An Open Letter to Cancer Researchers

OVER THE PAST TWO YEARS, PANEL DISCUSSIONS have been held to propose recommendations to the National Cancer Institute (NCI) regarding critical initiatives in cancer biology. One outcome was a proposal for a new initiative, the Human Cancer Genome Project (HCGP) (*1*). This program involves systematically analyzing genomic alterations in large numbers of human tumors to determine both common genetic and epigenetic alterations and to identify changes that characterize different tumor subtypes. The price tag would be $1,500,000,000 over 10 years, the equivalent of 1000 R01 grants.

We have three questions concerning the project: (i) Would the project, as proposed, achieve its goals? (ii) Would it impact ongoing or future funding for investigator-initiated cancer research? (iii) Is this the best application of funds toward the objective of hastening the discovery of cures for cancer?

A major goal of the HCGP is the identification of new cancer genes through genome resequencing, specifically to find mutations that occur with 5% or greater frequency across a broad range of human tumors. The implication is that such mutations would lead to new therapeutic targets. In support of this strategy, much has been said about the recent identification of activating mutations in the EGF receptor, EGFR, in lung cancer and the ability of patients with such mutations to respond dramatically to certain kinase inhibitors, such as Iressa. Mutations in EGFR exist in approximately 7% of small cell lung cancer patients, and over 50% of patients carrying mutations are responsive to Iressa, but as yet there is no increase in survival in this population. This partial success is being used as an example of the information that will emerge from the HCGP, implying that identification of many new mutations would lead to rapid cures. However, the vast majority of mutations identified by sequencing tumors would be loss-of-function mutations, not gain-of-function mutations as in EGFR, and thus would not be candidates for drug inhibition. Additionally, much of the excitement surrounding the identification of EGFR mutations was due to their ability to predict responses of lung cancers to a pre-existing drug, a situation unlikely to exist for mutations in new genes.

A pilot project that provides clues to the potential information yield from the sequencing component of the HCGP was recently published by Stephens *et al.* (*2*). Out of 72 breast tumors and 9 cell lines, only six kinases out of 518 sequenced had two mutations resulting in amino acid changes. Thus, mutations identified in the kinases fall below the 5% cut-off level for significance proposed for the HCGP, an already low bar. Similar results were obtained for lung and testicular cancer (*3*, *4*). These studies addressed a family of key signaling molecules. As such, alterations in these proteins might be expected to affect cell physiology in a sufficiently broad sense to contribute to tumor development. They are also among the most easily "drugable" proteins and are a family about which we have extensive knowledge. If we cannot find interpretable information here, what gene families are likely to yield such information? These results call into question whether a massive sequencing effort, estimated to be 75% of the entire $1.5-billion price tag, is going to produce a harvest of useful information that matches its huge budget.

A key stated component of the HCGP is that the money to fund this initiative would not be taken from the funds currently available for ongoing cancer research. We are skeptical of the assertion that such "new" money would not impact current or future funding for investigator-initiated cancer research. Although it is possible that NCI would be able to persuade Congress to allocate this money, in the current fiscal climate, the HCGP would likely be weighed against future allocations, as NIH funding is likely to be a zero sum game for the foreseeable future. In view of current budget constraints, it seems responsible to plan a more conservative effort that balances the cost and potential yield of current sequencing technologies against future advances that promise to greatly reduce sequencing costs.

The unstated goal of the HCGP is to accelerate the discovery of cures for cancers. The question we need to answer is not whether the information generated will be useful, but whether, if given $1.5 billion in "new" cancer money, would the HCGP be the best application of that money toward the goal of cancer cures. Some elements of the proposed HCGP represent sensible and cost-effective steps toward the goal of managing cancer. Identification of regions amplified in tumors can be achieved at a fraction of the cost of sequencing. Such efforts yield not only potential drug targets but also diagnostic information. However, there are also important approaches that are completely missing. One is a systematic exploration of the genetic alterations that could kill cancer cells, a Genetic Cancer Genome Project. With RNAi, we can now systematically identify genes that interfere with the growth and survival of tumor cells—both in cell culture and in animal models. These functional screens seem a highly plausible method to identify potential anticancer drug targets and a more direct approach than those contemplated within the current framework of the HCGP. A second and more immediate way to enhance cancer therapy in the short term is the development of biomarkers for early detection.

As cancer researchers, we have a special responsibility with respect to guiding resource allocation to fight cancer. We need to be able to look cancer patients and their families in the eye and say, "We are spending your money in the best way we know to find a cure for you." We must apply this standard in judging any large-scale proposal for dedicated research funding allocations. As currently configured, the HCGP needs to be reconsidered and reprioritized to produce a program that gives us the best chance for

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> –ELLEDGE AND HANNON

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fighting this disease. Therefore, because the most productive direction of research is still a debatable question, we propose that (i) sequencing be delayed until advances in sequencing technology are achieved; (ii) objective criteria be established to allow a go/no go decision for continued DNA sequencing based on pilot studies; and (iii) large-scale genetic screening to identify targets whose inhibition kills cancer cells should be incorporated into the HCGP.

STEPHEN J. ELLEDGE[1,3] AND GREGORY J. HANNON[2,3]
[1]Department of Genetics, Center for Genetics and Genomics, Harvard Medical School, Boston, MA 02115, USA. [2]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA. [3]Howard Hughes Medical Institute.

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1. A portion of this overall report has now been presented publicly at the recent symposium "Molecular Approaches to Controlling Cancer" at Cold Spring Harbor in the format of a panel discussion to solicit input from the broader community of cancer biologists.
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Evaluating Evidence for Aging

IN THEIR REPORT "MITOCHONDRIAL DNA mutations, oxidative stress, and apoptosis in mammalian aging" (15 July, p. 481), G. C. Kujoth *et al.* present data showing that mice with a mutant form of mitochondrial DNA polymerase accumulate mitochondrial mutations, die at a very early age, and exhibit multiple forms of pathology that the authors interpret as "accelerated aging." The evidence for this last claim, however, needs to be evaluated more critically. The mutant mice show a dramatic anemia, with erythrocyte counts falling by more then 50% by 10 months of age. In normal aging mice, however, red blood cell counts fall only by about 10% (*1*), unless the mice become ill. The mutant mice show a dramatic loss of intestinal crypts, but in normal mice, crypt numbers of very old mice remain at levels of 80 to 90% of those seen in young mice (*2*). The mutants show hearing loss, but apparently without the loss in cochlear hair cells that underlies late-life hearing deficits in normal mice. The animals show many phenotypes, such as grey hair, spontaneous alopecia, kyphosis, and weight loss, that are uncommon in healthy aged mice, although the latter two are commonly seen in chronically ill mice of any age.

There are two ways to try to show that a mutant exhibits accelerated aging. The primrose path, selected by nearly all enthusiasts of "accelerated aging" models, is to list symptoms seen in a mutant, note that some of these are seen in normal aged mice [or aged humans; see (*3*)], and declare the case closed. The thornier approach, which is more convincing but seldom attempted, is to start with a set of traits shown by authentic aging mice and then determine how many of these are seen in the mutant.

Kujoth *et al.* have developed an exciting system for the analysis of how mitochondrial mutations can affect erythropoiesis, gastrointestinal homeostasis, and muscle function. Whether the rate of aging depends critically on mitochondrial mutations is still very much an open question.

RICHARD A. MILLER
Department of Pathology, University of Michigan and Ann Arbor VAMC, Geriatrics Center, 1500 East Medical Center Drive, Ann Arbor, MI 48109–0940, USA.

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THE REPORT "MITOCHONDRIAL DNA mutations, oxidative stress, and apoptosis in mammalian aging" by G. C. Kujoth *et al.* (15 July, p. 481) has flawed reasoning and conclusions. Basing conclusions regarding life-span–determining factors on a model of shortening the life-span of organisms rather than extending it is misleading. The authors achieved a technical feat in introducing genetic instability into mitochondria by reducing proofreading of the mitochondrial genome. By their own calculations, this uncontrolled, introduced mutability resulted in 4 to 10 mitochondrial mutations per mitochondrial genome. Considering that the mitochondrial genome contains only 37 genes, all essential, such a rate of mutation is highly detrimental. Much lower levels of mitochondrial mutations occur in cells of "normal" aging mice [e.g., (*1*)]. This catastrophic mutation rate can be discerned from the precipitous mortality of the engineered mice (see fig. 1C), which is rarely observed in wild-type mice. Also, the homozygous engineered mice are likely infertile because of severe problems in their germ cells caused by faulty mitochondria. It is a false premise that if a certain genotype containing detrimental alleles mimics some features found in aging organisms, such as hair graying, that it is a good model for studying biological aging.

DAVID GERSHON
Professor emeritus, Technion-Israel Institute of Technology, and Redox Pharmaceutical Corp., 3960 Broadway, New York, NY 10032, USA. E-mail: dgershon@redoxpharm.com

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Response

MILLER DISPUTES OUR CONCLUSION THAT aging phenotypes in mice carrying a mutation in the exonuclease domain of POLG (D257A mice) are relevant to normal aging and also questions the validity of animal models of accelerated aging. Because multiple biological processes are likely to contribute to aging in complex organisms, interventions that significantly extend maximum life-span in mammals are likely to be rare. Large (over 50%) increases in life span in mice are only observed with caloric restriction or dwarfism (*1*), both of which result in overt metabolic and hormonal alterations and multiple secondary effects. In contrast, interventions that result in accelerated aging phenotypes have provided information on how the alteration of a given pathway or individual gene impacts aging (*2*, *3*). The accumulation of mitochondrial DNA (mtDNA) mutations is a hallmark of aging in multiple species, and we have clearly demonstrated that such mutations can lead to aging phenotypes. Many of these, such as hearing loss, graying, bone loss, and sarcopenia, are associated with aging in multiple species. Miller's argument is based on a misconception: The severity of phenotypes in D257A mice need not be present in aging of normal mice for this animal model to be relevant to our understanding of aging mechanisms. The severe phenotypes associated with mtDNA mutations in D257A mice can be explained by stem cell depletion through increased apoptosis, which is unlikely to occur to a similar extent in normal aging. However, progressive accumulation of mtDNA mutations is likely to lead to physiological impairments and a decline in tissue regenerative capacity. We believe that interventions that result in either accelerated aging phenotypes or extend life-span have contributed to our understanding of general aging mechanisms. This is clearly demonstrated by the analysis of the mouse klotho gene, first identified as resulting in accelerated aging when mutated and recently shown to extend survival when overexpressed (*4*).

Gershon questions the relevance of high mtDNA mutation rates observed in D257A mice to normal aging, but misrepresents our findings in the process. We observed high levels of mtDNA mutations in both wild-type and D257A mice by 5 months of age. Specifically, the quoted estimation of 4 to 10 mtDNA mutations/mtDNA was the number observed for wild-type mice. D257A mice show mutation frequency increases above this baseline on the order of three- to eight-fold, depending on the tissue under study. Thus, mtDNA mutations are surprisingly high in wild-type, relatively young animals.

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Further age-related accumulation of mutations is likely to contribute to age-related declines in physiological function. Several features of aging in D257A mice, such as sarcopenia, bone loss, and hearing loss, are commonly observed in aging. More severe phenotypes, such as anemia and severe loss of intestinal crypts, are likely to be secondary to complete stem cell depletion, which is not observed in normal aging.

TOMAS A. PROLLA[1] AND R. H. WEINDRUCH[2]
[1]Department of Genetics and Medical Genetics, University of Wisconsin–Madison, 445 Henry Mall, Madison, WI 53706, USA. [2]Department of Medicine and Veterans Administration Hospital, University of Wisconsin–Madison, Madison, WI 53705–2286, USA.

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Tracing Contaminants with $\delta^{15}N$ Measurements

IN THEIR BREVIA "ARCTIC SEABIRDS TRANSport marine-derived contaminants" (15 July, p. 445), J. M. Blais *et al.* showed that contaminant concentrations increased exponentially with stable isotope ratio of nitrogen ($\delta^{15}N$) values in sediments of high Arctic pools associated with guano input from a seabird (fulmar) colony. However, this nice result may mask complexities associated with the use of $\delta^{15}N$ as a proxy for trophic level and as a direct tracer in contaminant studies. First, the $\delta^{15}N$ value of 20 per mil for fulmar guano far exceeds that expected from fulmar diet, tissue, and isotopic mass balance considerations (*1*). In fact, these values approximate those expected for polar bears from the same area (*2*). Rather, such elevated $\delta^{15}N$ values in guano derive, in part, from ammonia volatilization, as noted previously for soils in several Antarctic seabird rookeries, a factor fairly independent of the trophic level of the bird species involved (*3*–*5*). In addition, $\delta^{15}N$ values in foodwebs reflect not only baseline nutrient values, but also nutrient concentration and rate of growth of primary production. Plotting $\delta^{15}N$ values versus chlorophyll a, total phophorus, and dissolved organic carbon from the authors' data shows threshold responses involving high sensitivity of sediment $\delta^{15}N$ values at low nutrient concentrations and a plateau at higher concentrations. Future studies using stable isotopes to track ornithogenic origins of contaminants should consider nonlinear effects of nutrient concentrations and variable effects of ammonification on foodweb $\delta^{15}N$ values using multiple stable isotopes (*6*).

KEITH A. HOBSON
Canadian Wildlife Service, 115 Perimeter Road, Saskatoon, SK S7N 0X4, Canada.

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Response

WE THANK HOBSON FOR RAISING VALID points about the use of stable isotopes in contaminant studies, and we fully acknowledge these concerns. Stable isotopic composition of nitrogen can be altered by many external factors and thus is not a flawless tracer for nitrogen sources. For example, ammonification and denitrification are well known to increase $\delta^{15}N$ in dissolved inorganic nitrogen species and in dissolved and particulate organic nitrogen in water and sediment [e.g., (*1*), footnote number 14]. However, as we indicated in our study (see Fig. 1 caption), if we expressed our contaminant data relative to other indicators of guano input (e.g., total phophorus, total nitrogen, dissolved organic carbon and cadmium), we would see similar relationships with contaminant concentrations in sediments, as we showed with the isotope data. The purpose of these chemical tracers was to link contaminants to the guano produced from the seabirds, which could have been accomplished with any of the tracers mentioned above. Furthermore, we could have shown the same patterns simply using field observations. A simple ranking of bird influence from 1 to 11 for the 11 study ponds would have matched the same ranking we described with the isotope data (or the same general ranking we would achieve using our other proxies of bird influence). The fact that all tracers produced a similar pattern is further evidence that the source was adequately identified by the isotope data in our study.

Hobson is mistaken in saying that the contaminant concentrations increased exponentially with $\delta^{15}N$ values in all cases for the sediments of our study ponds. This interpretation is perhaps prompted by our logarithmic axis in Fig. 1. The distribution of these data was log-normal, so a transformation was considered necessary for regression analysis. There is also the implication that we used $\delta^{15}N$ measurements in sediments to directly infer trophic position. That was not our intention; nitrogen isotopes were used as one of several possible proxies to infer bird influence. We believed that the isotope data on our figure would be more easily interpreted by a general readership. Finally, we agree with Hobson's last point that, ideally, multiple stable isotopes should be used, if possible, in future food web studies.

JULES M. BLAIS,[1]* LYNDA E. KIMPE,[1] DOMINIQUE MCMAHON,[1] BRONWYN E. KEATLEY,[2] MARK L. MALLORY,[3] MARIANNE S. V. DOUGLAS,[4] JOHN P. SMOL[2]
[1]Centre for Advanced Research in Environmental Genomics, Department of Biology, University of Ottawa, Ottawa, ON K1N 6N5, Canada. [2]Paleoecological Environmental Assessment and Research Laboratory, Department of Biology, Queen's University, Kingston, ON K7L 3N6, Canada. [3]Canadian Wildlife Service, Environment Canada, Iqaluit, NU X0A 0H0, Canada. [4]Department of Geology, University of Toronto, Toronto, ON M5S 3B1, Canada.
*To whom correspondence should be addressed. E-mail: jblais@science.uottawa.ca

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Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 6 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

CORRECTIONS AND CLARIFICATIONS

2005 Visualization Challenge: "Noninteractive media" by C. Gramling (23 Sept., p. 1992). The print version of this article contained the following errors: The winning entry, "Return of the 17-Year Cicadas," was credited in the subtitle to Roger Hangarter; it should have been credited to Roger Hangarter and Samuel Orr. In the subtitle for the honorable mention "Rip Currents: Nearshore Fundamentals," the name of credited contributor Dan Riter was misspelled. The honorable mention, "Forces of Nature," was credited in both the subtitle and the text to Leslie Ann Aldridge of National Geographic TV & Film, Washington, D.C.; it should have been credited to National Geographic TV & Film, Washington, D.C.; Evan Ricks, Pixel Play Studios, Los Angeles, California; and Tim Sassoon, Sassoon Film Design, Santa Monica, California. The honorable mention "Evolutionary Morphing" was credited in the subtitle to Nina Amenta, University of California, Davis; it should have been credited to Nina Amenta and David Wiley, University of California, Davis; Eric Delson, City University of New York; F. James Rohlf, State University of New York, Stony Brook; and colleagues. (These credits are all correct in the HTML version of the article on *Science* Online.)

2005 Visualization Challenge: "Interactive media" by C. Gramling (23 Sept., p. 1993). In the text, Tracy Sterling's first name was misspelled in the subtitle and she was described as an entomologist and plant pathologist; she is actually a weed pathologist.

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