In this paper I will consider an aspect of carcinogenesis that needs further in-depth study because it could be a major cause of human cancers. I am referring to the Damoclean possibility that certain normal endogenous cellular processes are inherently mutagenic and that this intrinsic mutagenesis is a significant factor in the etiology and pathogenesis of some human cancers. Moreover, I will prospectively consider the impact of molecular biology on cancer research. Our ability to determine the composition of genes at the molecular and atomic levels is likely to change radically many facets of cancer research. New techniques in molecular biology not only have highlighted critical deficiencies in our understanding of the differences between normal and cancer cells but also have lent us confidence that new and deeper, more fundamental insights will soon be within our grasp. I am pleased to report that our Association has taken new initiatives to incorporate recent advances in molecular biology into the purview of cancer researchers and thereby will be a leading facilitator of the transport of molecular oncology into the 21st century.

Environmental Carcinogens

A number of environmental agents are now classified as human carcinogens. By far the most significant is tobacco smoke, the cause of 30% of cancer deaths in the United States (1). Currently, the risk of the most common deadly cancer in the United States, cancer of the lung, is 22 and 12 times greater in male and female smokers, respectively, than in nonsmokers (2). The prevalence of cigarette smoking among males in the United States has decreased steadily in the last 10 years, from a high of 50% in 1965 to the current level of 31% (3). In conjunction, after 40 years of relentless increase there has been a plateauing in the rate of lung cancer deaths in males and a significant decrease in the 45-55-year-old age group (3). Unfortunately, smoking incidence has not decreased among American females, and there has been a substantial elevation in cancer of the lung among males and females, and there has been a substantial elevation in cancer of the lung among males and females (2). The other significant environmental carcinogen is the indoor radon gas, a product of the radioactive decay of radium. Radon gas can be quite toxic and is the leading cause of lung cancer in women (4). However, despite extensive studies, many human cancers have no apparent risk factors and do not appear to have widely varying incidences among individuals of diverse genetic backgrounds or among those living in different geographical areas. Human cancers with minimal variation in incidence include many childhood malignancies, as well as carcinoma of the pancreas, ovary, and colon (4). Wilms' tumor has been recommended as an index for consistency in tumor registries (5). Even in breast (6) and colon cancers, where a large number of risk factors have been established, they account for only about one-fourth of the incidence.

DNA Damage by Chemical Carcinogens

In the last few years we have created a veritable explosion in knowledge about how chemical carcinogens damage DNA (7, 8). We have extended concepts originally formulated by the Millers (9); among chemical carcinogens are already activated electrophiles or those that are converted to electrophiles by cellular activating enzymes. It is the resultant activated chemical species that react with many cellular macromolecules including DNA. The interaction with DNA marks cancer as a disease of genes—not a classically inherited disease, but rather a disease that is transmitted from parent cell to daughter cell with each division cycle. It is a disease that is associated invariably with altered nucleotide sequences in DNA and/or with changes in gene transcription.

A grossly simplified model that attempts to relate damaged DNA to mutagenesis is presented in Fig. 1. Mutagenesis results from unrepaired DNA damage. During each cell division cycle, DNA polymerases copy past the damaged DNA and insert noncomplementary nucleotides opposite the site of damage. In this model, mutations do not result from error-free DNA repair or from unrepaired lesions that are copied without a change in sequence of the newly replicated DNA. Furthermore, mutations do not occur in cells that fail to undergo DNA replication. A mutational cause of malignancy presupposes that among the dispersed mutations in the genome are mutations in key genes that alter the properties of cells, allowing them to escape homeostatic mechanisms that regulate cell division, invade, and metastasize. In this model, important forces in determining whether or not mutations are expressed are the stimuli for cell division which include increase in autocrine and paracrine growth factors and regenerative stimuli brought about by the death of adjacent cells and tissues.

Spontaneous Mutations as a Cause of Cancer

Since mutagenesis by environmental carcinogens appears to be a key causal event in the etiology of certain cancers, might not spontaneous mutagenesis also be carcinogenic? Many processes that damage DNA and could contribute to background or so-called spontaneous mutagenesis have been identified. As in the case of DNA damage by exogenous chemical agents, spon-
Unrepaired DNA Damage → Mutations

DNA Damage → Quiescent Cells → Increased Cell Proliferation

Error-Free Repair → DNA Replication → No change in sequence

Error-Prone (SOS) Repair → Erroneous Incorporation → Mutations

Fig. 1. Schematic representation of the relationship of DNA damage to mutagenesis. This scheme emphasizes the importance of increased cell proliferation in the generation of mutations.

Spontaneous damage would need to occur at a sufficiently high frequency to exceed the capacity of the cell for DNA repair (Fig. 2). It should be noted that human cells possess an unusually high efficiency in repairing DNA damage (10). This repair capacity has been postulated to be responsible for the comparatively long lifespan of our species (11). Nevertheless, not all DNA damage is repaired, as judged by the production of both germ line and somatic mutations. The spectrum of mutations resulting from DNA damage by either exogenous chemicals or spontaneous lesions would be that damage that is not repaired (Fig. 2). In a similar manner, errors made by DNA polymerases would be those that sieve through the mechanisms of the cell for the correction of mismatches during DNA replication (12). Spontaneous mutations could have the same potential for inducing cancer as those caused by exogenous environmental agents.

Mutation Rate and Cancer

Clues about the relationships between mutations and cancer can be gleaned from quantitative analyses. There are “hot spots” for mutagenesis within genes; however, until one identifies the sites of mutations within critical genes that code for malignant changes, the least prejudicial assumption is that DNA damage is random. Diverse studies suggest that the mutation rate in somatic cells is $10^{-9}$ to $10^{-12}$ events per nucleotide per cell division (13). However, higher rates may occur when cells are maintained in tissue culture. The human body contains some $10^{14}$ cells and it is estimated that within a life span our cells undergo a total of $10^{16}$ division cycles (14). If one assumes that a single dominant mutation is oncogenic, then the spontaneous mutation rate is sufficient to produce millions of cancer cells during one's lifetime. If mutations at more than one site can activate a cancer gene (consider the multiplicity of activated ras gene (15)) or if there are a large number of tumorigenic target genes, then an even greater number of tumor cells could be formed. However, it can be argued that many mutations with the potential to cause cancer may not do so, since cell proliferation may be held in check by the homeostatic mechanisms that regulate cell growth and behavior.

Alternatively, many mutations may be required to produce a clinically detectable malignant tumor. The numerology for spontaneous mutations suggests either two dominant mutations on separate genes or two recessive mutations on the same allele. Assuming two dominant mutations, each in a different gene within the same cell, then the rate of spontaneous mutagenesis would be adequate for 1 cancer in 100 individuals. This estimate is based on $10^{10}$ cells divided by the square of the spontaneous mutation rate. A frequency approximating one cancer per individual would be predicted on the basis of allelic recessive mutations, because of the greater number of sites that potentially could inactivate a critical gene. Recessive oncogenes as a cause of human cancer have been most intensively studied in retinoblastoma (16) and have been observed in a variety of human tumors (17, 18). Considering the inaccuracy of our estimates for rates of spontaneous mutagenesis and the known “hot spots” for mutagenesis at different genetic loci in prokaryotes (19), either of these possibilities seems reasonable. It is of interest that the frequency of spontaneous mutations is in the same range as the number of DNA lesions per genome produced upon exposure of cells in culture and animals to many chemicals at carcinogenic doses (20). Exposures of animals and cells in culture to higher doses of chemical carcinogens are frequently lethal, and treatment at lower doses fails to yield significant numbers of oncogenic transformations.

A number of observations suggest that multiple and perhaps sequential events are required for carcinogenesis (21, 22). Of importance is age dependence in the incidence of many cancers; in different species, including humans, the incidences of cancer increase with the fourth to sixth power of age (23). The analysis I have presented on the rate of spontaneous mutagenesis and the number of alterations in DNA induced by a single exposure to a chemical carcinogen could set limits on the number of mutagenic events. Either many of these age-dependent events are not mutagenic or one of these mutations results in the induction of a mutator phenotype (22, 24). A mutator phenotype could result from mutations in genes such as DNA polymerase, with the production of an altered enzyme that is error prone in catalyzing the synthesis of DNA (25). A mutator phenotype would account for the chromosomal instability that is known to characterize tumor progression (21). An abnormally high frequency of spontaneous deletions has been observed in tumor-derived cells maintained in tissue culture (17). Considering the multiplicity of proteins involved in DNA replication, DNA repair, and chromosomal segregation, the number of potential targets that could generate mutator phenotypes may be larger than the number of known oncogenes (26). Nevertheless, the question of whether or not the chromosomal instability that characterizes tumor progression results from increased errors in DNA replication remains an important yet unsettled problem for future study (27–29).

Potential Sources of Spontaneous Mutations

Until fairly recently, DNA was universally considered to be an essentially unchanged molecule within a cell. Exceptional
stability is presumably required in germ line cells so that genetic information can be passed from generation to generation with few if any alterations. We have assumed that a similar unalterability prevails in somatic cells. However, this assumption has been repeatedly challenged by measurements on the chemical instability of DNA in solution and by observations of DNA rearrangements within cells. I will consider three processes that alter DNA. Each process is known to be mutagenic both in vitro and in vivo and to occur at a sufficiently high frequency to account for spontaneous mutagenesis. In model systems, it has been possible to determine the types, or spectrum, of mutations produced by each of these processes. A comparison of the spectra in model systems with the spectrum of spontaneous mutations occurring in somatic cells may help to quantitate the contribution of each of these processes to spontaneous mutagenesis. As we identify mutated genes associated with cancers, it may then be possible to determine further whether spontaneous mutagenesis is responsible for mutations in those genes associated with individual types of cancers.

The Chemical Instability of DNA

DNA is not entirely stable in aqueous solution. Of the covalent changes that have been documented, depurination is the most frequent (30). Depurination of DNA results from the cleavage of the N-glycosylic bond that connects the purine base to the deoxyribose sugar. As a result, the DNA backbone is left intact; and, during DNA replication, DNA polymerases would encounter a site without a base, i.e., an abasic site (31). Lindahl and Nyberg (30) first measured the rate of release of purine bases from DNA in aqueous solution as a function of time, pH, and ionic strength. From these experiments, they calculated that, under physiological conditions, depurination occurs at a rate of 3 \times 10^{-11} \text{ events/base/s}. We have confirmed their measurements under a variety of conditions and have also observed that the rate of depurination is accelerated by the presence of several divalent metal ions (32). Since the human nuclear genome contains some 3 \times 10^9 purine bases, each cell would undergo 10,000 depurinations per day. Hydrolysis of the glycosylic bond connecting pyrimidine bases to the deoxyribose sugar occurs at a rate 100-fold slower and also results in abasic sites in DNA (33).

Because depurination is such a frequent event, it is not surprising that cells possess multiple pathways for the repair of apurinic sites (7). Nevertheless, it seems probable that many abasic sites would filter through this DNA repair screen. Sequstration from DNA repair may be afforded by histones that are tightly complexed to DNA in eukaryotic cells (34). Consequently, DNA polymerases may frequently encounter abasic sites; replication and misincorporations opposite these sites could be a major source of spontaneous mutations. Evidence for the mutagenic nature of abasic sites is the increased misincorporation by DNA polymerases in copying DNA templates containing abasic sites (35–37). Surprisingly, DNA polymerases do not randomly insert nucleotide substrates opposite abasic sites; misincorporation is highly specific; deoxyadenosine is most frequently inserted as a single base substitution (35, 38, 39). Deoxyadenosine is preferentially incorporated by a variety of DNA polymerases using an analogue of an abasic site present at a single position within a variety of nucleotide sequences (40). Accordingly, if depurination is a principal source of spontaneous mutations, then the spectrum of spontaneous mutations should be heavily biased toward substitutions by deoxyadenosine. However, other lesions in DNA including bulky adducts may also direct the incorporation of deoxyadenosine (41).

Deamination of cytidine to uridine occurs at one five-hundredth of the rate of depurination (8). Uridine base pairs with deoxyadenosine during DNA replication and thus would produce a direct alteration in the sequence of nucleotides in the newly replicated DNA. However, human cells have a high level of uracil glycosylase activity (42) and thereby have the potential to excise uridine and reduce mutagenesis. The eukaryotic genome contains 5-methylcytosine, which is believed to function in the control of gene transcription. Depurination of 5-methylcytosine yields thymidine, a normal nucleoside, that presumably if not repaired, provide a potent pathway for mutagenesis via the formation of C:G $\rightarrow$ T:A transitions (43).

A number of normal, active cellular metabolites are able to form stable covalent adducts on DNA. Such covalent modifications include methylation of DNA by $S$-adenosylmethionine (44) and the glycosylation of DNA by reducing sugars (45). The frequencies of DNA alterations by these normal cellular metabolites and their mutagenic potential remain to be determined in order to evaluate their potential contribution to spontaneous mutagenesis. Indeed, this field is likely to be a fruitful area for investigation, not only for carcinogenesis but also for other age-dependent disease processes.

Mutagenesis by Free Radicals of Oxygen

The rate of damage to DNA by oxygen free radicals produced in cellular metabolism (46) may be of the same order of magnitude as nucleotide alteration in DNA caused by depurination. In cells, oxygen is metabolized by a series of one electron reductions with the generation of highly active free radical intermediates (47). Among these radicals, hydroxyl ions appear to be the most damaging. Processes that generate oxygen free radicals include respiration, phagocytosis, and cell injury. The resultant free radicals modify RNA, proteins, membranes, and DNA. A multiplicity of nucleotide alterations have been demonstrated by exposing DNA to systems that generate oxygen free radicals. From measurements of 8-hydroxydeoxyguanosine and thymine glycol in urine, it can be estimated that approximately 10,000 oxygen free radical-induced alterations occur in DNA per human cell per day (48). Thus, damage to DNA occurs at sufficiently high frequency to be a source of spontaneous mutations. In counteracting this genotoxicity, cells have evolved a multiplicity of systems to scavenge oxygen free radicals and to excise some of the nucleotides from DNA that have been damaged by these radicals. Damage to DNA that escapes these repair mechanisms would provide a mechanism for the generation of mutations and, in fact, the mutagenicity of oxygen free radicals has been demonstrated in eukaryotic cells after exposure to elevated concentrations of oxygen or upon incubation with systems that generate oxygen free radicals (49).

Even though oxygen metabolism has been well recognized as a potential source of spontaneous mutations, the contribution of oxygen-induced mutation to the spontaneous mutation rate has been very difficult to assess. This is because multiple pathways exist in cells for the production of oxygen free radicals, a variety of reactive species are generated, a multiplicity of alterations occur in DNA, and until recently there has not been available a sensitive system for evaluating the mutagenicity of each type of nucleotide alteration. However, new molecular systems are sensitive enough to allow one to measure the mutagenic potential of single lesions at a defined site in DNA (40, 50, 51). Thus it should be possible to test each of the
species produced by free radical damage for their potential mutagenicity.

The mutations caused by incubating DNA with Fe²⁺ in air arise via the generation of oxygen free radicals (52), providing a simple and defined chemical system to analyze the mutagenic lesions in DNA induced by oxygen free radicals. In this system, DNA from bacteriophage φX174, containing a single base change, is incubated with Fe²⁺ and then transfected into Escherichia coli spheroplasts. Substitutions of other nucleotides at this single site result in the production of a wild type progeny phage that can be quantitated on appropriate indicator bacteria (53). Mutagenesis requires the presence of oxygen and can be abolished by catalase and manganese, suggesting the involvement of H₂O₂ and hydroxyl radicals, respectively (52). In E. coli, mutagenesis is dependent on the induction of the “error-prone” SOS pathway (54). The DNA sequence of mutants produced by base substitutions at the target site indicates that deoxyadenosine is inserted preferentially opposite a deoxyadenosine in the template strand. This preferential insertion of deoxyadenosine during replication of the damaged DNA template does not appear to result from the formation of an abasic site caused by oxygen free radical damage. Mutagenesis by Fe²⁺-treated DNA is not abolished by incubation of the damaged DNA with an apurinic endonuclease that would cleave DNA containing abasic sites and render the DNA biologically inactive. The chemical nature of the deoxyadenosine that is altered by exposure of DNA to the oxygen free radicals remains to be determined.

Preliminary results indicate that the generation of oxygen free radicals by other mechanisms is also mutagenic in this system. Mutagenesis has been observed with myeloperoxidase, cytochrome oxidase, activated polymorphonuclear leukocytes, as well as Cu²⁺. The advantage of this molecular approach is that DNA damage and mutations can be produced in vitro using a defined system in which all components are present as chemically pure species. With Fe²⁺ or Cu²⁺, the extent of mutagenesis per lethal event at the target site is greater than that observed with any other agent thus far tested that damages DNA. This high level of mutagenicity indicates that it will be feasible to use a forward mutation assay to establish the spectrum of mutations produced by oxygen free radicals.

Mutagenesis Due to Errors in DNA Replication

In order for DNA replication not to be a key factor in spontaneous mutagenesis, DNA synthesis must be an incredibly accurate process. During each cellular replicative cycle, some 3 x 10⁹ nucleotides are copied. Errors in this copying (if not corrected) are by definition mutations. The primary enzyme responsible for the high accuracy is DNA polymerase; it catalyzes the sequential addition of deoxynucleoside triphosphates. The fidelity of this process is governed by a multistep process: (a) by the exactness of base pairings between the template nucleotides and the incoming deoxynucleoside triphosphate substrates; (b) by the capacity of the polymerase to increase the free energy difference between the correct and incorrect basepairings; and (c) by the ability of some, but not all, DNA polymerases to “proofread” via a 3'→5' exonuclease that excises an incorporated noncomplementary nucleotide before addition of the next complementary nucleotide. Furthermore, in bacteria, there is a postsynthetic pathway for removing incorrectly incorporated nucleotides after DNA replication (12), and it is likely, but not proven, that a similar mechanism is present in eukaryotic cells (55).

The primacy of DNA polymerases in guaranteeing the fidelity of DNA replication not only is apparent from their central role in DNA replication but is also substantiated by genetic studies. Prokaryotic (56) and eukaryotic (57) mutants in DNA polymerases exhibit a mutator phenotype, i.e., one that increases in the rate of spontaneous mutations throughout the genome. Additional evidence that errors by DNA polymerase increase mutagenesis includes experiments demonstrating enhanced mutagenesis due to alterations in the relative concentrations of deoxynucleoside triphosphates, the substrates of DNA polymerases (58).

In order to measure frequencies and types of misincorporations by DNA polymerases or by DNA replicating complexes, we designed a genetic assay (53). A single-stranded circular φX174 DNA template containing an amber mutation is primed with a complementary oligonucleotide and copied in vitro with a purified DNA polymerase. Incorporation of any noncomplementary nucleotide at position 587 opposite the adenosine in the amber codon results in reversion to the wild type phenotype. After copying proceeds past the amber site, the partially double-stranded DNA product is transfected into E. coli spheroplasts where synthesis is completed. The error rate of the DNA polymerase is determined from the reversion rate of the progeny phage after plating on bacteria that are permissive or nonpermissive for the amber colony. This assay is able to detect misincorporations at a frequency of 10⁻⁸ when the four deoxynucleoside triphosphate substrates are at equal concentrations in the reaction mixture and even at a lower frequency of 10⁻⁸ when one noncomplementary nucleotide substrate is present at a very high concentration (59).

The frequencies of misincorporation by various DNA polymerases have been analyzed using the φX fidelity assay (60) and assays to measure forward mutations (61). The combined data indicate that DNA polymerases are highly error prone in comparison with the low frequency of spontaneous mutations exhibited by human cells. DNA polymerase α, the major replicating DNA polymerase found in eukaryotic cells, has an error rate of approximately 1/30,000 when purified as a monomeric species (60) and 1/200,000 when purified by antibody affinity chromatography as a 4-subunit complex containing DNA primase (62). The most frequent errors are single-base substitutions and, of these, transitions are more frequent than transversions. DNA polymerase β, the enzyme presumed to function primarily in DNA repair, has an in vitro error rate of about 1/5000 (61). Its most frequent errors are single-base substitutions, particularly involving T:G and C:A mismatches, followed by single-base deletions opposite stretches of identical template nucleotides. DNA polymerase δ, an enzyme also postulated to be involved in DNA replication, shares many properties with DNA polymerase α but contains a 3'→5' proofreading exonuclease (25, 63). As a result, this DNA polymerase is more accurate; the error frequency has been estimated at 1/500,000 (64). One current model for eukaryotic DNA replication involves coordinate synthesis by DNA polymerases α and δ, of the lagging and leading strand, respectively (65), and it has been proposed that the exonuclease activity associated with polymerase δ is able also to excise errors produced by DNA polymerase α on the opposite, newly replicated strand.

Studies on the fidelity of DNA synthesis by eukaryotic DNA polymerases may not be adequate. Many of the purified en-

---


3 F. W. Perrino and L. A. Loeb, unpublished observations.
zymes could be unphysiological in that they lack a proofreading exonuclease present in cells but lost during purification. Also in bacteria, there is a potent system for the postsynthetic correction of errors during DNA replication, and there is some evidence for such a system in eukaryotes (55). Nevertheless, the combined studies on the fidelity of DNA synthesis in vitro indicate that misincorporations by eukaryotic DNA polymerases are very frequent events; they occur at a much higher frequency than spontaneous mutations. The most frequent errors are single-base substitutions and "minus one" base frameshifts (66). These results are in accord with studies on spontaneous DNA replication mutations in bacteria. Schaaper and Dunn (67) determined the mutation spectrum of errors in DNA replication by using bacteria lacking the genes for postreplicative mismatch repair. Of all mutants, 25% were single-base deletions and 75% were single-base substitutions. Among the single-base substitutions, 96% were transitions (67).

The Spectrum of Spontaneous Mutations in Eukaryotic Cells

Detailed comparisons of the frequencies and types of mutations produced by singular pathways in vitro with actual spontaneous mutations in eukaryotic cells may yield important clues concerning the contribution of a specific pathway to spontaneous mutagenesis. In principle, the insertion of recombinant shuttle vectors into eukaryotic cells should allow one to sample the eukaryotic environment for the production of spontaneous mutations. However, these studies have been complicated by a high frequency of mutagenesis, presumably due to DNA damage produced during the DNA transfection process (68, 69). In contrast, recent studies in which chromosomal genes from mutant cells are cloned or amplified using the polymerase chain reaction are beginning to yield information on the nucleotide sequence alterations that characterize spontaneous mutagenesis. Studies with cells containing a single copy of the nonessential adenine phosphoribosyltransferase locus (APRT) have been particularly informative. Results from two laboratories (58, 70) indicate that the majority of spontaneous mutations are single-base substitutions; in one study, 22 of 27 mutants were G:C to A:T transitions (70). These limited studies are in accord with the hypothesis that infidelity of DNA synthesis is a major factor in spontaneous mutagenesis. There is, however, a caveat; if genes adjacent to the hemizygous APRT gene are essential, then many deletion and frameshift mutants in APRT may go undetected, since these alterations might inactivate the essential neighboring genes.

Mutations within the three ras genes have been observed in a significant percentage of all human tumors (15, 71–74). Even though the role of these mutations in carcinogenesis remains to be established, these genes provide an interesting probe for sampling the spectrum of spontaneous mutations associated with tumorigenesis (75). However, the interpretation of results obtained with mutant ras genes should be restricted, since only a subset of all possible activating ras mutations are found in tumors; thus, those detected may be mutational "hot spots" and not representative of random mutagenesis. As many as 65% of human colon cancers contain mutant ras genes (71). In one study, 71% of mutations in codon 12 in the K-ras gene were transitions (74), while in another 25 of the 26 mutations were G to A transitions (71, 76). The high frequency of transitions favor errors by DNA replication as the source of spontaneous mutations within the K-ras gene. On the other hand, studies of K-ras gene mutations in human pancreatic adenocarcinoma yielded a different result; the predominant changes were A→T transitions (73), suggesting depurination as a possible cause for these mutations. However, in another study, the most frequent mutation was a G→A transition (72). Even though these early studies are only beginning to yield data about the origin of spontaneous mutations, they indicate that recent molecular techniques are applicable, and they offer evidence that the source(s) of spontaneous mutations will soon be identified.

I have considered the possibility that spontaneous mutations contribute causally to human cancer. This contribution is likely to be most significant for cancers with no known risk factors and/or for cancers the incidence of which is age dependent and similar, with respect to genetic background or geography. Molecular methods to identify mutations in genes that determine the malignant phenotype should allow us to evaluate the contribution of spontaneous mutagenesis to carcinogenesis in different human cancers. Thus, for each individual it could be only a matter of time before a random mutation occurs in a key gene, the thread that holds the sword of Damocles breaks, and the carcinogenic process starts. A superficial analysis would suggest that many cancers are inevitable. A deeper inspection would highlight our lack of understanding of the molecular differences between normal and cancer cells and the mechanisms of the cell for accurate replication of DNA; this knowledge is required before we can even begin to consider how to manipulate these factors. Thus, molecular biology offers both the power and the promise to unravel the mysteries of human cancer.

Molecular Biology within the AACR

I have used studies on the origin of spontaneous mutations and their possible relationships to human cancer to illustrate the potential of molecular biology to define differences between normal and malignant cells. I believe we are currently witnessing a quantum leap in our understanding of how cells work. This revolution is spearheaded by molecular biology. It seems to me that many facets of cancer research will be redirected. As a practitioner of molecular biology, I find it difficult to be objective. Yet, despite my zeal, let me consider, "How should the American Association for Cancer Research respond to this new discipline? What have we so far accomplished?"

The history of cancer research is a record of considerable accomplishments. Most childhood malignancies are now potentially curable; but as recently as 20 years ago, they were invariably fatal. The years of productive lives saved by just this one of many successes have more than justified the cost of cancer research on economic grounds, to say nothing about humanitarian grounds. Members of our Association have been major contributors to this success. In this regard the Association salutes the awarding of the Nobel Prize in Medicine this year to Gertrude Elion and George H. Hitchings, two very active members of our Association. (Dr. Elion served as President of the American Association for Cancer Research in 1983, and Dr. Hitchings was elected an Honorary Member in 1981.)

Even though advances in research have furthered our understanding of the malignant process and have resulted in improvements in the treatment of specific adult cancers, all too frequently new directions in cancer research have not met our expectations. Is the new emphasis on molecular biology simply another chapter in this uneven ascent? I submit not. Previous new pathways in cancer research were limited at the start by methodology; and perhaps the absence of this limitation is the promise of molecular biology.

Molecular biology has been classified as a discipline with its
own curriculum, rules, and practitioners. But this definition may be short-sighted. Instead, I submit that molecular biology constitutes a series of new experimental approaches of unprecedented power—approaches that will increasingly change most aspects of cancer research. Therefore, it is imperative that this new technology become part of the armamentarium of current and future cancer investigators. To take full advantage of this technology, our society has initiated a series of new programs. These new directions are not limited to molecular biology. They are designed to provide new avenues to enable a large society such as ours to stay at the forefront of science and yet also to maintain a forum for interdisciplinary discussion. We must maintain this interdisciplinary approach to cancer research, since this disease has so many ramifications.

This year, the American Association for Cancer Research has inaugurated a series of satellite meetings focused on molecular oncology. The first of these meetings, "Oncogenes and Transcription," was organized by Phillip Sharp. At this meeting was presented for the first time a series of reports documenting that two oncogenes, fos and jun, are transcription factors. These discoveries foretell a new direction in cancer research, i.e., the association of oncogenes with the control of transcription. The second meeting, "Human DNA Tumor Viruses," was organized by Harald zur Hausen. The thematic question was, "To what extent are common pathogenic human DNA viruses responsible for human tumors?" This year's national meeting was preceded by two short satellite meetings. One, organized by B. Singer and D. Patel, addressed the structure of DNA adducts and their mutagenic potential. Another, organized by Inder Verma, considered the relationship of oncogenes to growth factors. Our annual meeting was followed by three workshops in molecular biology and a joint U.S./Japanese Meeting on "Growth Control and Cancer." I should emphasize that even though many of these meetings have been focused on molecular approaches to human cancer, other meetings that will focus on a diversity of topics are planned. We will choose the topics of future meetings based in part on new exciting findings and the potential impact of these fields on future cancer research.

In January 1990, the American Association for Cancer Research will publish a new journal, "Cell Growth and Differentiation." The Journal will be edited by Dr. George Vande Woude and a cadre of distinguished scientists. Our goal is to expeditiously publish exciting papers that merge the fields of molecular and cellular biology with cancer research.

Perhaps the three workshops following the most recent Annual Meeting best convey the power of this new molecular technology. The workshop on cloning of genes considered current methods for identifying, recovering, and expressing genes in both prokaryotic and eukaryotic cells. We now have the ability to isolate a single cellular gene, insert it into a recombinant DNA vector, and amplify it into a million functional copies. Thus, we can approach the question of how genes in cancer cells are altered during carcinogenesis. The workshop on the polymerase chain reaction (77) presented a new technology allowing one to choose a region of a gene in a single cell, hybridize onto it oligonucleotide primers and then, with DNA polymerase, synthesize a million copies of that region in vitro in a single day. Moreover, one can use archival material as a source of DNA and thus reconstruct the genetic history of a tumor (71, 78). I predict that this in vitro technique is likely to replace DNA cloning. The third workshop focused on techniques for the production of transgenic mice. It is now possible to insert genes or artificial constructs into embryos and produce mice containing these transgenes in many tissues. By the use of a specific promoter, one can study the expression of oncogenes in selective tissues and do so at different times during development (79). Thus, one has the potential to determine how mutations in genes and inappropriate gene expression lead to malignant changes in vivo.

The molecular biology approaches already at hand will have profound effects as molecular oncology enters the 21st century. I predict that not only will this new discipline alter fundamental cancer research, but its tentacles will also extend into most related disciplines. Let us consider some fields and examples.

Cancer Genetics. It has long been recognized that some cancers occur more frequently in individuals with deficits in DNA repair (7). Our ability to transplant genes from one human cell to another, or from a human cell to an animal cell deficient in DNA repair, should allow us to carry out biochemical studies on human DNA repair enzymes. The identification of the altered genes and their functions will give us clues into the molecular basis for carcinogenesis.

Cancer Diagnoses. In most cases the diagnosis of tumors and the origin of metastasis is based on tissue histology and cell morphology. However, 5 to 10% of tumors are too inadequately differentiated to establish the tissue of origin. For pathologists, new molecular probes are becoming available to unequivocally establish the tissue of origin, a diagnosis increasingly relevant to the choice of appropriate chemotherapy.

Cancer Prognoses. Current cancer therapy can be debilitating. Thus, a major dilemma for the clinician is to choose a therapy based on prognosis. If the multiplicity of mutations in certain human cancers determines the tumor phenotype, methods are at hand to predict the ability of individual tumors to spread and metastasize. Also, the early recurrence of tumors can be established by using the polymerase chain reaction to detect rare tumor cells with known changes in DNA sequence among a large population of normal cells.

Cancer Therapy. Most, but not all, effective chemotherapeutic agents are directed against DNA replication. They bind to the DNA template, inhibit DNA replication, or interfere with chromosome segregation. The identification of genes involved in cancers should yield a new approach to cancer therapy, i.e., gene therapy. This approach might include the use of antisense DNA or RNA to interfere with gene expression in cells likely to become malignant and even methods to inactivate selectively cells harboring specific mutations.

Acknowledgments

I would like to thank Drs. Sam Sorof, Ann Blank, and Keith Cheng for generous advice in reviewing and Mary Whiting for preparing this manuscript.

Now that I have completed my term as President of the American Association for Cancer Research, I can state unequivocally that this is an exciting position, a conclusion shared by the 79 presidents who preceded me. I can highly recommend this office to Drs. Busch and Weinstein, who will succeed me, as well as to future presidents. I have had the support and tolerance of two families: my wife, Phyllis, and my children who endured the many trips to Philadelphia, as well as a
second family of students, postdoctoral fellows, and colleagues who have provided ongoing counsel. I wish I could thank them all by name; in particular, let me again thank Sam Sorof, as well as Richmond Prehn, Michael Sirover, Bea Singer, and Brad Preston. Every recent president has thanked Marge Foti and her staff; these tributes are genuine and are not phatic statements. We are indeed very fortunate to have our organization staffed by such competent and dedicated people.

Prehn, Michael Sirover, Bea Singer, and Brad Preston. Every recent have provided ongoing counsel. I wish I could thank them all by name;

References


Endogenous Carcinogenesis: Molecular Oncology into the Twenty-first Century—Presidential Address

Lawrence A. Loeb


Updated version  Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/49/20/5489.citation

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.