Stem Cells and Neurological Disorders

CONSTANCE HOLDEN'S ARTICLE "VERSATILE cells against intractable diseases" (News Focus, 26 July, p. 500) is fascinating, but also somewhat misleading. I believe that it is important to point out that most stem cells transplanted into humans to treat neurological disorders do not develop into differentiated cells with neuronal profiles. About 90 to 98% of the initial grafts disappear over time, and those that do remain seem to migrate from the initial zone of implantation and assume glial rather than neuronal morphology. Nonetheless, it is interesting to note that in many cases, functional recovery can still be obtained and sustained over long periods of time in the absence of new neuronal circuits being formed between the host and transplanted tissue.

Holden also seems to suggest that the only way to obtain functional repair is through inherent neurogenesis or by the transplantation and development of "precursor" cells into neurons. The "plasticity" of the nervous system is not "newfound" at all. Even the great neuroanatomist, Santiago Ramon y Cajal, writing at the beginning of the 20th century, was aware of the inherent capacity of the nervous system to show limited repair by axonal and dendritic collateral sprouting (*1*). Whether damaged nerve cells can actually develop new branches as a form of regeneration has been more controversial; nonetheless, there has been an existing literature for more than 30 years on this topic.

Because there is so much financial interest in developing stem cell therapies, one has to be very careful about overinterpreting claims about their safety and efficacy. Another recent *Science* article ("Stem cells not so stealthy after all," G. Vogel, News of the Week, 12 July, p. 175) points out that, as they differentiate, stem cells may be rejected by patients. Under such circumstances, it seems unwise to promote stem cell therapies too vigorously. Much more needs to be known about what the cells are and what they actually do in the damaged or diseased brain.

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Reference
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Taxonomic Bias and Vulnerable Species

THE TAXONOMIC BIAS IN CONSERVATION research noted by J. A. Clark and R. M. May ("Taxonomic bias in conservation research," Letters, 12 July, p. 191) (*1*) is not unexpected, but their letter neglects an important aspect of this disparity—its inverse relation with extinction risk across taxonomic groups. Although a disproportionate amount of research focuses on vertebrates in general, and birds and mammals in particular, our analyses of the conservation status of U.S. organisms find birds and mammals to have the lowest extinction risk levels among 14 taxonomic groups (n = 20,897 species) examined (*2*). Only 14% and 16% of U.S. birds and mammals, respectively, are classified as extinct, imperiled, or vulnerable, whereas 69% of unionid mussels, 51% of crayfishes, 43% of stoneflies, 37% of freshwater fishes, and 33% of flowering plants are included in those risk categories (*3*, *4*). These figures are consistent with emerging data at a global level (*5*). Although a global amphibian assessment is currently under way, birds and mammals, not surprisingly, are the only two major groups so far comprehensively assessed worldwide. We agree with Clark and May that there may be useful reasons for focusing research on charismatic organisms, such as garnering public support and funds for conservation, but our data suggest that such an approach underrepresents the vast majority of species and those organisms at greatest risk of extinction and thus in greatest need of conservation attention.

51% of crayfish species are classified as extinct, imperiled, or vulnerable.

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References and Notes
1. J. A. Clark, R. M. May, *Conserv. Practice* 3, 28 (2002).
2. B. A. Stein *et al.*, *Precious Heritage: The Status of Biodiversity in the United States* (Oxford Univ. Press, New York, 2000).
3. Risk assessments are based on conservation status ranks in use by NatureServe and its natural heritage program members. Definitions, assessment criteria, and current status ranks for each species are available at www.natureserve.org/explorer.
4. See pp. 100–104 of Stein *et al.* (*2*).
5. C. Hilton-Taylor, *2000 IUCN Red List of Threatened Species* (IUCN, Gland, Switzerland, and Cambridge, UK, 2000).

Response

WE WARMLY WELCOME STEIN *ET AL.*'S comments, which we think reinforce our message and also add an important further dimension. Our primary hope in documenting taxonomic bias in the conservation literature was to begin a dialogue in which this bias is both acknowledged and addressed. In fact, there are opportunities as well as liabilities inherent in such bias—witness, for example, World Wildlife Fund's success in using the panda on its logo as a means of inspiring interest and donations for broad biodiversity protection. Nevertheless, as Stein *et al.* note, the goal of preservation of all biodiversity cannot be realized as long as we continue to bias our research toward birds and mammals.

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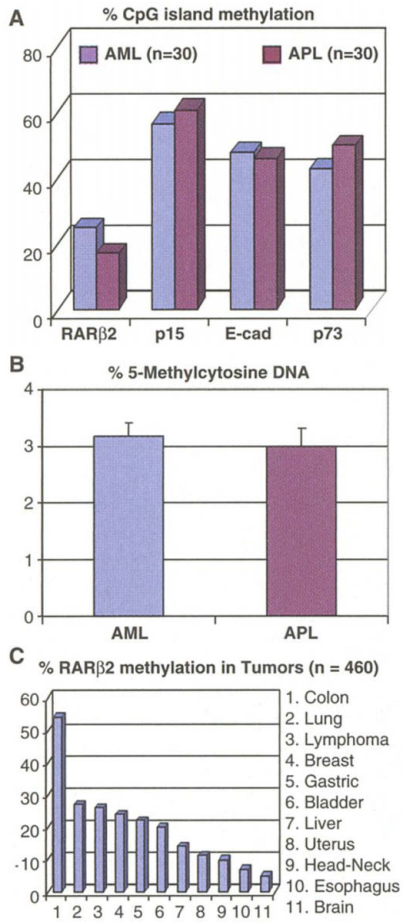
Cancer Epigenetics and Methylation

AN IMPORTANT QUESTION IN THE FIELD OF cancer epigenetics involves the causes of CpG island hypermethylation in tumor suppressor genes leading to transcriptional silencing. L. Di Croce *et al.* recently shed

some light on this subject ("Methyltransferase recruitment and DNA hypermethylation of target promoters by an oncogenic transcription factor," Reports, 8 Feb., p. 1079). They report that the leukemia-promoting PML-RAR fusion protein may be an important cause of this epigenetic aberration. However, several lines of evidence suggest that this experimental model does not explain the accumulating data concerning promoter region methylation.



(A) Frequency of CpG island hypermethylation of the *RARβ2*, $p15^{INK4b}$ E-cadherin, and p73 genes in AML versus APL. (B) Global genomic methylation content in AML versus APL. (C) Frequency of RARβ2 promoter hypermethylation in human cancer.

In this experimental model, expression of the PML-RAR transcript with an exogenous construct leads to recruitment of DNMT1 and DNMT3a and increased methylation of a RARβ-Luc fusion construct or the endogenous promoter. Consistent with this, seven of nine primary acute promyelocytic leukemias (APLs) had methylation of the 5′ region of the endogenous *RARβ2* gene. Our own analysis suggests that the frequency of methylation of *RARβ2* is the same in APLs that have the PML-RAR translocation as it is in other subtypes of acute myelogenous leukemia (AML) that do not have this alteration (see figure). This suggests that the presence of the PML-RAR translocation is neither necessary nor sufficient to induce *RARβ2* methylation. In fact, many other malignancies have *RARβ2* methylation without this translocation (see figure), in some cases more commonly than APL. We have also observed that APL patients with the PML-RAR translocations have the same frequency of CpG island hypermethylation of $p15^{INK4b}$, CDH1, and p73 (all of them with potential RAR elements in their promoters) and global genomic methylation as other AML subtypes without the translocation (see figure). These observations prompt us to caution the extension of the experimental studies described into the more complex genetic and epigenetic alterations observed in primary human malignancies.

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Response

WE RECENTLY DEMONSTRATED THAT, IN APLS, PML-RAR promotes specific methylation of its target gene *RARβ2* by recruiting DNA methyltransferases (DNMTs) to the promoter region. On the basis of these findings, we have proposed a general mechanism for the specificity of DNA methylation in cancer cells, e.g., aberrant recruitment of DNMTs by oncogenic transcription factors to specific regulative loci. Esteller *et al.* now report that the frequency of *RARβ2* methylation (and other RA-target genes) is similar for APLs, which express PML-RAR, and other subtypes of AMLs, which do not express this fusion protein, and they question the importance of the role of PML-RAR in *RARβ2* methylation.

The mechanism(s) responsible for *RARβ2* methylation in AMLs is presently unknown. On the basis of our proposed model, *RARβ2* methylation in AMLs might be triggered by AML-specific fusion proteins. Indeed, one of the two components of each fusion protein is generally a transcription factor, so that AML-associated fusion proteins function as aberrant transcriptional regulators (similar to PML-RAR in APLs). We have recently shown that transcriptional repression of RA signaling is a common feature of AMLs. In particular, we have shown that *AML1-ETO*, the most common AML-associated fusion protein, is an HDAC-dependent repressor of RA signaling, thereby suggesting that RA target genes (such as *RARβ2* and potentially the $p15^{INK4b}$, CDH1, and p73 genes mentioned by Esteller *et al.*) are deregulated by AML-fusion proteins (*1*). Thus, the data reported by Esteller *et al.* do not contradict our model. Rather, they suggest that methylation of RA target genes is a frequent event in leukemias. Further investigation is required to decipher the mechanistic roles of AML-associated fusion proteins in establishing specific DNA methylation in AMLs.

A similar scenario (aberrant recruitment of DNMTs by oncogenic transcription factors) can be envisioned for *RARβ2* methylation in other cancers. Indeed, we are currently investigating the interactions between DNMTs and general transcription factors, both in normal and transformed cells. However, we cannot rule out the possibility that, in some tumors, *RARβ2* methylation is caused by secondary mechanism(s).

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Consequences of Siberian Traps Volcanism

M. K. REICHOW *ET AL.* ("$^{40}Ar/^{39}Ar$ DATES from the West Siberian Basin: Siberian flood basalt province doubled," Reports, 7 June, p. 1846) report that the areal extent and volume of the Siberian Traps volcanism of 250 million years ago were much greater than previously thought. Traps volcanism was already known to be the largest eruptive sequence of the past 540 million years and was synchronous with (*1*) and likely linked to the great end-Permian biotic crisis, when 90 to 95% of then-existing species perished.

The vastly greater Traps magmatism may have had biological consequences well beyond the "climate change [caused] by the injection of volatiles and aerosols into the atmosphere," suggested by Reichow *et al.* These more important consequences would have resulted from the erupted basalts being extruded into and onto permafrost regions of Siberia (then at about the same high northern latitude as today) (*2*) and the adjacent underwater continental margin (*3*) in the West Siberian Basin.

This direct heating would have produced