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DNA Lesions, Inducible DNA Repair, and Cell Division: Three Key Factors in Mutagenesis and Carcinogenesis

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DNA lesions that escape repair have a certain probability of giving rise to mutations when the cell divides. Endogenous DNA damage is high: 10^6 oxidative lesions are present per rat cell. An exogenous mutagen produces an increment in lesions over the background rate of endogenous lesions. The effectiveness of a particular lesion depends on whether it is excised by a DNA repair system and the probability that it gives rise to a mutation when the cell divides. When the cell divides, an unrepaired DNA lesion has a certain probability of giving rise to a mutation. Thus, an important factor in the mutagenic effect of an exogenous agent whether it is genotoxic or non-genotoxic, is the increment it causes over the background cell division rate (mitogenesis) in cells that appear to matter most in cancer, the stem cells, which are not on their way to being discarded. Increasing their cell division rate increases mutation and therefore cancer. There is little cancer from nondividing cells. Endogenous cell division rates can be influenced by hormone levels, decreased by calorie restriction, or increased by high doses of chemicals. If both the rate of DNA lesions and cell division are increased, then there will be a multiplicative effect on mutagenesis (and carcinogenesis), for example, by high doses of a mutagen that also increases mitogenesis through cell killing. The defense system against reactive electrophilic mutagens, such as the glutathione transferases, are also almost all inducible and buffer cells against increments in active forms of chemicals that can cause DNA lesions. A variety of DNA repair defense systems, almost all inducible, buffer the cell against any increment in DNA lesions. Therefore, the effect of a particular chemical insult depends on the level of each defense, which in turn depends on the past history of exposure. Exogenous agents can influence the induction and effectiveness of these defenses. Defenses can be partially disabled by lack of particular micronutrients in the diet (e.g., antioxidants).

Endogenous DNA Damage and Mutagenesis

Endogenous rates of DNA damage are enormous. Mutagens are often thought to be only exogenous agents, but endogenous mutagens cause extensive DNA damage (oxidative and other lesions), some of which is converted to mutations during cell division. Four endogenous processes leading to significant DNA damage are oxidation (*1–3*), methylation, deamination, and depurination (*2*). The importance of these processes is supported by the existence of specific DNA repair glycosylases for oxidative, methylated, and deaminated adducts and a repair system for apurinic sites that are produced by spontaneous depurination (*3*).

DNA damage produced by oxidation appears to be the most significant endogenous damage (*4*). We estimate that the DNA hits per cell per day from endogenous oxidants are normally 10^5 in the rat and 10^4 in the human (*5–7*). These oxidative lesions are effectively but not perfectly repaired; the normal steady-state level of oxidative DNA lesions is about 10^6 per cell in the young rat and about twice this in the old rat (*6,8*). Oxidants are produced as byproducts of mitochondrial electron transport, various oxygen-utilizing enzyme system, peroxisomes, and other processes associated with normal aerobic metabolism, as well as by lipid

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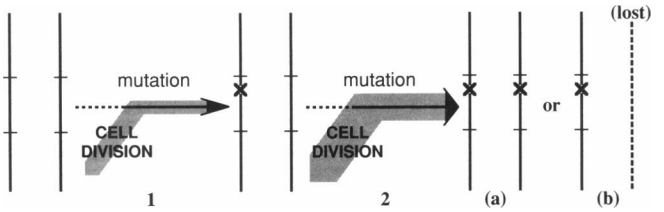

FIGURE 1. Mitogenesis (induced cell division) is a major multiplier of endogenous (or exogenous) DNA damage leading to mutation. The pathway to inactivating (x) both copies of a recessive tumor-suppressor gene is shown (two vertical lines represent the pair of chromosomes carrying the genes). Cell division increases mutagenesis due to DNA adducts converted to mutations before they are repaired (1 and 2*a*); mutations due to DNA replication (1 and 2*a*); replicating DNA is more vulnerable to damage (1 and 2*a*). Mitotic recombination (2*a*), gene conversion (2*a*), and nondisjunction (2*b*) are more frequent, and the first two give rise to the same mutations on both chromosomes. This diagram does not attempt to deal with the complex mutational pathway to tumors (*164,165*).

peroxidation. Oxidants that escape the numerous antioxidant defenses can damage cellular macromolecules, including DNA, and such damage can lead to mutations and cancer.

We have argued that this oxidative DNA damage is a major contributor to aging and the degenerative diseases associated with aging (*4,9*). Because of the high background of endogenous DNA lesions, any agent causing chronic mitogenesis can be indirectly mutagenic, and consequently carcinogenic, by increasing the probability of these endogenous DNA lesions being converted to mutations (Fig. 1). Furthermore, endogenous rates of DNA damage are so high that at low doses that do not increase mitogenesis, it may be difficult for exogenous mutagens to make a significant increment in the total DNA damage. Dietary micronutrients, e.g., antioxidants, are necessary for cellular defense systems; deficiency of micronutrients can alter the balance between damage and defense and thus can increase DNA lesion rates. Oxidants are also important in mitogenesis (wound healing) and progression (see below).

Mitogenesis Increases Mutagenesis

When the cell divides, an unrepaired DNA lesion has a certain probability of giving rise to a mutation. Thus, an important factor in the mutagenic effect of an exogenous agent, whether it is genotoxic or nongenotoxic, is the increment it causes over the background cell division rate (mitogenesis) (*10*). There is little cancer from nondividing cells. The time interval for DNA repair during cell division is short, and lesions can be converted to point mutations or gaps during replication. In support of higher "spontaneous" mutation rates in dividing cells is the observation that background hypoxanthine phosphoribosyltransferase mutations that arise *in vivo* in human T-lymphocytes arise preferentially in dividing T-cells (*11–14*).

During cell division, single-stranded DNA is without base-pairing, nucleosomes, or histones and is thus more sensitive to damage than double-stranded DNA. Cell division triggers mitotic recombination, gene conversion, and nondisjunction, which together seem more effective than an independent second mutation (*15–19*) in converting a heterozygous recessive gene (e.g., p53, a tumor-suppressor gene) to homo- or hemizygosity. Thus, the second mutational step toward cancer is more dependent on mitogenesis than the first. Heterozygotes at the human HLA-A gene are spontaneously converted to homozygotes during cell division (*20*). The above mechanisms could account for gross chromosomal alterations that occur frequently in human tumors (*21–27*). Cell division allows gene duplication, which can increase expression of oncogenes that are otherwise weakly expressed (*28*).

Cell division (i.e., through mitotic recombination) and DNA damage (*29*) would be expected to increase the rate of loss of 5-methylcytosine. Epigenetic changes in DNA, such as 5-methylcytosine levels, appear to be important in turning off genes in differentiation and could play a role in both cancer (*30–34*) and aging (*31,35*). It has been observed that the 5-methylcytosine level decreases with age (*36*), and it is known that cells dedifferentiate with age (*37,38*).

Those cells that appear to be most important for cancer are the stem cells, which are not on their way to being discarded. Increasing the division rate of stem cells increases mutation and therefore cancer. In dividing cells such as in epithelial tissues, one important unknown factor is to what extent there is a queue of reserve stem cells to replace stem cells that are programmed to die (apoptosis) after a certain number of divisions. A recent study on transformation of ovarian cells examined various factors and emphasized the role of cell division (*39*). Thus, understanding cell division rates, apoptosis, cell lineages, and differentiation are all relevant to mutagenesis and carcinogenesis (*40*). Endogenous cell division rates can be influenced by hormone levels, decreased by calorie restriction, or increased by high doses of chemicals. If the rates of both DNA lesions and cell division are increased, there will be a multiplicative effect on mutagenesis (and carcinogenesis), for example, by high doses of a mutagen that also increase mitogenesis through cell killing.

Mitogenesis and Carcinogenesis

Epidemiological studies, a large body of experimental evidence, and theoretical work on the mechanisms of carcinogenesis point to mitogenesis as a major contributor to cancer.

Suppression of Intercellular Communication Causes Mitogenesis. At near-toxic doses, some chemicals interfere with cell-cell communication in quiescent tissues (e.g., the liver, the major target site for carcinogenesis in rodents), thereby causing mito-

genesis and carcinogenesis (*41–43*). Trosko and his associates (*41,42*) have shown that suppression of gap junctional intercellular communication, which is associated with an increase in Ha-*ras* expression in contact-inhibited cells, leads to mitogenesis (*44*). It is of great interest to identify chemicals causing mitogenesis at doses much below the maximum tolerated dose [e.g., certain peroxisome proliferators (*45*)].

Mitogenesis from Exogenous and Endogenous Factors. Chronic toxicity can cause injury to tissues, resulting in replacement cell division (*46–50*). In an experimental cancer model, the surgical removal of part of the liver causes neighboring cells to proliferate (*46–48*). The incidence of liver cancer is low in humans (but not in some strains of mice) unless the liver is chronically damaged. Viruses or alcohol excess, for example, cause damage to the liver, which is a risk factor for cancer. Salt excess is a risk factor in human stomach cancer because it causes mitogenesis (*51–58*). Chronic toxicity can also cause an inflammatory reaction because phagocytic cells unleash a barrage of oxidants in destroying dead cells at a wound. The oxidants produced are in part the same as those produced by ionizing radiation; therefore, chronic inflammation may be equivalent to irradiating the tissue (*59*). Nitrogen oxides from inflammation are also mutagens. The oxidants produced as a result of inflammation can stimulate proto-oncogenes and cell division (*60–63*). Chronic irritation and inflammation cause cancer in animals (*64*). Chronic inflammation is, as expected, a risk factor for human cancer (*65–67*); the carcinogenic effect of asbestos (*68*) and cigarette smoke, for example, may be due primarily to inflammation, which increases both mitogenesis and mutagenesis.

Chronic infection from viruses, bacteria, schistosomes, and other organisms that cause cell killing and consequent mitogenesis can be risk factors for cancer. Two examples are the human virus hepatitis B, a major cause of liver cancer in the world (*69,70*), and human papilloma virus 16, a risk factor for cervical cancer and one of whose major effects is to increase mitogenesis (*71*). In transgenic mice, overproduction of one protein of the hepatitis B virus, a surface antigen, results in cell turnover that causes all of the mice to develop hepatocellular carcinomas (*72*). Human T-cell lymphotropic virus type 1 causes constitutive expression of the T-cell interleukin-2 receptor. This may commit the cell to unremitting *in vivo* cell division with an increased likelihood for the occurrence of critical mutations leading to T-cell leukemia/lymphoma (*73,74*). Chronic *Helicobacter (Campylobacter)* infection is a risk factor for stomach cancer (*75,78*). Chronic schistosome infection is a risk factor for bladder and colorectal cancer (*79*). Dietary antioxidants would be expected to decrease both the mutagenic and proliferative effects of oxidants and therefore to lower tumor incidence.

Hormones can also cause mitogenesis, and hormone imbalances are major risk factors for a number of human cancers [e.g., breast cancer (*80,82*)]. Thus, agents causing chronic mitogenesis can be proper carcinogens and are important in human cancer (*80,82*).

A calorie-restricted diet, compared to an *ad libitum* diet, significantly increases the life span of rats and mice and markedly decreases the cancer rate. It is striking that in calorie-restricted animals, mitogenesis rates are markedly lowered in a variety of tissues (*83,84*): this could in principle account for much of the decrease in the cancer rate (see below).

Thinking of chemicals as "initiators" or "promoters" confuses mechanistic issues of carcinogenesis (*85*). The idea that promoters are not in themselves carcinogens is not credible on mechanistic grounds and is not correct on experimental grounds (*85–87*). Every classical promoter that has been tested in a high-dose cancer test is a carcinogen (e.g., phenobarbital, catechol, TPA (*88,89*). A promoter has been viewed as an agent that facilitates the development of tumors by selected growth of an initiated cell; however, a promoter can be viewed more accurately as inducing cell division and thus stimulating the rate of accumulation of key mutations that are needed to acquire a transformed phenotype. Thus, the very word promoter is confusing because mitogenesis is caused by one dose of a chemical and not by a lower dose. Nongenotoxic agents such as saccharin can be carcinogens at high doses just by causing cell killing and inducing chronic mitogenesis and inflammation, and the dose response would be expected to show a threshold (*86,90–92*).

Chronic mitogenesis alone can be a risk factor for cancer: theory predicts this and a large literature supports it (*79,86*). Work on radiation has also supported the idea that both mutagenesis and mitogenesis are important in tumor induction (*93–96*).

DNA Repair and Other Inducible Defenses

A variety of DNA repair defense systems, almost all inducible, protect the cell against any increment in DNA lesions by both exogenous and endogenous mutagens (*97*). The defense systems against reactive electrophilic mutagens, such as the glutathione transferases, are also almost all inducible and buffer cells against increases in active forms of chemicals that can cause DNA lesions (*98,99*). Therefore, the effect of a particular chemical insult is dependent on the level of each defense, which in turn is dependent on the past history of exposure. Thus, a small dose of a mutagen can protect against a subsequent challenge from a large dose, as has been shown for radiation (*100*) and a variety of other mutagens.

Different adducts are repaired with different effectiveness. Oxidative lesions, being so common in all aerobic creatures, may be particularly well repaired. However, even though radiation is an oxidative mutagen, it still can add to the background of DNA lesions to give increased mutation and cancer. Exogenous agents can influence the induction and effectiveness of

these defenses. Defenses can be partially disabled by lack of particular micronutrients in the diet [e.g., antioxidants (*101*)].

Mitogenesis and Animal Cancer Tests

Animal cancer tests are conducted at the maximum tolerated dose (MTD) and 1/2 the MTD of the test chemical for long periods of time—both high doses that can cause chronic mitogenesis (*86,90,102*). Future experimental work measuring mitogenesis will show how often chronic dosing at the MTD is like chronic wounding, which is known to increase tumor yields in rodent tests and to be a risk factor for cancer in humans (*103*). Our theoretical arguments (*87,104,105*) for taking mitogenesis into account in animal cancer tests help to explain many of the results that have been found in analyzing these tests, as discussed below.

To the extent that increases in tumor incidence in rodent studies are due to the secondary effects of administering high doses, then any chemical that increases mitogenesis (e.g., by chronic cell killing and cell replacement or by suppression of intercellular communication) may be a rodent carcinogen; thus, one would expect that a high proportion of chemicals tested at the MTD would be positive, and this is indeed what is found. In our large Carcinogenic Potency Database (CPDB), approximately half of the chemicals are positive in at least one test, and this proportion is similar for a variety of subsets of the CPDB (*106–110*). It is unlikely that the high proportion of carcinogens in rodent studies is due simply to selection of suspicious chemical structures because most chemicals were selected because of their use as industrial compounds, pesticides, drugs, or food additives. Moreover, historically, our knowledge to predict carcinogenicity has been inadequate (*104*).

Because mitogenesis indirectly increases mutation, one would expect that in animal tests at the MTD, nongenotoxic carcinogens are likely to be acting by this mechanism (*86*). Indeed, among chemicals in the CPDB that have been tested adequately in rats and mice, we find that half of the nonmutagens in *Salmonella* are carcinogens, and approximately 45% of the carcinogens are not mutagenic (*110*).

Mitogenesis at the MTD is an important, and possibly the dominant, factor in carcinogenesis for mutagens. Mutagenic chemicals, because they directly damage DNA, are generally more effective at killing cells at high doses (with consequent cell replacement) than nonmutagens and thus are more effective at causing mitogenesis. Mutagens, unlike nonmutagens, can damage DNA as well as increase mitogenesis at high doses, giving a multiplicative interaction for carcinogenesis. This would lead one to expect three results that are, in fact, found in analyses of animal cancer tests: Mutagens, compared to nonmutagens, are *a*) more likely to be carcinogenic (*104,111,112*), *b*) more likely to be positive in both rats and mice (*104,111,112*), and *c*) more likely to induce tumors in multiple organs (*112*).

One would not expect the mutagenicity of a chemical in *Salmonella* to always indicate the mechanism in a rodent. In the CPDB, of 384 chemicals tested for both carcinogenicity in rats and mice and mutagenicity in *Salmonella*, 26% of the noncarcinogens are mutagens in *Salmonella*; these presumably are not acting as significant mutagens in the rodents (*110*). Additionally, some nonmutagens in *Salmonella* may be indirectly mutagenic in higher organisms, for example, peroxisome proliferators. Even those mutagens that are carcinogens may not all be acting as genotoxins in animals because of detoxification and other processes. The importance of mitogenesis, even for mutagens, has been shown in experiments with pairs of mutagenic isomers (1 versus 2-nitropropane and 2,4, versus 2,6-diaminotoluene). In each pair only one chemical was a carcinogen, and only the carcinogen was mitogenic (*113,114*).

Several recent analyses of dose response in animal tests are consistent with the idea that mitogenesis from cell killing and cell replacement at the high doses tested is important. In the usual experimental design of dosing at the MTD and 1/2 MTD, both dose levels are high and may result in mitogenesis. Even at these two high doses, we have found that 44% of the positive sites in National Toxicology Program bioassays are statistically significant at the MTD but not at 1/2 MTD (among 365 positive sites). Moreover, the proportion positive only at the high dose is similar for mutagens and nonmutagens (Gold et al., unpublished data). Another analysis of the shape of dose response curves indicates that a quadratic dose response is compatible with more of the data than a linear one for both mutagens and nonmutagens (115). That mitogenesis at near-toxic doses is important in the carcinogenic response at the MTD is also suggested by the lack of chemicals that are highly carcinogenic relative to their MTD. Such chemicals would be expected to produce very high tumor incidence rates at the MTD; however, in animal cancer tests at the MTD it is uncommon to find 100% of the animals developing tumors (*102*). Moreover, if chemicals were highly carcinogenic relative to their MTD, then the dose-response curve might plateau well below the MTD. However, we found that only 10% of the dose-response functions indicate a possible plateau (a leveling off of the dose response). Moreover, even for these 10% there was a lack of consistency: for the compounds in which an apparent plateau was observed in one site, the result was generally not replicated in other target sites in the same experiment, in other sex of the same species, or in other species (*102*).

In this paper, we have mainly discussed theoretical issues. The pioneering work on this subject by experimentalists is discussed in detail in other pages in this issue.

It is clear that the mechanisms of action for all rodent carcinogens are not the same. For some chemicals there is evidence to support mitogenesis effects

unique to high doses, for example, formaldehyde, melamine, and saccharin. For others (e.g., butadiene), carcinogenic effects have been found considerably below the MTD. Further studies of mechanism in rodent bioassays should help to clarify such differences. Adding routine measurements of mitogenesis to the 13-week toxicology study and the 2-year bioassay would provide information that would improve dose setting, interpretation of experimental results, and risk assessment.

As currently conducted, standard rodent bioassays do not provide sufficient information to assess carcinogenic risk to humans at doses thousands of times below the MTD. If mitogenesis is a dominant factor in carcinogenesis at the MTD, then at low doses where mitogenesis is not generally induced, the hazards to humans of rodent carcinogens may be much lower than commonly assumed. Defenses are inducible at low doses, and even for mutagens the increment in DNA damage over the enormous rate of endogenous background damage may not be significant.

Toxicological examination of synthetic chemicals such as pesticides and industrial pollutants, without similar examination of chemicals that occur naturally, has resulted in an imbalance in both data and perception about chemical carcinogens. About 80% of the chemicals tested in both rats and mice are synthetic. Yet, there is an enormous background of natural chemicals in the diet such as plant pesticides and the products of cooking that have not been a focus of carcinogenicity testing (*105*). Regulatory policy to prevent human cancer has primarily addressed synthetic chemicals, yet similar proportions of natural chemicals and synthetic chemicals test positive in rodent studies, as expected from an understanding of toxicological defenses (*116*). The vast proportion of human exposures are to natural chemicals [e.g., 99.99% by weight of pesticides humans ingest are natural (105)]. Thus, rodent carcinogens that humans are exposed to are ubiquitous (*105,117*). Natural chemicals are the experimental control for evaluating cancer-regulatory strategies for synthetic pollutants. Possible hazards from residues of synthetic chemicals should be routinely compared to the possible hazards from natural chemicals. We have found that when the same index is used for natural and synthetic chemicals, possible carcinogenic hazards from current levels of pesticide residues or water pollution are likely to be of minimal concern relative to the background levels of natural substances (*117,118*). Extrapolation to low dose human exposure from results of animal cancer tests done at high dose should be based on knowledge about mechanisms of carcinogenesis for each chemical, particularly mitogenic effects that are present at high but not at low doses.

Progression and Clonal Instability

One of the hallmarks of latter stages of cancer is a genetic instability that leads through a succession of stages ("progression") to a metastasizing, aggressive tumor (*119*). Numerous authors have discussed progression in terms of a possible clonal instability (*119–121*). Because there is a balance between a high endogenous oxidant production and extensive oxidative defenses, a plausible way to get a clone with a high mutation rate is to upset this balance. Because oxidants are both mitogens [the wound healing response (*122–124*)] and mutagens, they furnish the selective growth and instability needed for progression to a malignant tumor. Recent work supports this idea: tumor cell lines from various organs produce excess H_2O_2 (*125*); breast tumors show high levels of oxidative DNA damage (*126*), and a number of tumors have altered antioxidant defenses (*127–133*). There is a large literature on antioxidants as anticarcinogens (*134,135*). Antioxidants have been shown to be effective in suppressing all stages of carcinogenesis (*135,136*).

Factors Decreasing Mitogenesis, Mutagenesis, Carcinogenesis

Dietary Imbalances and Degenerative Diseases. The high endogenous level of oxidative DNA lesions reinforces evidence from epidemiology that deficiency of antioxidants (*137–139*) is likely to be an important risk factor for cancer. Epidemiologists have been accumulating evidence that unbalanced diets are major contributors to heart disease and cancer and are likely to be as important as smoking. It has been estimated that approximately 30% of all cancers are related to diet (*140*). The main culprit appears to be a dietary imbalance of too few fruits and vegetables and too much fat (*137–139*). Particular micronutrients in fruits and vegetables that appear to be important in disease prevention are antioxidants (carotenoids, tocopherols, ascorbate) and folic acid, but many more vitamins and essential minerals may also be of interest (*137–139, 141*). Micronutrients are components of defenses against oxidants and other endogenous mutagens that contribute to the degenerative diseases associated with aging, such as cancer, heart disease, and cataracts. Because endogenous oxidative DNA damage is enormous, there are good theoretical reasons for thinking that antioxidants should be as important as they are being found to be. Surveys have indicated that 91% of the U.S. population is not eating sufficient fruits and vegetables; for example, almost half the population had eaten neither fruits nor vegetables on the day of the survey (*142*).

A study of sperm DNA (*101*) indicates that levels of oxidative DNA damage are inversely correlated with ascorbate concentrations in seminal fluid which, in turn, are related to the amount of dietary ascorbate consumed. In 10 individuals whose dietary intake of ascorbate was started at 250 mg/day for 2 weeks and then reduced to 10 or 20 mg/day, the level of ascorbate in seminal fluid fell to one-fourth, and the levels of oxidative lesions in sperm DNA were increased 2.5

times; repletion of dietary ascorbate led to a decrease in lesion levels closer to those before ascorbate deprivation. In a separate group consisting of 24 free-living subjects, the steady-state levels of lesions were also found to correlate inversely with content of ascorbate in seminal plasma. These results demonstrate the protective effect of dietary ascorbate against oxidative damage to human sperm DNA. This protective effect may be especially relevant to about one third of the male population who have low ascorbate levels because of poor diets or smoking (*139,143,144*). Their progeny may be at a higher risk for birth defects and childhood cancer. It seems likely that the men's somatic cells are being mutated as well as their sperm.

Work showing that folate deficiency in mice results in chromosome breakage (*145*) reinforces studies indicating that folate deficiency is an important cause of chromosome breaks, cancer, and birth defects (*137,138*). Again, a sizeable proportion of the population (30% or more) may not be ingesting sufficient folate. Hypomethylation of DNA, which is associated with folate deficiency, might also contribute to epigenetic effects, such as dedifferentiation of cells, which occurs during tumorigenesis (*146,147*).

In the quest to delay aging and prevent cancer and heart disease, it is important to understand what level of each micronutrient is optimal for long-term effects. The RDA (U.S. Recommended Daily Allowance) for micronutrients is based on the level necessary to prevent an immediate pathological effect, but the optimal levels that maybe needed to suppress tumor formation are likely to be much higher. The great genetic variability of the human species makes it likely that the optimal RDA for particular micronutrients will be higher than average for many people. Techniques for noninvasive measurement of DNA damage in humans are clearly relevant (*148,149*).

Calorie Restriction Lowers Mitogenesis Rates. In rodents, a high-calorie diet appears to be carcinogenic (*150–152*). A calorie-restricted diet, compared to an *ad libitum* diet, significantly increases the life span of rats and mice and dramatically decreases the cancer rate. Though a causative mechanism to account for the beneficial effects of calorie restriction has not been established, several physiological indexes are altered in rodents that are fed restricted diets. Calorie restriction activates the pituitary adrenocorticotropic axis, resulting in a decrease in the release of reproductive and mitogenic hormones. It has been suggested that Darwinian fitness will be increased if reproductive function is delayed during periods of low food availability (*153*) and that the saved resources will be invested in maintaining the body until food resources are available for successful reproduction (*154*). If one accepts the concept of a trade-off between reproduction and maintenance as predicted by evolutionary biology (*155*), it becomes evident why calorie restriction is so effective in reducing cancer. Decreases in mitogenic hormones such as insulin, thyroid-stimulating hormone, growth hormone, estrogen, and prolactin decrease the likelihood of hormone-induced cancers, as has been shown in various animal studies (*156*). The markedly lowered mitogenesis rates observed in a variety of tissues of calorie-restricted relative to *ad libitum* rodents (*83,84*) is consistent with suppression of mitogenic hormones (*157*) and decreased protooncogene expression (*158*). The lowered incidence of mammary tumors observed in calorie-restricted rats has been attributed to reduced circulating levels of the mammotropic hormones estrogen and prolactin (*159*). Thus, the decrease in mitogenesis rates in calorie-restricted rats is likely to account for much of the decrease in tumor incidence.

The suggestion that maintenance functions are enhanced in calorie-restricted rats is supported by the findings of more efficient DNA repair (*160*), better coupled mitochondrial respiration (*161*), and a delay in the age-dependent decline of antioxidant defenses (*162*). The overall effect of these enhanced maintenance activities would be a reduction in oxidative damage to cellular macromolecules, and a decrease in somatic mutations that are induced by endogenous oxidative mutagens. The higher levels of antioxidant defenses in calorie-restricted rodents could account for an enhanced immune response (*163*) that sometimes suppresses tumor formation.

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