyroid hormone receptor	chicken	(supplements action of v-*erb*-B)
erb-B	protein kinase (tyrosine); epidermal growth factor (EGF) receptor	chicken	erythroleukemia; fibrosarcoma
ets	nuclear protein	chicken	(supplements action of v-*myb*)
fes/fps	protein kinase (tyrosine)	cat/chicken	sarcoma
fgr	protein kinase (tyrosine)	cat	sarcoma
fms	protein kinase (tyrosine); macrophage colony-stimulating factor (M-CSF) receptor	cat	sarcoma
fos	nuclear transcription factor	mouse	osteosarcoma
jun	nuclear protein; AP-1 transcription factor	chicken	fibrosarcoma
kit	protein kinase (tyrosine)	cat	sarcoma
mil/raf	protein kinase (serine/threonine)	chicken/mouse	sarcoma
mos	protein kinase (serine/threonine)	mouse	sarcoma
myb	nuclear protein	chicken	myeloblastosis
myc	nuclear protein	chicken	sarcoma; myelocytoma; carcinoma
H-*ras*	G protein	rat	sarcoma; erythroleukemia
K-*ras*	G protein	rat	sarcoma; erythroleukemia
rel	nuclear protein	turkey	reticuloendotheliosis
ros	protein kinase (tyrosine)	chicken	sarcoma
sea	protein kinase (tyrosine)	chicken	sarcoma; leukemia
sis	platelet-derived growth factor, B chain	monkey	sarcoma
ski	nuclear protein	chicken	carcinoma
src	protein kinase (tyrosine)	chicken	sarcoma
yes	protein kinase (tyrosine)	chicken	sarcoma

distinct families, the largest group being the protein kinase gene family to which the oncogene of the Rous sarcoma virus (the *src* oncogene) belongs (Figure 21–22).

There are two ways in which a proto-oncogene can be converted into an oncogene upon incorporation into a retrovirus: the gene sequence may be altered or truncated so that it codes for a protein with abnormal activity, or the gene may be brought under the control of powerful promoters and enhancers in the viral genome, which cause its product to be made in excess or in inappropriate circumstances; often both effects occur together. Retroviruses can also exert similar oncogenic effects in another way that does not involve picking up host genes and carrying them from cell to cell: DNA copies of the viral RNA may simply insert themselves into the host cell genome at sites close to, or even within, proto-oncogenes. The resulting genetic disruption is called **insertional mutagenesis,** and the altered genome is inherited by all the progeny of the original host cell. Random insertion of DNA copies of the viral RNA into the host DNA is a part of the normal retroviral life cycle, and insertion anywhere within about 10,000 nucleotide pairs from a proto-oncogene is liable to cause abnormal activation of that

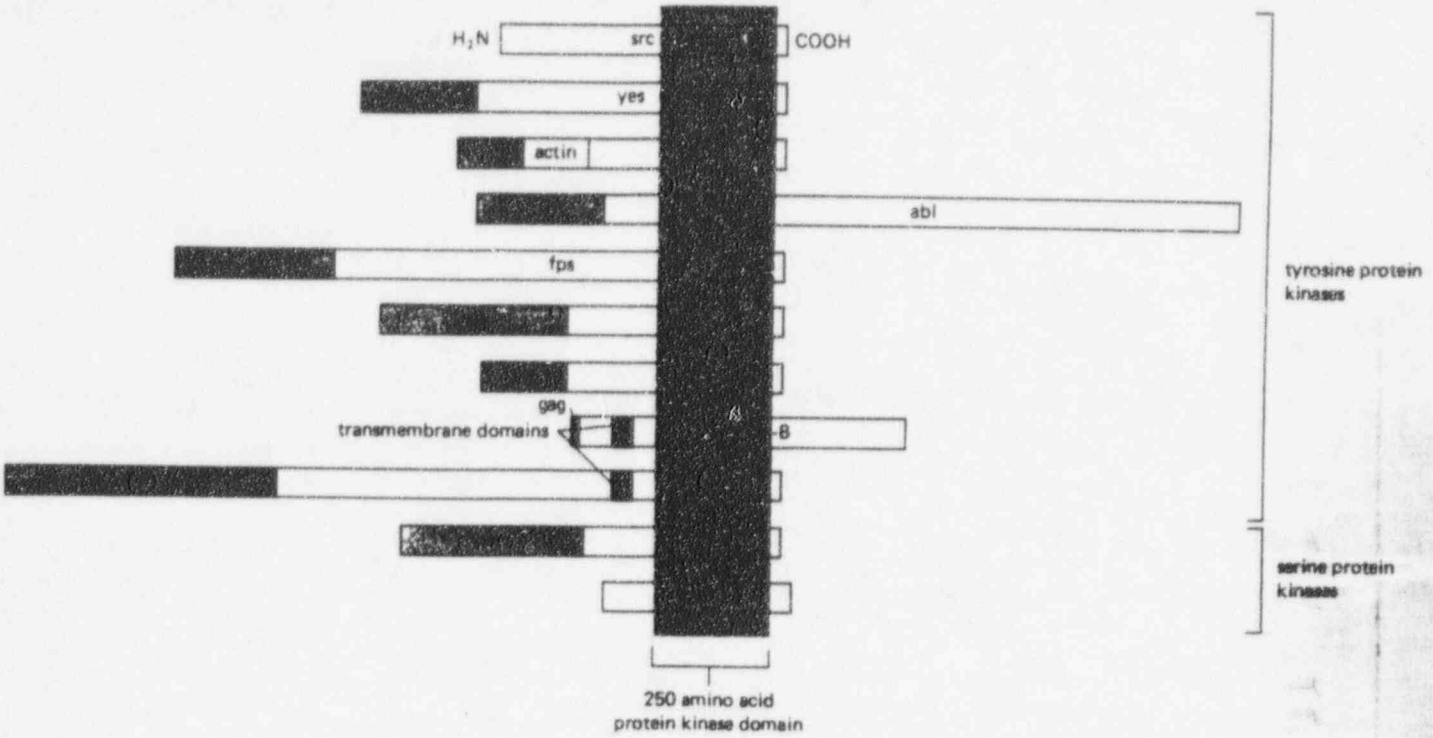


Figure 21–22 Many of the proteins produced by the oncogenes carried by transforming retroviruses are protein kinases; the diagram compares their primary structures schematically. Note that most of the oncogenes have disrupted normal viral genes and thereby produce fusion proteins that contain the amino terminus of the viral gag protein. (After T. Hunter, *Sci. Am.* 251(2):70–79, 1984.)

gene. Insertional mutagenesis provides an important means of identifying proto-oncogenes, which can be tracked down by their proximity to the inserted retrovirus. Proto-oncogenes identified in this way often turn out to be the same as those discovered in the other way, as counterparts to oncogenes that retroviruses carry from cell to cell, but some new ones have been discovered as well (Table 21–5). An example is the *int*-1 gene, activated by insertional mutagenesis in breast cancers in mice infected with the mouse mammary tumor virus (Figure 21–23). When cloned and sequenced, this gene turned out to be closely homologous to the *Drosophila* gene *wingless* (see p. 929), which is apparently involved in cell-cell communications that regulate details of the segmental pattern of the fly.

Different Searches for the Genetic Basis of Cancer Converge on Disturbances in the Same Small Set of Proto-oncogenes[15,19]

While some researchers pursued the line of investigation leading from retroviruses to oncogenes, others took a more direct approach and searched for DNA sequences in human cancer cells that would provoke uncontrolled proliferation when introduced into noncancerous cells. The assay was done in cell culture, using a particular line of mouse-derived 3T3 cells—NIH 3T3 cells—as the noncancerous hosts and transfecting them with DNA taken from human tumor cells (see p. 755 and Figure 13–33). The findings were dramatic: oncogenes were detected in many lines of human cancer cells, and in several cases these oncogenes turned out to be mutant alleles of some of the same proto-oncogenes that had been identified by the retroviral approach, or of genes very closely related to them. For example, about one in four human tumors was found to contain a mutated mem-

Figure 21–23 Insertional mutagenesis that activates a gene called *int*-1 and produces breast cancer in mice infected with the mouse mammary tumor virus (MMTV). The sites of MMTV integration observed in 19 different tumor isolates are indicated by arrows. Note that the insertions can activate transcription of the *int*-1 gene from distances of more than 10,000 nucleotide pairs away and from either side of the gene. This effect is attributed to a powerful enhancer DNA sequence present in the terminal repeats of the MMTV genome.

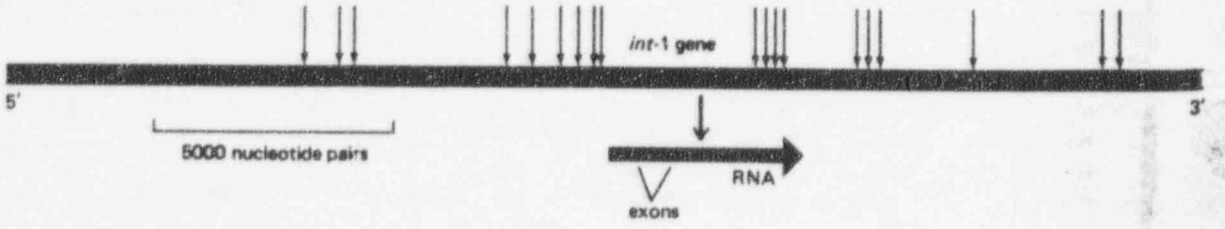


Table 21–5 Some Oncogenes Originally Identified by Means Other Than Their Presence in Transforming Retroviruses

Means of Detection	Oncogenes
Amplification:	*L-myc, N-myc, Gli*
Transfection:	*mas, met, neu, N-ras, trk, onc-F, "thy"*
Translocation:	*bcl-1, bcl-2, tcl-1, tcl-2, tcl-3a, tcl-3b*
Insertional mutation:	*evi-1, int-1, int-2, int-3, int-4, Mlvi-2, Mlvi-3, Pim-1, Flvi-1, Gin-1, fis-1, lck, dsi-1, fim-1, ahi-1, mis-1, mis-2, mis-3, mis-4, spi*

ber of the *ras* gene family (see p. 705), first discovered as oncogenes carried by retroviruses that cause sarcomas in rats. Thus two independent lines of enquiry converged on the same genes.

Yet another approach that led to some of the same proto-oncogenes was based on the karyotyping of tumor cells. As mentioned earlier (see p. 1190), in almost all patients with chronic myelogenous leukemia, the leukemic cells show the same chromosomal translocation, between chromosomes 9 and 22; likewise, in Burkitt's lymphoma there is regularly a translocation between chromosome 8 and one of the three chromosomes containing the genes that encode antibody molecules. In both these types of cancer the translocation breakpoint, where part of one chromosome is joined to another, was found to coincide exactly with the location of a proto-oncogene already known from retroviral studies—c-*abl* in chronic myelogenous leukemia, c-*myc* in Burkitt's lymphoma. Analogous chromosome translocations are similarly associated with some other types of cancer. From DNA sequencing studies it seems that in some cases the translocation turns a proto-oncogene into an oncogene by fusing the proto-oncogene to another gene in such a way that an altered protein is produced (Figure 21–24); in other cases the translocation moves a proto-oncogene into an inappropriate chromosomal environment that activates its transcription so that the normal protein is produced in excess.

A Proto-oncogene Can Be Made Oncogenic in Many Ways[14,15,20]

So far, about 60 proto-oncogenes have been discovered (see Tables 21–4 and 21–5); each of these can be perverted into an oncogene that plays a dominant part in cancers of one sort or another. Most such genes have been encountered repeat-

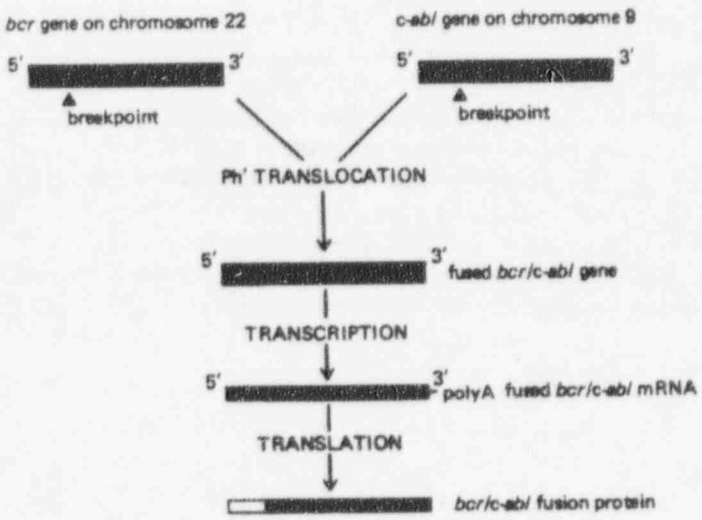


Figure 21–24 The conversion of th c-*abl* proto-oncogene into an oncoge in patients with chronic myelogenou leukemia. The chromosome translocation responsible joins the *b* gene on chromosome 22 to the c-*ab* gene from chromosome 9, thereby generating a Philadelphia chromoso (see Figure 21–4). The resulting fusi protein has the amino terminus of t bcr protein joined to the carboxyl terminus of the abl tyrosine protein kinase; in consequence, the abl kin domain presumably becomes inappropriately active, driving exce proliferation of a clone of hemopoi cells in the bone marrow.

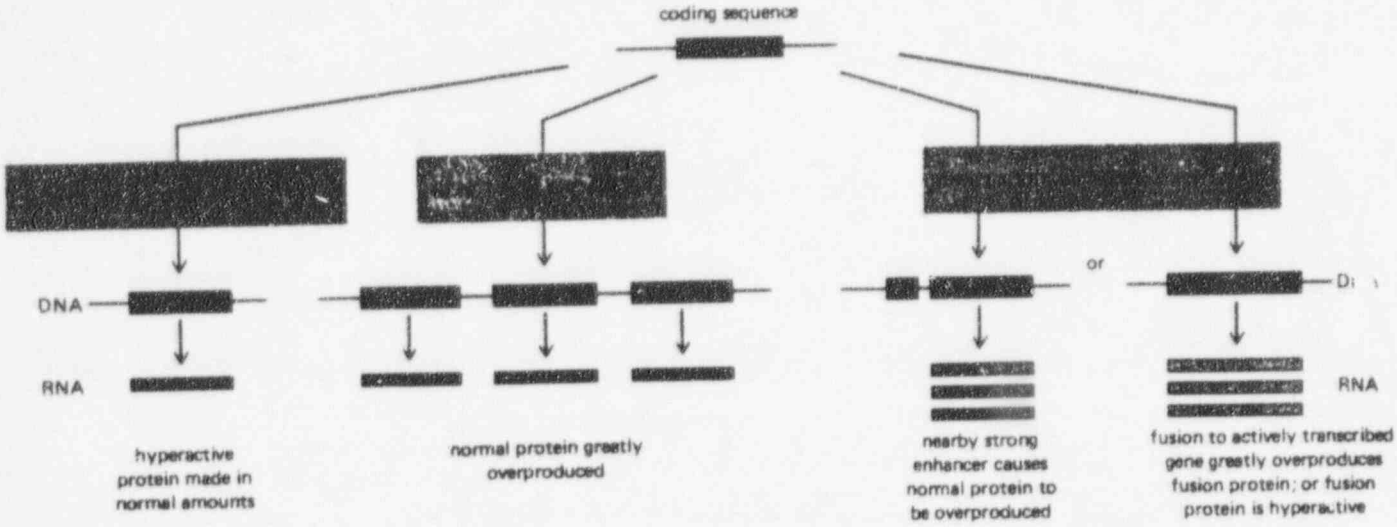


Figure 21–25 Three ways in which a proto-oncogene can be converted into an oncogene. A fourth mechanism (not shown) involves recombination between retroviral DNA and a proto-oncogene: this has effects similar to those of chromosome rearrangement, bringing the proto-oncogene under the control of a viral enhancer and/or fusing it to a viral gene that is actively transcribed.

edly, in a variety of mutant guises and in several different forms of cancer, suggesting that the majority of mammalian proto-oncogenes may already have been identified. There are many conceivable modes of genetic disruption that could convert a proto-oncogene into an oncogene, and examples of almost all of them are known (Figure 21–25). The gene may be altered by a point mutation, or through a chromosomal translocation, or by insertion of a mobile genetic element such as a retrovirus. The change can occur in the protein-coding region so as to yield a hyperactive product, or it can occur in adjacent control regions so that the gene is simply overexpressed. Alternatively, the gene may be overexpressed because it has been amplified to a high copy number through a process that probably begins with anomalous chromosome replication (Figure 21–26). Specific types of abnormality are characteristic of particular genes and of the responses to particular carcinogens. Members of the *myc* gene family, for example, are frequently overexpressed or amplified, while 90% of the skin tumors evoked in mice by the tumor initiator dimethylbenz(a)anthracene (DMBA) have an A-to-T alteration at exactly the same site in a mutant H-*ras* gene.

The molecular functions of the proto-oncogenes mutated in these various ways are beginning to be elucidated, and an increasing number of them can be assigned to particular cell-signaling pathways, as discussed in Chapters 12 and 13 (see p. 705 and p. 756). Many of the gene products appear to interact with one another as components of an elaborate control network (Figure 21–27). Thus some proto-oncogenes code for growth factors, some for growth-factor receptors and other protein kinases; still others code for G proteins of the *ras* family (see p. 705) or for gene regulatory proteins located in the nucleus. The molecular mechanisms of carcinogenesis are correspondingly beginning to make sense: through mutation of a gene (c-*sis*) for a growth factor (PDGF), leading to its inappropriate expression, cells become able to stimulate themselves continually to divide; through mutation of a gene (c-*erbB*) for a growth-factor receptor (the EGF receptor), they behave as though the corresponding growth factor were constantly present; through mutations that exaggerate expression of a gene (c-*myc*) whose product is thought to mediate nuclear changes required for cell proliferation, cells become committed to proliferate indefinitely. A great deal still remains unclear, however. In spite of a wealth of information about the DNA sequences and molecular structures of proto-oncogenes and oncogenes and their products, we still have only a vague notion of the parts that most of them play in the physiology of the cell.

The Actions of Oncogenes Can Be Assayed in Transgenic Mice[21]

The concept of an oncogene is paradoxical. As we have argued at length in the first part of this chapter, a single mutation is not enough to cause a cancer; yet an oncogene is defined as a dominantly acting gene and is typically assayed by its ability to cause neoplastic transformation of cultured cells on its own. This

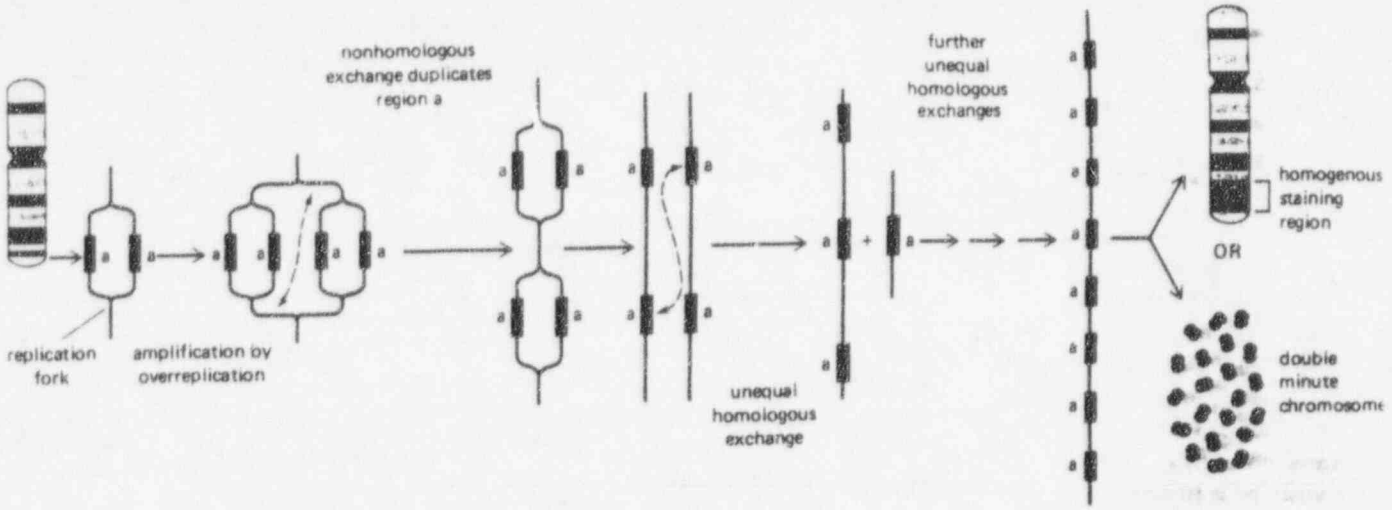


Figure 21–26 A possible mechanism for gene amplification leading to overproduction of a protein. The process begins with a gene-duplication event whose mechanism is not well understood but must involve illegitimate recombination. The mechanism illustrated here incorporates the suggestion that the illegitimate recombination may be a consequence of a destabilizing effect of illegitimate over-replication of the DNA. Once a gene duplication has occurred, unequal sister-chromatid exchanges due to recombination between identical gene copies in the course of DNA replication can further amplify the number of gene copies (see p. 600), until the chromosome contains tens or hundreds of them. When many DNA repeats are present, the segment containing them may become visible as a *homogeneously staining region* in the chromosome, or it may—probably through another recombination event—become excised from its original locus and give rise to independent *double minute chromosomes* (see p. 1202). The DNA sequence that becomes repeated by such an amplification mechanism is generally more than 100,000 nucleotide pairs long.

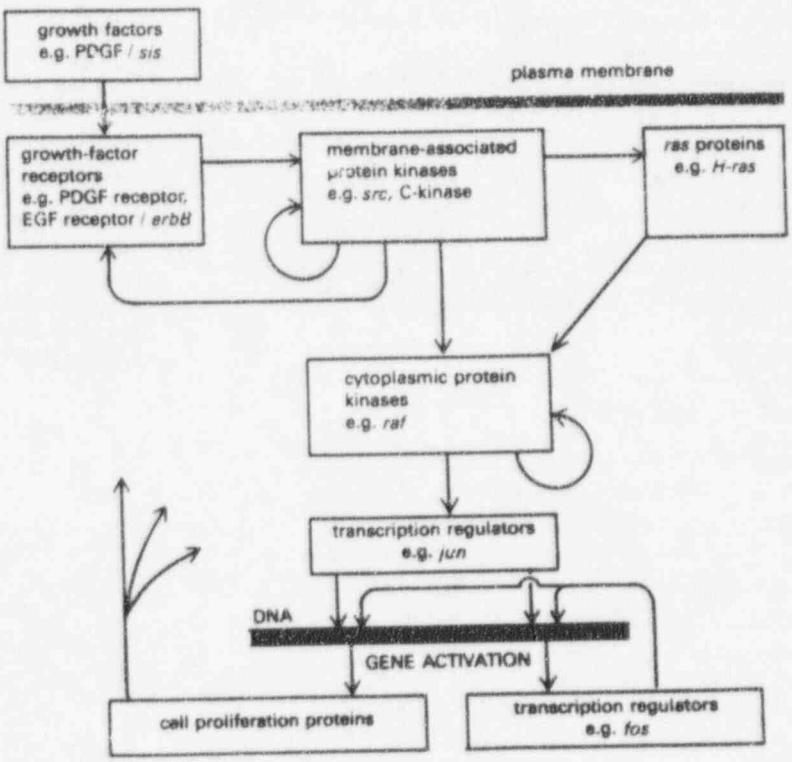


Figure 21–27 A tentative outline of the relationships of the major classes of proto-oncogenes in the intracellular control network through which external signals stimulate cell proliferation. A single representative proto-oncogene is indicated for each class. An arrow from A to B indicates that in a normal cell the activation of A exerts its effect by activating B (directly or indirectly). The diagram is based partly on studies of the molecules in cell-free systems and partly on studies of cells in which particular components are activated by introducing oncogenes or inactivated by microinjecting appropriate antibodies. Note that each of the classes of regulatory molecules has many members, so that each arrow in the diagram potentially stands for many parallel arrows linking the individual members of one class to those of another. Moreover, the members of a given class in many instances can interact with one another—for example by mutual phosphorylation—as well as with the members of the classes upstream and downstream from them. The existence of multiple parallel interconnected pathways presumably helps to make the cell fail-safe, so that a single oncogenic mutation will not normally suffice to turn it into a cancer cell; but this complexity, and the presence of feedback loops, makes the system hard to analyze experimentally. For further details, see pp. 566, 705–7 and 756–758.

apparent contradiction reflects the gulf between the simplified models of cancer most widely studied by molecular biologists and the complexity of the actual human disease. The standard assay for identification of oncogenes does not test their effects on normal human somatic cells but on a mouse-derived 3T3 cell line; and the 3T3 cells, through establishment in culture, have already undergone mutations that make them abnormally easy to transform by a single further genetic change. Moreover, as explained on page 1192, mice, with their shorter life-spans and smaller cell numbers, run an intrinsically smaller risk of cancer than do human beings, so that their cells may be less securely protected against the consequences of carcinogenic mutations than are human cells.

Even in a mouse, however, a single oncogene is not usually sufficient to turn a normal cell into a cancer cell. This can be strikingly demonstrated by studies of transgenic mice. An oncogene in the form of a DNA fragment, derived from either a virus or a tumor cell, can be linked to a suitable promoter DNA sequence and then injected into a mouse egg nucleus. Often this recombinant DNA molecule will become integrated into a mouse chromosome, leading to the generation of a strain of transgenic mice that carry the oncogene in all their cells. The oncogene introduced in this way may be expressed in many tissues or only in a select few, according to the tissue specificity of the associated promoter. Typically, in mice that are thus endowed with a *myc* or H-*ras* oncogene, some of the tissues that express the oncogene grow to an exaggerated size; and occasional cells, with the passage of time, undergo further changes and give rise to cancers. The vast majority of the cells in the transgenic mouse that express the *myc* or H-*ras* oncogene, however, do not give rise to cancers, showing that the single oncogene is not enough to cause neoplastic transformation.

This approach has been taken a step further by breeding from a pair of transgenic mice, one carrying a *myc* oncogene, the other carrying an H-*ras* oncogene, so as to obtain progeny that carry both oncogenes together. These offspring develop cancers at a much higher rate than either parental strain (Figure 21–28), but again the cancers originate as scattered isolated tumors among noncancerous cells. Thus, even with two expressed oncogenes, the cells must undergo further, randomly generated changes to become cancerous.

Although this is a typical finding, some exceptions have been reported where a type of transgenic mouse carrying a single oncogene has all the cells of a particular tissue transformed simultaneously into cancer cells. But in these cases the oncogene apparently has to be doubly activated, through a mutation in its coding sequence as well as through coupling to an abnormal control sequence that drives it to be overexpressed, or inappropriately expressed, in the affected tissue. Thus, even though a single oncogene may sometimes be enough to cause transformation of a normal cell into a cancer cell, a single genetic lesion probably is not.

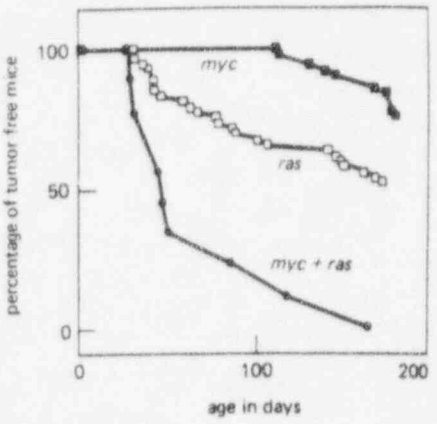


Figure 21–28 The occurrence of tumors in three types of transgenic mice, one carrying a *myc* oncogene, one carrying a *ras* oncogene, and one carrying both oncogenes. For these experiments, two lines of transgenic mice were constructed. One carries an inserted copy of an oncogene created by fusing the proto-oncogene c-*myc* with the mouse mammary tumor virus promoter/enhancer (which then drives c-*myc* overexpression in specific tissues such as the mammary gland). The other line carries an inserted copy of the oncogene v-H-*ras* under control of the same promoter/enhancer. Both strains of mice develop tumors much more frequently than normal, most often in the mammary or salivary glands. Mice that carry both oncogenes together were obtained by crossing the two strains. These hybrids develop tumors at a far higher rate still, much greater than the sum of the rates for the two oncogenes separately. Nevertheless, the tumors arise only after a delay and only from a small proportion of the cells in the tissues where the two genes are expressed: some further accidental change, in addition to the two oncogenes, is apparently required for the development of cancer. (After E. Sinn, et al., *Cell* 49:465–475, 1987.)

Cancer Involves the Loss of Tumor Suppressor Genes as Well as the Acquisition of Oncogenes[22]

From all the evidence, it seems that the control system that regulates cell proliferation must be fail-safe, in the sense that no single genetic accident is disastrous for its proper operation: proliferation is usually kept within safe bounds even if any single component goes awry. As a rule, a single oncogene can exert its dominant effect in provoking cancerous behavior only if the control system is already severely disturbed; the discoverers of oncogenes were fortunate in the choice of suitable cell lines for their assays.

It has proved considerably harder to identify and isolate tumor suppressor genes—the genes that normally act to inhibit excessive cell proliferation. There are, however, several lines of evidence that the loss of such genes plays a part in cancer. As we saw on page 755, for example, when a transformed cell in culture is fused with a nontransformed cell, the resulting hybrid cell often behaves as though nontransformed; only if a specific chromosome derived from the nontransformed partner is lost from the hybrid does the cell revert to cancerous behavior. In such a case the specific chromosome presumably contains the cell's only copy of a particular tumor suppressor gene.

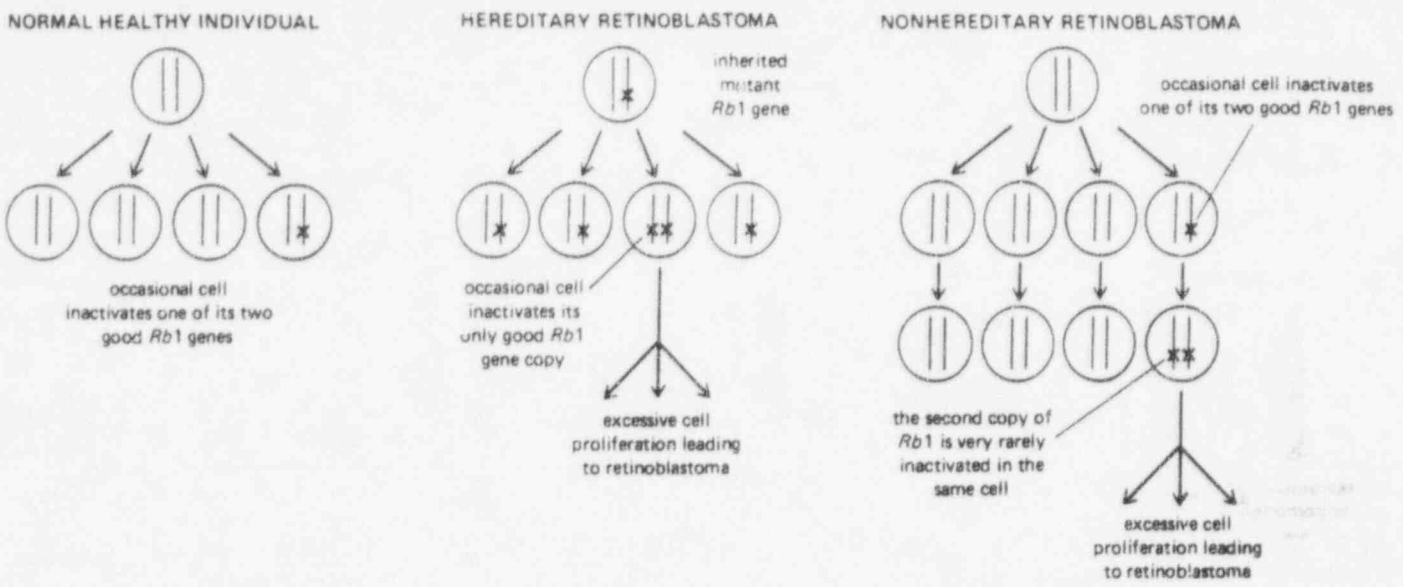


Figure 21–29 The genetic mechanisms underlying retinoblastoma. In the hereditary form, all cells in the body lack one of the normal two functional copies of a tumor suppressor gene, and tumors occur where the remaining copy is lost or inactivated by a somatic mutation. In the nonhereditary form, all cells initially contain two functional copies of the gene, and the tumor arises because both copies are lost or inactivated through the coincidence of two somatic mutations in one cell.

In an ordinary diploid cell, both copies of a tumor suppressor gene will generally have to be lost or inactivated before there is a loss of growth control: a mutation that contributes to cancer by deleting or inactivating the gene will be recessive. A mutation that creates an oncogene, by contrast, can have an effect on cell behavior even in the presence of a normal copy of the corresponding proto-oncogene. Thus, in principle, defective tumor suppressor genes can be recognized and distinguished from oncogenes by simple genetic criteria. An illustration is provided by a rare childhood cancer known as *retinoblastoma*, in which tumors develop from neural precursor cells in the immature retina. There are two forms of the disease, one hereditary, the other not. In the hereditary form, multiple tumors usually arise independently, affecting both eyes and sometimes occurring also in bone; in the nonhereditary form, only one eye is affected, and only by one tumor. Moreover, some hereditary sufferers from retinoblastoma are found to have a visibly abnormal karyotype, with a deletion of a specific band on chromosome 13; and deletions of this same locus are also seen in tumor cells from patients with the nonhereditary disease. This points to an explanation of the cancer in terms of loss of a tumor suppressor gene rather than acquisition of an oncogene. Specifically, the observations suggest that the cells of patients with the hereditary disease have a predisposition to become cancerous because they all lack one of the two normal copies of a certain tumor suppressor gene, called the retinoblastoma or *Rb*1 gene; a single somatic mutation that spoils the remaining good copy of the gene in one of the million or more cells in the growing retina will then suffice to initiate a cancer. In children without the hereditary predisposition, retinoblastomas would be very rare because they require the coincidence of two somatic mutations in a single retinal cell to destroy both copies of the *Rb*1 gene (Figure 21–29). The *Rb*1 gene has now been cloned and shown to encode a protein that is normally expressed in the retina (and in many other tissues), contains "zinc fingers" similar to those found in many DNA binding proteins (see p. 490), and also binds to the proteins encoded by the oncogenes of some DNA viruses. DNA probes prepared from the cloned *Rb*1 gene have been used to confirm that the tumor cells differ from their nontransformed neighbors in having deletions or inactivating lesions of *Rb*1 in both their maternal and their paternal chromosome sets; the loss can occur in a variety of ways (Figure 21–30). Similar mechanisms (involving other genes) have been shown to be responsible for some other cancers of early childhood, such as Wilms' tumor of the kidney, that have a hereditary form. Although these cancers are rare and somewhat exceptional, there is increasing evidence to suggest that loss or inactivation of tumor suppressor genes also plays a part in many common cancers occurring in adult life.

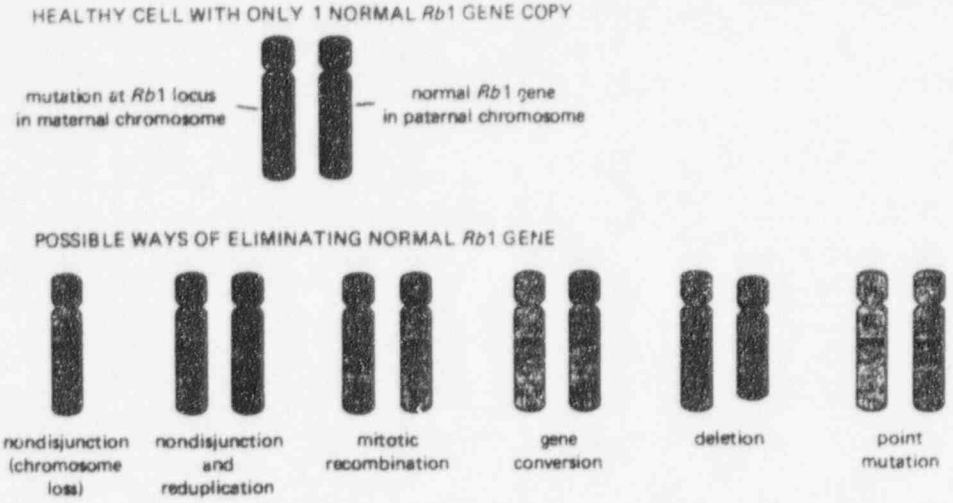


Figure 21–30 Possible mechanisms by which a cell that is defective in one of its two copies of a tumor suppressor gene may come to lose the function of the other copy as well, and thereby progress toward cancer. Cloned DNA probes can be used in conjunction with restriction-fragment length polymorphisms (see p. 270) to analyze the tumor DNA and thereby discover which type of event has occurred in a given patient. Note that most of the mechanisms result in a cell that totally lacks either the maternal or the paternal copy of the tumor suppressor gene. The remaining version is itself defective and may be present in duplicate or in a single copy. Such *reduction to homozygosity or hemizygosity* is a characteristic hallmark of a cancer dependent on loss of the function of a tumor suppressor gene. (After W.K. Cavenee et al., *Nature* 305:779–784, 1983.)

Molecular Analysis of Lung Tumors Highlights the Complexity and Variability of Human Cancer[23]

Each year, about 140,000 new cases of lung cancer are diagnosed in the United States. At present, very little can be done to save these patients, and more than 90% of them die within a year of diagnosis. To get some insight into the molecular biology of the disease, detailed studies have been made of one of its forms, *small-cell carcinoma of the lung*, which accounts for about 20% of all lung cancers. The small-cell carcinomas are thought to be derived from pulmonary neuroendocrine cells, which secrete *gastrin-releasing peptide* (*GRP*, also known as *bombesin* from its similarity to an amphibian neuropeptide of that name) and other local chemical mediators. More than a hundred cultured cell lines have been established from the tumors of different patients with this cancer and subjected to molecular analysis. They display a rich array of anomalies, differing from one cell line to another. There are instances of disturbances in at least ten known oncogenes and tumor suppressor genes, numerous chromosome deletions and translocations, and disorders that cause secretion of signaling molecules that affect cell proliferation.

Thus, for example, many of the cell lines show overexpression of the *myc* proto-oncogene or of the related genes N-*myc* and L-*myc*, often as a result of gene amplification, manifest in the karyotype as double minute chromosomes or homogeneously staining chromosome regions (Figure 21–31; see also p. 1202). In the same cells, genes of the *ras* family are often found to have undergone specific point mutations. Amplification of *myc* is regularly correlated with especially rapid growth of the tumor cells in culture and with reduced expression of differentiated properties; and patients who have tumors with this gene amplification have a particularly short life expectancy.

Together with other chromosomal abnormalities, several of the tumor cell lines have gross chromosomal defects in the region of the *Rb*1 gene, and more than half of the lines make no detectable *Rb*1 messenger RNA, even though this is made in normal lung cells and in most other types of cancers. In addition, many of the tumor cell lines have grossly visible deletions that include a specific band on chromosome 3, hinting that loss of a gene in this region may play a part in development of the cancer. Cloned DNA probes complementary to chromosome 3 in this region provide a precise and reliable way to test for deletions of the region and to identify any remaining copies of it according to whether they are versions inherited from the patient's mother or the patient's father. (The technique depends on exploiting restriction-fragment length polymorphisms—see p. 270.) This approach reveals that in almost every case (of small-cell carcinoma and of the other major forms of lung cancer) one of the two parental copies of the identified region of chromosome 3 has been lost. Noncancerous cells from the same patients do not show such a loss, and it is not generally observed in other types of cancers in other patients. There is a strong suggestion, therefore, that a crucial step in the development of lung cancer is the elimination of a specific tumor suppressor gene

Figure 21–31 Karyotype of a cell from a small-cell lung cancer, showing double minute chromosomes (*see arrows*) reflecting amplification of a *myc* gene. (Courtesy of J. Whang-Peng and J.D. Minna.)

on chromosome 3, by one of the same types of mechanisms outlined for retinoblastoma in Figure 21–30: presumably, in a typical lung cancer cell, one of the two original good copies of the lung cancer suppressor gene is lost by deletion of the chromosome segment that contains it, while the other is inactivated by a less conspicuous, more pointlike mutation. The analogy with retinoblastoma suggests that some people may inherit a recessive defect at this locus, predisposing them to lung cancer; because lung cancer probably requires changes in other genes as well and is strongly dependent on environmental factors, a hereditary predisposition of this sort might be less marked than in retinoblastoma and could easily have gone unnoticed in the past (although there are in fact some reports of genetic variation in the susceptibility to lung cancer from smoking). Similar circumstantial evidence has recently been obtained for loss of tumor suppressor genes at a different locus in another very common cancer, *colorectal carcinoma* (carcinoma of the large intestine); in this case there is known to be a rare hereditary form of the disease, although its molecular relationship to the nonhereditary form is unclear. Molecular studies such as those just described should help in identifying individuals who are at high risk for certain specific types of cancer and who may benefit from appropriate preventive measures.

Another clinically important finding from the molecular analysis of small-cell lung cancers centers on the GRP/bombesin peptide, which acts as a growth factor, stimulating proliferation of the tumor cell lines. Some, but not all, of the cell lines both respond to this factor and secrete it, thereby creating an autocrine loop through which they stimulate themselves to divide. In culture the proliferation of such cells can be inhibited by an antibody that binds to the peptide, blocking the stimulation. This holds out the hope that it may be possible to inhibit tumor growth by a similar growth-factor blockade in patients who have this particular variant of small-cell lung cancer.

Each Case of Cancer Represents a Separate Experiment in Cell Evolution

All medical progress depends on accurate diagnosis. If one cannot identify a disease correctly and distinguish it from others, one cannot discover its causes, predict its outcome, select the appropriate treatment for a given patient, or make trials on a population of patients to judge whether a proposed treatment is effective. As we have just seen for small-cell carcinoma of the lung, the traditional classification of cancers is inadequate: a single one of the conventional categories turns out on close scrutiny to be a heterogeneous collection of disorders, each characterized by its own array of genetic lesions. Molecular biology is beginning to provide tools to find out precisely which genes are amplified, which deleted, and which mutated in the tumor cells of any given patient. Such information may prove to be as important for the management and prevention of cancer as is the identification of microorganisms in patients with infectious diseases.

The discovery of oncogenes and, more recently, of tumor suppressor genes has marked the end of an era of groping in the dark for clues to the biochemical basis of cancer. But it is still sobering to turn from the simplified laboratory models of neoplasia that have made these triumphs possible to the complexity of the common human cancers. We are far from understanding these diseases fully, and there has been painfully little progress toward devising effective rational treatments for them. We know the DNA sequences of many oncogenes and proto-oncogenes but the precise physiological functions of only a few. We need better understanding of how these and other molecules interact to govern the behavior of the individual cell, better understanding of the sociology of cells in tissues, and better understanding of the cell population genetics that govern the genesis of cancer cells through mutation and natural selection.

Each patient with cancer represents an independent, unfortunate natural experiment in cell evolution. The complexity of the evolutionary possibilities accounts for the complexity of the phenomenon of cancer, and at the same time the principles of evolution provide a unifying perspective from which that complexity can be surveyed and understood. Thus cancer returns us to the theme with which this book began: as it has been said, nothing in biology makes sense except in the light of evolution.

Summary

The proliferation of normal cells is regulated by both inhibitory and stimulatory control molecules, corresponding to tumor suppressor genes and proto-oncogenes, respectively. A tendency toward cancerous behavior can result from loss or inactivation of both copies of a tumor suppressor gene or from amplification or hyperactivation of one of the two copies of a proto-oncogene. The control of cell proliferation may also be heritably upset by foreign, viral genetic material. Retroviruses can become oncogenic by picking up a copy of a proto-oncogene from a host cell and, in the process, converting it into an oncogene; they can also create an oncogene by acting as an insertional mutagen when they integrate next to a proto-oncogene in the host genome. Although most human cancers are thought not to be caused by viruses, they frequently involve mutations that affect the same proto-oncogenes identified through studies of retroviruses. In human cancers, proto-oncogenes have been found to give rise to oncogenes through point mutations, through amplification of the number of gene copies, or through chromosome translocations that either upset the control of their expression or join them to another gene to produce a fusion protein. Likewise, tumor suppressor genes may be functionally lost through a variety of types of mutations; a person who inherits a deletion or a defective copy of one of these genes may show a strong predisposition to a specific type of cancer, a notable example being retinoblastoma. Molecular analysis of tumor cells from patients sharing one of the common forms of cancer reveals a complex and heterogeneous collection of genetic lesions, including both oncogene activation and tumor suppressor gene loss. These observations reflect the randomness of the evolutionary process by which cancers arise, and suggest that each case of cancer is likely to represent a molecularly unique illness.

References

General

Cairns, J. Cancer: Science and Society. San Francisco: Freeman, 1978.

Cancer Biology: Readings from Scientific American. New York: W.H. Freeman, 1986.

Darnell, J.E.; Lodish, H.F.; Baltimore, D. Molecular Cell Biology, pp. 1035–1080. New York: W.H. Freeman/Scientific American Books, 1986.

De Vita, V.T.; Hellman, S.; Rosenberg, S.A., eds. Cancer: Principles and Practice of Oncology, 2nd ed. Philadelphia: Lippincott, 1985.

Farmer, P.B.; Walker, J.M., eds. The Molecular Basis of Cancer. New York: Wiley, 1985.

Franks, L.M.; Teich, N., eds. Introduction to the Cellular and Molecular Biology of Cancer. Oxford, U.K.: Oxford University Press, 1986.

Prescott, D.M.; Flexer, A.S. Cancer: The Misguided Cell, 2nd ed. Sunderland, MA: Sinauer, 1986.

Watson, J.D.; Hopkins, N.H.; Roberts, J.W.; Steitz, J.A.; Weiner, A.M. Molecular Biology of the Gene, 4th ed., pp. 962-1096. Menlo Park, CA: Benjamin-Cummings, 1987.

Cited

1. Cairns, J. Mutation selection, and the natural history of cancer. *Nature* 255:197–200, 1975.

   Nowell, P.C. The clonal evolution of tumor cell populations. *Science* 194:23–28, 1976.

2. Doll, R.; Peto, R. Epidemiology of cancer. In Oxford Textbook of Medicine (D.J. Weatherall, J.G.G. Ledingham, D.A. Warrell, eds.), 2nd ed., pp. 4.95–4.123. Oxford, U.K.: Oxford University Press, 1987.

   Parkin, D.M.; Laara, E.; Muir, C.S. Estimates of the worldwide frequency of sixteen major cancers in 1980. *Int. J. Cancer* 41:184–197, 1988.

   Ritchie, A.C. The classification, morphology, and behaviour of tumours. In General Pathology (H. Florey, ed.), 4th ed., pp. 668–719. London: Lloyd-Luke, 1970.

   Robbins, S.L.; Cotran, R.S.; Kumar, V. Pathologic Basis of Disease, 3rd ed., pp. 214–272. Philadelphia: Saunders, 1984.

3. Fearon, E.R.; Hamilton, S.R.; Vogelstein, B. Clonal analysis of human colorectal tumors. *Science* 238:193–197, 1987.

   Fialkow, P.J. Clonal origin of human tumors. *Biochim. Biophys. Acta* 458:283–321, 1976.

   Groffen, J.J.; et al. Philadelphia chromosomal breakpoints are clustered within a limited region, *bcr*, on chromosome 22. *Cell* 36:93–99, 1984.

   Nowell, P.C.; Hungerford, D.A. A minute chromosome in human granulocytic leukemia. *Science* 132:1497, 1960.

4. Ames, B.; Durston, W.E.; Yamasaki, E.; Lee, F.D. Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection. *Proc. Natl. Acad. Sci. USA* 70:2281–2285, 1973.

   Ashby, J.; Tennant, R.W. Chemical structure, *Salmonella* mutagenicity and extent of carcinogenicity as indicators of genotoxic carcinogenesis among 222 chemicals tested in rodents by the U.S. NCI/NTP. *Mutation Res.* 204:17–115, 1988.

   Case, R.M.P. Tumours of the urinary tract as an occupational disease in several industries. *Ann. R. Coll. Surg. Engl.* 39: 213–235, 1966.

   Doll, R. Strategy for detection of cancer hazards to man. *Nature* 265:589–597, 1977.

   Hay, A. How to identify a carcinogen. *Nature* 332:782–783, 1988.

   Walker, J.M. Testing for carcinogens. In The Molecular Basis of Cancer (P.B.Farmer, J.M. Walker, eds.), pp. 181–200. New York: Wiley, 1985.

5. Armitage, P.; Doll, R. The age distribution of cancer and a multi-stage theory of carcinogenesis. *Br. J. Cancer* 8:1–12, 1954.

   Peto, R. Cancer epidemiology, multistage models, and short-term mutagenicity tests. In Origins of Human Cancer (H.H. Hiatt, J.D. Watson, J.A. Winsten, eds.), pp. 1403–1428. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1977.

6. Cairns, J. Cancer: Science and Society, pp. 144–156. San Francisco: Freeman, 1978.

   Campion, M.J.; McCance, D.J.; Cuzick, J.; Singer, A. Progressive potential of mild cervical atypia: prospective, cytological, colposcopic, and virological study. *Lancet* 2:237–240, 1986.

   Farber, E.; Cameron, C. The sequential analysis of cancer development. *Adv. Cancer Res.* 31:125–225, 1980.

   Foulds, L. The natural history of cancer. *J. Chronic Dis.* 8:2–37, 1958.

   McIndoe, W.A.; McLean, M.R.; Jones, R.W.; Mullins, P.R. The invasive potential of carcinoma in situ of the cervix. *Obstet. Gynecol.* 64:451–464, 1984.

7. Albanes, D.; Winick, M. Are cell number and cell proliferation risk factors for cancer? *J. Natl. Cancer Inst.* 80:772–775, 1988.

   Schnipper, L.E. Clinical implications of tumor-cell heterogeneity. *N. Engl. J. Med.* 314:1423–1431, 1986.

8. Berenblum, I. A speculative review: the probable nature of promoting action and its significance in the understanding of the mechanism of carcinogenesis. *Cancer Res.* 14: 471–477, 1954.

   Berenblum, I.; Shubik, P. The role of croton oil applications associated with a single painting of a carcinogen in tumour induction in the mouse's skin. *Br. J. Cancer* 1:379–383, 1947.

   Dolberg, D.S.; Hollingsworth, R.; Hertle, M.; Bissell, M.J. Wounding and its role in RSV-mediated tumor formation. *Science* 230:676–678, 1985.

   Lamph, W.W.; Wamsley, P.; Sassone-Corsi, P.; Verma, I.M. Induction of proto-oncogene JUN/AP-1 by serum and TPA. *Nature* 334:629–631, 1988.

   Reddy, A.L.; Fialkow, P.J. Influence of dose of initiator on two-stage skin carcinogenesis in BALB/c mice with cellular mosaicism. *Carcinogenesis* 9:751–754, 1988.

9. Cairns, J. The treatment of diseases and the war against cancer. *Sci. Am.* 253(5):51–59, 1985.

   Cohen, L.A. Diet and cancer. *Sci. Am.* 257(5):42–48, 1987. Doll, R.; Peto, R. The Causes of Cancer. New York: Oxford University Press, 1981. Enstrom, J.E. Cancer and mortality among active Mormons. *Cancer* 42:1943–1951, 1978.

   Pike, M.C.; Ross, R.K. Breast cancer. *Br. Med. Bull.* 40:351–354, 1984.

10. Fentiman, I. The local treatment of cancer. In Introduction to the Cellular and Molecular Biology of Cancer (L.M. Franks, N. Teich, eds.), pp. 350–362. Oxford, U.K.: Oxford University Press, 1986.

    Malpas, J. Chemotherapy. In Introduction to the Cellular and Molecular Biology of Cancer (L.M. Franks, N. Teich, eds.), pp. 363–377. Oxford, U.K.: Oxford University Press, 1986.

11. Sachs, L. Growth, differentiation, and the reversal of malignancy. *Sci. Am.* 254(1):40–47, 1986.

12. Fidler, I.; Hart, I. Biological diversity in metastatic neoplasms: origins and implications. *Science* 217:998–1003, 1982.

    Liotta, L.A. Tumor invasion and metastases—role of the extracellular matrix: Rhoads Memorial Award lecture. *Cancer Res.* 46:1–7, 1986.

    McCarthy, J.B.; Skubitz, A.P.N.; Palm, S.L.; Furcht, L.T. Metastasis inhibition of different tumor types by purified lam-

inin fragments and a heparin-binding fragment of fibronectin. *J. Natl. Cancer Inst.* 80:108–116, 1988.

Nicholson, G. Cancer metastasis. *Sci. Am.* 240(3):66–76, 1979.

Schirrmacher, V. Cancer metastasis: experimental approaches, theoretical concepts, and impacts for treatment strategies. *Adv. Cancer Res.* 43:1–73, 1985.

13. German, J., ed. Chromosome Mutation and Neoplasia. New York: Liss, 1983.

Hanawalt, P.; Sarasin, A. Cancer-prone hereditary diseases with DNA processing abnormalities. *Trends Genet.* 2:124–129, 1986.

Lindahl, T. Regulation and deficiencies in DNA repair. *Br. J. Cancer* 56:91–95, 1987.

Nowell, P.C. Mechanisms of tumor progression. *Cancer Res.* 46:2203–2207, 1986.

14. Alt, F.W.; Kellems, R.E.; Bertino, J.R.; Schimke, R.T. Selective multiplication of dihydrofolate reductase genes in methotrexate-resistant variants of cultured murine cells. *J. Biol. Chem.* 253:1357–1370, 1978.

Gerlach, J.H.; et al. Homology between P-glycoprotein and a bacterial haemolysin transport protein suggests a model for multidrug resistance. *Nature* 324:485–489, 1986.

Roninson, I.B.; Abelson, H.T.; Housman, D.E.; Howell, N.; Varshavsky, A. Amplification of specific DNA sequences correlates with multi-drug resistance in Chinese hamster cells. *Nature* 309:626–628, 1984.

Schimke, R.T. Gene amplification in cultured cells. *J. Biol. Chem.* 263:5989–5992, 1988.

15. Klein, G. The approaching era of the tumor suppressor genes. *Science* 238:1539–1545, 1987.

Watson, J.D.; Hopkins, N.H.; Roberts, J.W.; Steitz, J.A.; Weiner, A.M. Molecular Biology of the Gene, 4th ed., Chapters 26–27. Menlo Park, CA: Benjamin-Cummings, 1987.

Wyke, J.A. Viruses and Cancer. In Introduction to the Cellular and Molecular Biology of Cancer (L.M. Franks, N. Teich, eds.), pp. 176–199. Oxford, U.K.: Oxford University Press, 1986.

16. Kripke, M.L. Immunoregulation of carcinogenesis: past, present, and future. *J. Natl. Cancer Inst.* 80:722–727, 1988.

17. Lazo, P.A. Human papillomaviruses in oncogenesis. *Bioessays* 9:158–162, 1988.

Steinberg, B.M.; Brandsma, J.L.; Taichman, L.B. Cancer Cells 5 / Papillomaviruses. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1987.

18. Bishop, J.M. Oncogenes. *Sci. Am.* 246(3):80–92, 1982.

Rijsewijk, F.; et al. The *Drosophila* homolog of the mouse mammary oncogene int-1 is identical to the segment polarity gene wingless. *Cell* 50:649–657, 1987.

Varmus, H.E. The molecular genetics of cellular oncogenes. *Annu. Rev. Genet.* 18:553–612, 1984.

19. Bishop, J.M. The molecular genetics of cancer. *Science* 235: 305–311, 1987.

Croce, C.M.; Klein, G. Chromosome translocations and human cancer. *Sci. Am.* 252(3):44–50, 1985.

Weinberg, R.A. A molecular basis of cancer. *Sci. Am.* 249(5): 126–142, 1983.

20. Bradshaw, R.A.; Prentis, S. Oncogenes and Growth Factors. Amsterdam: Elsevier, 1987. (An anthology of articles from *Trends Biochem. Sci.*, *Trends Genet.*, and *Immunol. Today*.)

Hunter, T. The proteins of oncogenes. *Sci. Am.* 251(2):70–79, 1984.

Kahn, P.; Graf, T., eds. Oncogenes and Growth Control. Berlin: Springer, 1986. (An excellent collection of short reviews.)

Quintanilla, M.; Brown, K.; Ramsden, M.; Balmain, A. Carcinogen-specific mutation and amplification of Ha-ras during mouse skin carcinogenesis. *Nature* 322:78–80, 1986.

Smith, M.R.; DeGudicibus, S.J.; Stacey, D.W. Requirement for c-ras proteins during viral oncogene transformation. *Nature* 320:540–543, 1986. (Illustrates how antibodies can be used to analyze the organization of the proto-oncogene control network.)

21. Hanahan, D. Dissecting multistep tumorigenesis in mice. *Annu. Rev. Genet.* 22(in press), 1988.

Land, H.; Parada, L.F.; Weinberg, R.A. Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. *Nature* 304:596–602, 1983.

Quaife, C.J.; Pinkert, C.A.; Ornitz, D.M.; Palmiter, R.D.; Brinster, R.L. Pancreatic neoplasia induced by *ras* expression in acinar cells of transgenic mice. *Cell* 48:1023–1034, 1987.

Sinn, E.; et al. Coexpression of MMTV/v-Ha-*ras* and MMTV/c-*myc* genes in transgenic mice: synergistic action of oncogenes *in vivo*. *Cell* 49:465–475, 1987.

22. Hansen, M.F.; Cavenee, W.K. Retinoblastoma and the progression of tumor genetics. *Trends Genet.* 4:125–129, 1988.

Harris, H. The genetic analysis of malignancy. *J. Cell Sci. Suppl.* 4:431–444, 1986.

Knudson, A.G. Hereditary cancer, oncogenes, and antioncogenes. *Cancer Res.* 45:1437–1443, 1985.

Lee, E.Y.-H.P.; et al. Inactivation of the retinoblastoma susceptibility gene in human breast cancers. *Science* 241:218–221, 1988.

Weinberg, R.A. Finding the anti-oncogene. *Sci. Am.* 259(3):34–41, 1988.

23. Bodmer, W.F.; et al. Localization of the gene for familial adenomatous polyposis on chromosome 5. *Nature* 328:614–627, 1987.

Harbour, J.W.; et al. Abnormalities in structure and expression of the human retinoblastoma gene in SCLC. *Science* 241: 353–357, 1988. (SCLC = small-cell lung cancer.)

Kok, K.; et al. Deletion of a DNA sequence at the chromosomal region 3p21 in all major types of lung cancer. *Nature* 330: 561–578, 1987.

Leppert, M.; et al. The gene for familial polyposis coli maps to the long arm of chromosome 5. *Science* 258:1411–1413, 1987.

Minna, J.D.; et al. Molecular genetic analysis reveals chromosomal deletion, gene amplification, and autocrine growth factor production in the pathogenesis of human lung cancer. *Cold Spring Harbor Symp. Quant. Biol.* 51: 843–853, 1986.

DNA repair and aging in basal cell carcinoma: A molecular epidemiology study

(skin cancer/case–control study/molecular epidemiology)

QINGYI WEI*†, GENEVIEVE M. MATANOSKI*, EVAN R. FARMER‡, MOHAMMAD A. HEDAYATI†, AND LAWRENCE GROSSMAN†§

Departments of *Epidemiology and †Biochemistry, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD 21205; and ‡Department of Dermatology, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

Communicated by Evelyn M. Witkin, November 20, 1992

ABSTRACT **This molecular epidemiology study examines the DNA-repair capacities (DRCs) of basal cell carcinoma (BCC) skin cancer patients (88) and their controls (135) by using a plasmid/host-cell reactivation assay. In this assay UV-damaged expression vector plasmid is transfected into peripheral blood T lymphocytes from the subjects. The host-cellular repair enzymes repair the photochemical damage in the plasmid, and 40 hr later the plasmid-encoded reporter chloramphenicol acetyltransferase is measured. An age-related decline in this DRC, amounting to ≈0.61% per yr occurred in the controls from 20 to 60 yr of age. Reduced DRC was a particularly important risk factor for young individuals with BCC and for those individuals with a family history of skin cancer. Young individuals with BCC repaired DNA damage poorly when compared with controls. As the BCC patients aged, however, differences between cases and controls gradually disappeared. The normal decline in DNA repair with increased age may account for the increased risk of skin cancer that begins in middle age, suggesting that the occurrence of skin cancer in the young may represent precocious aging. Patients with reduced DRCs and overexposure to sunlight had an estimated risk of BCC >5-fold greater than the control group. Such a risk was even greater (10-fold) in female subjects.**

The role of DNA repair in cancer is exemplified by the paradigm of the DNA-repair-defective autosomal-recessive disease xeroderma pigmentosum (XP) (1). XP cells are deficient in those gene products required for catalyzing the incision step in nucleotide-excision repair of damaged DNA (2, 3). Repair-deficient patients develop their first sunlight-induced skin cancers at early ages and have a >2000-fold increased risk of cancer compared with normal subjects (4).

The relationship of the persistence of UV-induced DNA damage to mutation fixation is epitomized by recent molecular studies with human skin tumor cells. Activation of the Ha-*ras* oncogene, for instance, was noted in skin tumors on sun-exposed body sites (5). Further, invasive skin cancers show strong structural correlations between p53 tumor-suppressor gene mutations and CC → TT double-base changes and between pyrimidine dimer formation and C → T mutation in DNA (6). The relationship of the gene mutation to changes specific for UV damage implicates sunlight as a cause of the cancers according to the investigators. On the other hand, the initial plateau on a repair dose—response curve (7) reflects an individual's DNA-repair capacity (DRC), and its limits represent the extremes of saturation for the repair machinery of the organism or cell. The saturation point of this plateau may, then, predict individual susceptibility to sunlight-induced DNA damage. We conducted a molecular-epidemiological study to test the hypothesis that those individuals in the general population at risk for basal cell carcinoma (BCC) of the skin have impaired capacity to repair photochemically damaged DNA.

There are many different assays for measuring DNA repair, ranging from damage removal, damage or repair-dependent formation of single-strand breaks, and uptake of substrate for unscheduled DNA synthesis in response to DNA damage. Each assay may represent a unique rate-limiting step in the overall repair process. The test in this study used the subject's T lymphocytes as a surrogate cell to measure their overall DRC (7). The cellular DRC was determined by measuring the expression level of a nonreplicating recombinant plasmid DNA containing a UV-damaged chloramphenicol acetyltransferase (CAT) reporter gene in undamaged T lymphocytes. This CAT assay measures the entire progression of repair steps leading to the restoration of the biological properties of a reporter gene rather than a specific step within the process. Comparison of the overall process as well as identification of the rate-limiting step specific to a biological state should be of mechanistic importance.

METHODS

Subject. The study population consisted of 88 patients with histopathologically confirmed primary BCC and 135 cancer-free controls. All subjects are Caucasians 20–60 yr of age who lived in Baltimore City or its suburban surroundings for most of their lives and worked indoors. Skin biopsies taken in 1987–1990 were diagnosed as BCCs at the dermatopathology laboratory of the Johns Hopkins Hospital, which serves multiple practicing dermatologists in Maryland. The subjects did not receive chemotherapy, radiation therapy, or blood transfusions; nor did they have diseases known to be related to DNA-repair deficiency, such as XP, ataxia telangiectasia, or Cockayne syndrome. In addition, eligible cases and controls did not have other forms of cancer. About 55% of the cases are males with an average age of 48.7 yr. The corresponding figures for controls are 50% and 46 yr. The purpose of selecting young subjects was to maximize the differences in risk factors between cases and controls. The control group had diagnoses of mild skin disorders such as intradermal nevus (53%), seborrheic keratosis (31%), subacute eczematous dermatitis (5%), or others (11%).

Clinic Visit. All participants were seen at the dermatology clinic, at which time the subjects completed a structured questionnaire, had blood drawn for T lymphocytes, and were examined by a dermatologist. The questionnaire included information on demographics, history of sunlight exposure, and medical history, including recent medication. The extent

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: BCC, basal cell carcinoma; DRC, DNA-repair capacity; XP, xeroderma pigmentosum; CAT, chloramphenicol acetyltransferase.

§To whom reprint requests should be addressed.

of sunburns, including blistering, was listed to measure previous sunlight exposure. Family histories of all malignancies, as well as personal histories of cancers, were obtained from the questionnaire. The dermatologists examined the patients to verify current skin conditions, including skin lesions and signs of surgical removal of previous skin malignancies. Control individuals with a self-reported cancer history or clinical signs of skin cancer or other cancers were excluded.

Laboratory Assays. *CAT assay.* The peripheral blood lymphocytes isolated from each subject's peripheral blood were cryopreserved and assayed in batches, as described (7, 8). Briefly, the plasmid DNA containing the CAT gene was irradiated with UV of 254 nm at 0, 350, and 700 J/m^2 before transfection. The quick-thawed peripheral blood lymphocytes with a >95% viability were incubated for 72 hr with phytohemagglutinin and were then transfected with undamaged or damaged plasmid DNA. The standard assay for gene expression of CAT activity by measurement of [^{3}H]acetylchloramphenicol with a Beckman scintillation counter (LS 3801) was done after another 40-hr incubation. The DRC was calculated based on scintillation counts as the percentage of residual CAT gene expression (percentage CAT activity) after repair of damaged DNA compared with undamaged plasmid DNA (100%) (7). Lymphoblast cell lines from group A XP patients (GM2345; XP-A, most severe), group D XP patients (GM2253; XP-D, severe), and group C XP patients (GM2246; XP-C, classic form) were used to generate the standard DNA repair-deficient curves because one pyrimidine dimer can inactivate the transfected CAT gene in XP cells (9). In addition, lymphoblasts from normal individuals (GM0131 and GM0892) were assayed in parallel with each batch of assays, serving as normal standards as well as quality control tests. The dose curves of 700 J/m^2, 350 J/m^2, and zero were done in triplicate. Because the dose curves based on these three doses were linear, the repair capacity at 700 J/m^2 [30 J/m^2 produces one pyrimidine dimer per plasmid (7)] was taken as the repair capacity for that individual. The repeatability of the assay (intraassay variability) was tested by using the same blood sample at different times. There is an ≈5% fluctuation in CAT activities in which the rank order of each subject's CAT activity is maintained (Table 1). Neither blastogenic rate nor level of CAT gene expression correlate with CAT activity (Table 2).

CD4$^+$/CD8$^+$ lymphocyte test. An equal number of blood samples (n = 11) from both cases and controls spanning different ages were selected and assayed by S. Gore, Johns Hopkins School of Medicine, by using standard immunofluorescence methods with flow cytometry to count CD4- and CD8-positive lymphocytes. Although the control group had slightly higher blastogenic rates than the BCC group, the distribution of CD4$^+$ and CD8$^+$ cell types within T-lymphocyte fractions did not differ in BCC and control groups (Table 3). In addition, the CD4$^+$/CD8$^+$ ratios do not correlate with either CAT activity (r = 0.10; $P > 0.05$) or age (r = 0.35; $P > 0.05$). Therefore, we conclude that the CAT assay offers a DNA-repair measurement that is independent of immunological functions and culture conditions of the tested lymphocytes.

Table 1. Ranking of percentage CAT activity of repeated assays with the same blood sample from subjects

No.	Assay, % (rank)		
	First	Second	Third
51	5.4 (2)	6.1 (2)	5.8 (2)
121	8.2 (4)	9.8 (5)	
124	10.0 (6)	9.0 (4)	
152	8.9 (5)	11.2 (6)	
155	3.2 (1)	2.6 (1)	4.4 (1)
184	5.8 (3)	7.3 (3)	6.6 (3)
342	12.6 (7)	14.2 (7)	

CAT activity at UV dose of 700 J/m^2 measured by scintillation counts of radioactivity was standardized by CAT activity at zero UV dose. The assays were done in different weeks with one frozen 30-ml

Table 2. Correlations between age, lymphocyte blastogenic rate and CAT activity of undamaged and damaged plasmid (n = 223)

	Pearson correlation coefficient/P value		
	CAT_0 (counts)	CAT_{700} (%)	Blastogenic rate (%)
Age (in yr)	−0.091/0.174	−0.256/0.000	−0.058/0.387
CAT_0* (counts)		0.014/0.836	0.096/0.153
CAT_{700}† (%)		0.025/0.710	

Blastogenic rate represents the percentage of lymphocytes (blasts) responding to phytohemagglutinin within 72 hr.
*Original scintillation counts of radioactivity of CAT assays from lymphocytes transfected with undamaged plasmid DNA containing CAT gene.
†CAT activity at UV dose of 700 J/m^2 measured by scintillation counts of radioactivity was standardized by CAT activity at zero UV dose.

Statistical Analyses. Pearson correlation coefficients were calculated to evaluate the relationship between variables of interest. The group mean values of CAT activity were compared by using a two-tailed Student's t test. Simple and multiple linear-regression analyses were used to determine the best predictor for DRC. Logistic regression was used to calculate the adjusted odds ratio for estimating BCC risk. All computations were done with SAS Institute, Inc. statistical software on an International Business Machines mainframe computer.

RESULTS

Distributions of age, sex, smoking, and medicine use are similar for BCC and control groups. However, the BCC patients are more likely than controls to have blue eyes and fair skin, factors associated with BCC risk (Table 4).

Multiple linear-regression analysis revealed that the DRC of control subjects declined with age from 20 to 60 yr (Fig. 1). Based on the estimates from the linear-regression model for the 135 controls, the decline is 0.61% per yr between 20 and 60 yr of age (Table 5, model 1: the estimated coefficient (−0.071) for current age relative to estimated intercept (baseline, 11.725) gives a rate of decline of −0.61%). The four-

Table 3. Comparison of blastogenic rate, baseline gene expression, and CD4$^+$/CD8$^+$ ratio between BCC patients and cancer-free controls

Characteristic	BCC case, mean ± SD	Control, mean ± SD	t test, P
Mean of blastogenic rate, %*	61.7 ± 16.8	66.5 ± 16.5	0.037
Baseline CAT gene expression level, mean counts†	101,013 ± 40,266	102,019 ± 39,639	0.854
Mean of CD4, %	46.8 ± 6.4	45.2 ± 7.4	0.585
Mean of CD8, %	29.5 ± 7.7	27.3 ± 7.6	0.493
Mean of CD4/CD8 ratio	1.73 ± 0.7	1.79 ± 0.6	0.8

For the first two characteristics, n = 88 for BCC cases and 135 for controls; for the CD4 and CD8 studies, n = 11 BCC cases and 11 controls.
*Percentage of lymphocytes responding to phytohemagglutinin within 72 hr.
†CAT_0 equals the scintillation counts of radioactivity of CAT assay from lymphocytes transfected with undamaged plasmid DNA con-

Table 4. Association of selected host characteristics with BCC in a clinic-based case–control study, Baltimore, Maryland, 1987–1990

Characteristic	Number, % BCC case (n = 88)	Control (n = 135)	χ^2 test, P
Age, yr			
20–40	19 (21.6)	36 (26.7)	
41–50	26 (30.0)	51 (37.8)	
51–60	43 (48.9)	48 (35.6)	0.141
Sex			
Male	48 (54.6)	68 (50.4)	
Female	40 (45.4)	67 (49.6)	0.542
Skin complexion*			
Type I/II	61 (69.3)	69 (51.1)	
Type III/IV	27 (30.7)	66 (48.9)	0.007
Smoking (100 cigarettes in a lifetime)			
Smoker	58 (65.9)	85 (63.0)	
Nonsmoker	30 (34.1)	50 (37.0)	0.654
Recent medication (at least 1 mo)			
Yes	29 (33.0)	45 (34.4)	
No	59 (67.1)	86 (65.7)	0.844

*These data were recorded by a dermatologist according to Fitzpatrick skin typing: type I, severe burning (blistering) and no tanning; type II, moderate burning and mild tanning; type III, mild burning and moderate tanning; and type IV, no burning and intense tanning.

decade decline led to an accumulated 24.4% decrease in DRC. Although the age-related decline (apoptosis) in DNA-repair occurred in both BCC cases and controls, it only reached significance for the control group (Table 5, models 1 and 2). Model 2 is the only model that is not statistically different from the null model (P = 0.120).

In keeping with the XP paradigm, those cases with first onset of BCC at young ages repair DNA photoproducts poorly when compared with controls. Cases developing their first cancers at later ages show little difference in DRC compared with their controls (see Fig. 1). This result suggests that poor DNA repair is associated with precocious skin aging, as manifested by an early age of first BCC. After adjusting for age at onset, the age-related decline in the repair of UV damage among the BCC cases was, at least, as sharp as that of controls (P = 0.003). After controlling for age of subjects, the age at first onset of BCC correlated positively with DNA repair (P = 0.017), indicating that the earlier the age of onset of BCC, the lower was their DNA repair (Table 5, model 3). The group mean comparison revealed that the mean DRC of all BCC cases (n = 88) is 5% lower than that of all controls (n = 135), and the difference in means is of borderline significance (P = 0.103). When the controls with a family history of BCC and with actinic keratosis are removed from comparison (Table 6), the mean of the cases is 8% lower than that of controls, and the difference between means is statistically significant (P = 0.047). These findings suggest that heredity can influence DNA-repair levels in the general population. After further adjustment for age and sex, prior family history of BCC is a statistically significant indicator of the individuals' DRCs, regardless of whether they were BCC cases or control subjects (Table 5, model 4).

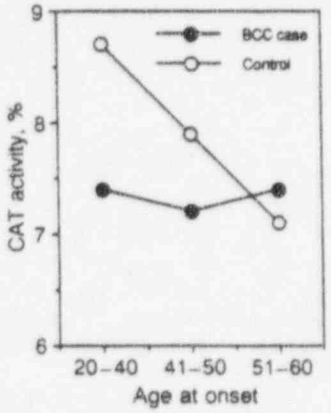


FIG. 1. Relationship between age at first BCC and DRC. The age-related decline in DRC among controls in comparison with that of age-matched cases is displayed. The linear-regression modeling and statistical tests of these data are presented in Table 5.

Table 5. Multiple linear-regression modeling of DNA repair related to risk factors among BCC and controls

Model*	Parameter	Estimate†	t value‡	P value§
1	Intercept	11.725	10.23	0.000
	Age in yr	−0.071	−3.47	0.000
	Sex	−0.409	−1.11	0.271
2	Intercept	10.445	6.96	0.000
	Age in yr	−0.047	−1.85	0.067
	Sex	−0.547	−1.26	0.211
3	Intercept	11.374	7.77	0.000
	Age in yr	−0.134	−3.09	0.003
	Sex	−0.360	0.84	0.404
	Age of onset	0.879	2.44	0.017
4	Intercept	11.569	12.81	0.000
	Age in yr	−0.067	−4.24	0.000
	Sex	−0.423	−1.51	0.133
	BCC FH	−0.645	−1.93	0.055

All models but model 2 significantly differ from a model with no risk factors according to the F test. The variables included in these linear-regression models are as follows: dependent variable is percentage of CAT activity at a UV dose of 700 J/m^2 (continuous variable); independent variables are age (at assay, in yr); age of onset (age at first BCC, in group): 1, <35; 2, 35–44; 3, 45–54; 4, 55–60; sex: 1, male; 2, female; BCC family history (FH): 1, without family history of BCC, 2, with BCC family history.

*Model: 1, control only (n = 135) with F value = 6.35 and R^2 = 0.088 and P = 0.002; 2, BCC case only (n = 88) with F value = 2.17 and R^2 = 0.049 and P = 0.120; 3, BCC case only (n = 88) with F = 3.51 and R^2 = 0.111 and P = 0.019; 4, all subjects (n = 223) with F = 7.44, R^2 = 0.092 and P = 0.0001.

†Least-square estimate of regression coefficient from multiple linear-regression models.

‡Student's t test for the null hypothesis that the estimate is equal to zero.

§Two-sided Student's t test.

When the relationship between age at first occurrence of BCC and family history of the disease was examined, ≈45% of cases first diagnosed with BCC between the ages of 20 and 44 yr (n = 38) reported that their relatives had had BCC, whereas only 10% of those cases who had their first BCC at ages 55–60 yr (n = 21) had relatives with BCC. This trend was

Table 6. Comparison of DCC between BCC patients and cancer-free controls

	n	Mean ± SD*	% difference†	t test‡
Controls without FH or actinic keratosis	106	8.00 ± 2.2	0.0	Reference
Controls with FH or actinic keratosis	29	7.28 ± 2.2	−9.0	0.126
BCC case	88	7.35 ± 2.0	−8.1	0.047

FH, family history of BCC.

*Mean percentage of CAT activity at UV dose to plasmid of 700 J/m^2 and its SD.

†Percentage reduction relative to controls (reference group), which were labeled as 0% difference.

‡Student's t test for comparison of means to reference group.

Table 7. Relationship of family history of BCC to the age of BCC onset

Subjects	*n*	BCC family history, no. (%)	χ^2 test for distribution,* *P*
Controls	135	21 (15.6)	
BCC onset			
20–44	38	17 (44.7)	
45–54	29	10 (34.5)	
55–60	21	2 (9.5)	0.022

*For BCC cases only.

statistically significant (Table 7). In contrast, only 16% of controls (n = 135) had a family history of BCC.

The proportion of those cases (36%) who experienced sunlight overexposure in their lifetime is double that of controls (17%). Those subjects who had six or more severe sunburns (blistering) in their lifetime had significantly lower DRCs in the BCC group than in the control group (a 1.3% difference, P = 0.044). Any measure of sunlight exposure affecting skin susceptibility—such as light skin type, poor tanning ability, or high burning tendency—showed a similar difference in DNA-repair between BCC cases and controls (8). These data suggest that if subjects are exposed to the genotoxic effects of environmental UV light at doses that traumatize skin, individuals with poor DRC are more likely to develop skin cancer than those with high DRC. These interactive effects between DNA-repair levels and UV exposure are shown graphically in Fig. 2. When the population is divided into high and low repair according to the median level of the controls, the estimated risk (odds ratio) of skin cancer after excessive sunlight exposure is almost five times higher in subjects with low repair but is only 1.9 times higher in those with high repair. Further stratification according to gender revealed that females with a history of six or more severe sunburns who had low DRC were at 10 times greater risk for BCC compared with those individuals with high DRC (Fig. 3). These data are consistent with the results in the previous small pilot study (7).

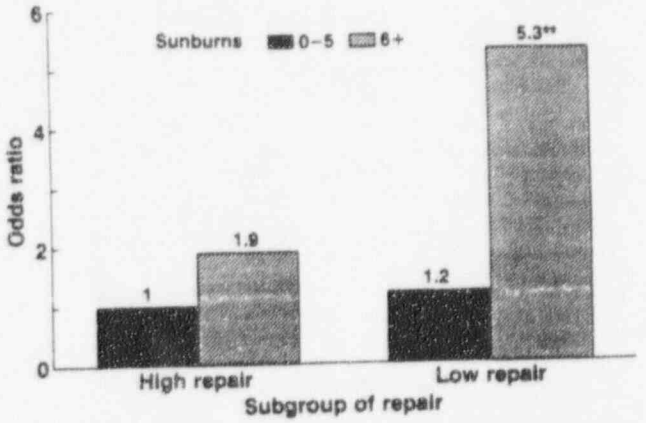


FIG. 2. Effect of DRC on risk of BCC: Relationship to number of severe sunburns in a lifetime. The reference group has an odds ratio of 1. The significantly increased age- and-sex-adjusted odds ratio [(5.32; 95% confidence interval (CI), 2.04–13.9)] is seen only in those who had both low DNA repair and six or more severe sunburns in their lifetime. **, $P < 0.01$.

DISCUSSION

In this study, we found that DRC decreases as age increases at a rate of 0.61% per annum. In a 40-yr period, there is a cumulative 25% loss in repair ability among controls. In addition to this physiological decline, a family history of skin cancer is associated with reduced DNA repair. This reduction is associated with an early onset of BCC. The occurrence of skin cancer in the aged is due to the interactive effects of both the natural decline in DNA repair and excessive sunlight exposure. Hence, reduced DNA repair is a host susceptibility factor for BCC.

The age-related decline in DNA repair may result in an accumulation of persistent DNA damage. In turn, this should be manifested in an increase in mutation fixation. The mutation rate in the *HPRT* gene in human lymphocytes with increased age is reportedly between 1.3 and 1.6% per yr (10), which is compatible with the observed rate of decline in DNA repair. There are genetically linked repair-deficiency diseases—e.g., Cockayne syndrome and XP—in which probands also manifest premature aging (11–15). In XP patients, the defect in DRC is associated with age-related skin changes and development of skin cancer 20 yr earlier than the normal population (14). Other workers have reported age-related changes in unscheduled DNA synthesis in lymphocytes (16, 17) and epidermal cells (18) from elderly blood donors. The age-related decline in DNA-repair activity of normal lymphocytes has been seen in repair of UV (16, 19), x-ray (17, 20), and γ irradiation (21). A 30% reduction in DNA repair was seen previously in normal peripheral

in DRC observed in this and other studies, although results have not been consistent (22–24).

A fundamental question is whether the genotoxicant–DNA-repair paradigm of XP is related to the carcinogenic response of individuals who possess marginal DRCs but who have oversaturated their repair system with excessive sunlight exposure. Having been exposed to sufficient sunlight to have caused six or more severe sunburns in a lifetime appear to be associated with an increased incidence of BCC in a large prospective study (25). In this study a high proportion of BCC patients had six or more severe sunburns, which probably reflects mutation fixation as a consequence of repair saturation and persistent photochemical DNA damage. After UV exposure, individuals with a family history of skin cancer and reduced DNA repair are likely to develop this disease sooner than others of the same age.

A few other studies have reported changes in the DNA repair of skin cancer patients. In an early BCC case–control study (26), a difference was seen in the UV-light induced unscheduled DNA synthesis of peripheral blood lymphocytes of cases (n = 29, aged 25–83 yr) and controls (n = 25, aged 25–83 yr). This difference was significant in patients with both BCC and squamous cell carcinoma (n = 10) but not in those

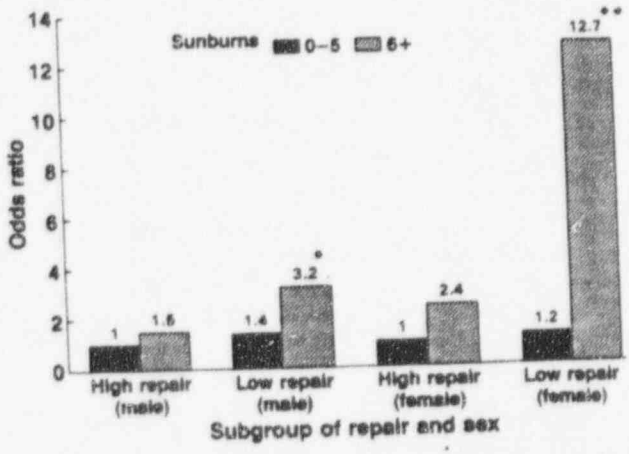


FIG. 3. Effect of DCC on risk of BCC by gender: Relationship to number of severe sunburns in a lifetime. The data in this graph are processed as for Fig. 2. The significantly increased age-adjusted odds ratio is seen in both males (3.22; 95% confidence interval, 0.97–10.7) and females (12.7; 95% confidence interval, 2.28–71.1) in those who

with BCC lesions only (n = 19). A recent study, in which the rate of pyrimidine dimer removal in fibroblasts from UV-irradiated skin biopsies was tested, found that reduced nucleotide-excision repair occurred in patients (n = 22) with BCC compared with cancer-free subjects (n = 19) (27). The small sample size in these studies did not permit age adjustment or stratification of known risk factors for skin cancer.

Reduced host DRC as a susceptibility factor is linked to a family history of BCC in this study. The risk of BCC is further increased as a consequence of sunlight exposure and low DNA repair. An alteration in the genes regulating DNA repair may be responsible for early onset of BCC, whereas BCC in the aged may be from mutation fixation as a result of accumulated DNA damage as a consequence of the age-related decline in DNA repair.

The assay used in this study has a number of technological advantages. One benefit of using the CAT assay to measure DNA repair is that it is the transfecting DNA that is damaged and not the host cell. This advantage minimizes any cytotoxic effects of these damaging agents that may indirectly compromise the endogenous repair mechanisms of the cell. The study focuses on skin cancer, a condition which is nonlethal and treated surgically without any confounding factors associated with chemotherapy or radiation treatment. In addition, this study emphasizes cancers in young individuals in whom the disease is rare and the effects of any risk factors can be maximized.

The findings from this study contribute to the understanding of the role of DNA repair in carcinogenesis in the general population. The probability of the early onset of mutation fixation because of reduced DRC suggests that the paradigm of XP applies to skin cancer in the general population. The age-related decline in DRC eventually will place all individuals at risk when their repair mechanisms can no longer accommodate the excess cumulative genotoxic damage. The maximum rate of pyrimidine dimer repair in a normal skin cell is reported to be 5×10^4 cell^{-1}·hr^{-1} (28), which is barely sufficient to cope with the rate at which damage is imposed on skin in full sunlight (29). Hence, a 25% decline in DNA repair in the 40-yr period between 20 and 60 yr places DNA repair at a sensitive focal point in the accumulation of persistent damage. This sensitivity may be responsible for tumor-suppressor gene mutations seen in skin cancer (6).

The consequences of accumulated damage may be particularly important in those postmitotic tissues where the dilution of damage by replicative mechanisms is severely diminished. There is a postnatal loss of DNA polymerases under developmental conditions in which repair enzymes remain relatively constant (30). Given the decline in DNA repair as a function of age, such tissues should be particularly vulnerable during the aging process because of their lack of regeneration. Therefore, physiological differences, such as those reported here, may serve to explain the elevated incidence of neurodegenerative diseases in many DNA-repair deficiency diseases (31).

The conclusions from our study are as follows. (*i*) The reduced host DRC of T lymphocytes correlates with the development of BCC in subjects overexposed to sunlight. (*ii*) The DRC of the normal population declines with increased age at an estimated rate of 0.61% per annum between ages 20 and 60 yr. Therefore, the age-related increased risk for BCC in the population may reflect a programmed decrease in the ability to repair photochemical DNA damage. (*iii*) A family history of BCC is associated with reduced DNA repair and early onset of this disease. (*iv*) The results of the study suggest that the persistence of DNA damage that leads to skin cancer may be directly attributed to reduced DRC, either from hereditary predisposition or from cumulative effects due to aging.

We are grateful to the many subjects who participated in this study. We thank Drs. Paul Strickland, Walter Stewart, Emily Harris, P. C. Huang, and John Groopman of the Johns Hopkins University for reviewing and criticizing this manuscript. We appreciate the assistance of Dr. Steve Gore for the $CD4^+/CD8^+$ tests, Ms. Deborah Lantry for subjects' recruitment, Ms. Correen Boucher and Ms. Gloria Taylor for their contribution during the clinic visits, and Ms. Jingrong Yan for her help in data management. Complete details for carrying out this assay and the target shuttle vector are available upon written request to L.G. This study was supported by a grant from the National Institutes of Health (GM-RO31110) to L.G. and by a grant from the National Institute of Environmental Health (2P30 ES0381906) to G.M.M.

1. Cleaver, J. E. (1968) *Nature (London)* **218**, 652–656.
2. Cleaver, J. E. (1968) *Proc. Natl. Acad. Sci. USA* **63**, 428–435.
3. Setlow, R. B., Regan, J. D., German, J. & Carrier, W. L. (1969) *Proc. Natl. Acad. Sci. USA* **64**, 1035–1041.
4. Kraemer, K. H., Lee, M. M. & Scotto, J. (1987) *Arch. Dermatol.* **123**, 241–250.
5. Ananthaswamy, H. N., Prince, J. E., Goldberg, L. H. & Bales, E. S. (1988) *Cancer Res.* **48**, 3341–3346.
6. Brash, D. E., Rudolph, J. A., Simon, J. A., Lin, A., McKenna, G. J., Baden, H. P., Halperin, A. J. & Pontén J. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 10124–10128.
7. Athas, W. F., Hedayati, M., Matanoski, G., Farmer, E. & Grossman, L. (1991) *Cancer Res.* **51**, 5786–5793.
8. Wei, Q. (1992) Doctoral dissertation (Johns Hopkins University, Baltimore, MD).
9. Protic-Sabljic, M. & Kraemer, K. H. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 6622–6626.
10. Cole, J., Green, M. H. L., James, S. E., Henderson, L. & Cole, H. (1988) *Mutat. Res.* **204**, 493–507.
11. Guzzetta, F. (1972) in *Handbook of Clinic Neurology*, eds. Vinken, P. J. & Bruyn, W. (North–Holland, Amsterdam), Vol. 13, pp. 431–440.
12. Martin, M. G. (1978) in *Genetic Effects on Aging*, eds. Bergsma, D. & Harrison, D. H. (Liss, New York), pp. 5–39.
13. Andrews, A. D., Barrett, S. F., Yoder, F. W. & Robbins, J. H. (1978) *J. Invest. Dermatol.* **70**, 237–239.
14. Wade, M. H. & Chu, E. H. Y. (1979) *Mutat. Res.* **59**, 49–60.
15. Cleaver, J. E. & Kraemer, K. H. (1989) in *The Metabolic Basis of Inherited Diseases II*, eds. Scriver, C. R., Beaudet, A. L., Sly, W. S. & Valle, D. (McGraw–Hill, New York), pp. 2949–2971.
16. Lambert, B., Ringborg, U. & Skoog, L. (1979) *Cancer Res.* **39**, 2792–2795.
17. Singh, N. P., Danner, D. B., Tice, R. R., Brant, L. & Schneider, E. L. (1990) *Mutat. Res.* **237**, 123–130.
18. Nette, E. G., Xi, Y. P., Sun, Y. K., Andrews, A. D. & King, D. W. (1984) *Mech. Ageing Dev.* **24**, 238–292.
19. Roth, M., Emmons, L. R., Haner, M., Muller, H. J. & Boyle, J. M. (1989) *Exp. Cell Res.* **180**, 171–177.
20. Harris, G., Holmes, A., Sabovijev, S. A., Cramp, W. A., Hedges, M., Hornsey, S., Hornsey, J. M. & Bennett, G. C. J. (1986) *Int. J. Radiat. Biol.* **50**, 685–694.
21. Licastro, F., Fransechi, C., Chiricolo, M., Battelli, M. G., Tabacchi, P., Cenci, M., Barboni, F. & Pallenzona, D. (1982) *Carcinogenesis* **3**, 45–48.
22. Kutlaca, R., Seshadri, R. & Morley, A. A. (1982) *Mech. Ageing Dev.* **19**, 97–101.
23. Tuner, D. R., Griffith, V. C. & Morley, A. A. (1982) *Mech. Ageing Dev.* **19**, 325–331.
24. Kovacs, E., Weber, W. & Muller, H. J. (1984) *Mutat. Res.* **131**, 231–237.
25. Hunter, D. J., Colditz, G. A., Stampfer, M. J., Rosner, B., Willett, W. C. & Speizer, F. E. (1990) *Ann. Epidemiol.* **1**, 13–23.
26. Munch-Petersen, B., Frentz, G., Squire, B., Wallevik, K., Horn, C. C., Reymann, F. & Faber, M. (1985) *Carcinogenesis* **6**, 843–845.
27. Alcalay, J., Freeman, S. E., Goldberg, L. H. & Wolf, J. E. (1990) *J. Invest. Dermatol.* **95**, 506–509.
28. Tice, R. R. & Setlow, R. B. (1985) in *Handbook of the Biology of Aging*, eds. Finch, C. E. & Schneider, E. L. (Van Nostrand Reinhold, New York), pp. 173–224.
29. Setlow, R. B. (1982) *Natl. Cancer Inst. Monogr.* **60**, 249–255.
30. Hübscher, U., Kuenzle, C. C. & Spadari, S. (1977) *Nucleic Acids Res.* **4**, 2917–2929.
31. Friedberg, E. (1985) *DNA Repair* (Freeman, New York), pp. 505–558.

Cancer linked to aging DNA repair ability

Despite decades of progress in learning how chemicals, sunlight, and other environmental factors can increase one's risk of developing cancer, the reasons that some people develop tumors and others do not remain mysterious.

Now, researchers studying people with skin cancer have found new evidence that individuals differ not just in their susceptibility to these factors, but also in their ability to fix damaged genes. In addition, adults lose about 1 percent of their capacity to repair DNA each year, says Lawrence Grossman, a molecular biologist at Johns Hopkins University in Baltimore.

This loss may help explain why aging is a risk factor for cancer, adds Qingyi Wei, a molecular epidemiologist at Johns Hopkins.

Wei, Grossman, and their colleagues studied 88 people with basal cell carcinoma, an easily observed and readily treated cancer. The researchers also looked at DNA repair in 135 individuals with mild skin problems but not skin cancer. All study participants were Caucasians between the ages of 20 and 60. Researchers took blood samples and medical histories from them and asked questions about the number of severe sunburns they had had and the incidence of cancer in their families. Dermatologists evaluated the participants' skin condition.

The researchers then take an unusual approach to assessing an individual's ability to fix broken genes, Grossman says. They inject a small piece of genetic material into white blood cells extracted from each participant. The genetic material contains a mutant bacterial gene that normally codes for an enzyme the white blood cells never make. If the cells fix the gene, the gene then causes the cells to produce the enzyme. Forty hours later, the researchers measure the activity of that enzyme, which signals how well the cells have mended the gene.

The study revealed that young people with this skin cancer had the repair capacity of someone 30 years older. They also tended to have relatives with the disease. "This gives a clue that DNA repair capacity might be genetically linked," says Wei.

Overall, people with low repair capacity were five times more likely to develop the skin cancer if exposed to intense sun than those who had high repair abilities and stayed out of the sun. Also, women seemed more susceptible than men, especially if they had six or more severe sunburns, the researchers reported in the Feb. 15 PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES.

As one of the first studies to combine laboratory and epidemiological data, "it verifies that effective DNA repair reduces the risk of cancer and that the magnitude [of that reduction] can be quite large," comments Richard Setlow of Brookhaven National Laboratory in Upton, NY.

Because of the body's gradual decline in repair capabilities, the researchers observed little difference in DNA repair capacities of older people. In them, differences in exposure to sunlight seemed critical to determining who developed tumors. "All that damage overcomes the repair system," says Wei.

"I think what people are starting to understand is that the link between aging and cancer is very, very close," says Grossman. "As you get older, you accumulate more and more persistent damage." He and his colleagues are now studying DNA repair in people with other types of cancer.

"It's an important paper that demonstrates that DNA repair plays a role in aging, which wasn't really demonstrated before," comments Vilhelm Bohr at National Institute on Aging's Geronto Research Center in Baltimore.

In addition, the differences between men and women suggest that hormones may influence DNA repair capacity. Grossman notes that, of the female participants, postmenopausal women who were receiving estrogen treatments retained 25 to 30 percent more repair capacity than other women their age. – *E. Pennisi*

Slow-motion slip may drive tsunami surprise

Because sudden jarring of the ocean floor or underwater landslides can trigger devastating waves, or tsunamis, people in earthquake-prone coastal regions have learned to run for high ground when the Earth begins to shake violently. Unfortunately, many people living on Nicaragua's Pacific coast barely felt the effects of a September 1992 earthquake. But just about 45 minutes after the main shock, say survivors, a tsunami reaching 10 meters high crashed onto a 300-kilometer-long stretch of the coastline.

More than 20 years ago, seismologist Hiroo Kanamori coined the term "tsunami earthquake" to describe such deceptively mild quakes that seem to spawn disproportionately large waves. Kanamori also proposed a possible explanation for the phenomenon, but the technical limitations of 1970s seismometers denied him compelling proof.

Now, based on seismic measurements taken during the Nicaraguan earthquake – the first event of its kind monitored with sensitive modern instruments – Kanamori and colleague Masayuki Kikuchi offer the clearest evidence to date that tsunami earthquakes stem from a "slow-slip" motion between oceanic plates. The seismic waves from this relatively gradual movement of the seafloor may scarcely be noticed by people on land.

Kanamori, of the California Institute of Technology in Pasadena, and Kikuchi, of Yokohama City University in Japan, report their findings in the Feb. 25 NATURE.

Judging from the seismic record of the Nicaraguan disaster, the researchers believe that the motion of oceanic plates in a deep trench off the Nicaraguan coast jiggled a 100-kilometer-long section of the ocean floor, moving it about 1 meter in a period of 2 minutes. The water set in motion by this event caused the surprise tsunami that destroyed 13,000 dwellings and killed more than 150 people.

Kanamori believes this slow slip – an event that releases great energy but in a way that can mask its true magnitude – may prove the hallmark of the tsunami earthquake. During a slow-slip earthquake, he suggests, an oceanic plate lubricated by soft ocean sediments slides under an adjoining plate relatively slowly. This produces a seismic spectrum rich in long-period waves, like a piece of music full of cellos and bass drums. Seismic readings show clearly that long-period waves dominated the Nicaraguan earthquake, the researchers report.

Unfortunately, humans are only sensitive to the electric-guitar thrash at the higher end of the seismic spectrum – the kind of short-period seismic waves that shake buildings to their foundations. Thus, during a tsunami earthquake, people on land fail to notice the malevolent bass drone of impending inundation.

Until recently, seismometers were largely insensitive to long-period waves, a technical problem solved in the early 1980s. However, calculations of earthquake magnitude – the famous Richter scale – are still based on relatively short-period waves and thus may underestimate the destructive potential of tsunami earthquakes, Kanamori says.

Based on short-period waves, the Nicaraguan quake comes out as a magnitude 7.0 event. However, by using long-period waves to calculate the earthquake's "seismic moment," a quantity that reflects the actual motion of the plates rather than their ground-level effects, the researchers peg the Nicaraguan earthquake at a significantly larger magnitude, 7.6.

Kanamori and others argue that use of long-period waves to calculate seismic moment, a task made quick and easy by modern technology, can provide an accurate determination of an earthquake's tsunami potential and perhaps offer sufficient warning to coastal communities at risk. Without such warning, the chance of surviving a tsunami traveling at jetway speed remain slim. "Once you see a tsunami coming towards you, it's usually too late," Kanamori notes. – *D. Pendick*