



"It comes as no surprise to plant biologists that attempts to clone mammals from single somatic cells are plagued by high frequencies of developmental abnormalities and lethality." A form of Lamarckian transmission of acquired traits to the next generation, specifically mutations in antibody genes, is mentioned. And the risk of emerging infectious diseases in threatened species as a consequence of certain conservation measures is discussed.

Cloning Problems Don't Surprise Plant Biologists

It comes as no surprise to plant biologists that attempts to clone mammals from single somatic cells are plagued by high frequencies of developmental abnormalities and lethality (News Focus, "Clones: A hard act to follow," E. Pennisi and G. Vogel, 9 June, p. 1722). The relative ease with which plants can be cloned from somatic cells belies the substantial developmental and morphological irregularities observed in populations of these plants (1). This phenotypic variability—termed somaclonal variation (2)—often thwarts efforts to obtain uniform populations of plants by asexual propagation (3).

Even when outwardly normal plants are regenerated from cultured cells, developmental problems at later stages can appear unpredictably. Examples are the fruit and floral abnormalities observed in cloned oil palms after a decade or more of growth (4). Although a certain amount of somaclonal variation

can be attributed to genetic changes (point mutations, DNA rearrangements, chromosome numerical changes) incurred during cell or tissue culture, it is increasingly appreciated that a strong epigenetic component is involved (5). Developmentally acquired epigenetic modifications that are normally erased during sexual repro-

duction are not always reset properly during asexual propagation, leading to stable silencing of the genes involved (6). Although somaclonal variation is a problem for applications requiring clonal uniformity, it can also be viewed as an opportunity to study the types of epigenetic and genetic changes that oc-

cur at the single-cell level as development unfolds.

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Support for the National Science Education Act

In the ScienceScope item "Late hit" (26 May, p. 1313), the chances of passage of Representative Vernon Ehlers's (R-MI) bill, H.R. 4271, the National Science Education Act, are described as "slim." But such a description is contrary to what the American Association of Engineering Societies (AAES) sees as strong momentum for this vital legislation, which strives to close the gap between current teacher skills and future student needs in science, mathematics, engineering, and technology education. AAES believes that this bill is a critical first step in improving education.

In our meetings at many congressional offices, AAES and member-society staff have found a broad base of support for H.R. 4271. Furthermore, there are already 42 cosponsors, ranging from conservative Republicans to progressive Democrats. Members of the House leadership have expressed interest, and some, including Congresswoman Deborah Pryce (R-OH) and Congressman Martin Frost (D-TX), have agreed to cosponsor the bill. Also, Senator Pat Roberts (R-KS) has recently introduced companion legislation in the Senate, creating momentum in both houses of Congress.

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Lamarck and Antibody Genes

In his News Focus article "Was Lamarck just a little bit right?" (7 Apr., p. 38), Michael Balter reviews some of the research on methylation-based epimutations (inherited changes in gene expression that are not associated with mutations of the DNA), which constitute a form of Lamarckian transmission of acquired characters in animals and plants.

A role for these epigenetic phenomena in evolution was outlined in 1994 in a book by Jablonka and Lamb (1). However, Balter does not discuss the possibility that selected, adaptive changes in gene sequences in somatic cells can be fed back to germ cells and transmitted to progeny animals, thus contributing in a Lamarckian and Darwinian manner to evolution. This concept, based on evidence from the immune system of vertebrates and the feedback of reverse transcripts of mutated antibody genes from lymphocytes to germ cells, was elaborated more than 20 years ago (2). Reports of uptake by mammalian sperm of DNA and RNA and of reverse transcriptase activity in sperm provide a mechanism for movement of somatic gene sequences to the germ line (3). Data from the immune system consistent with somatic to germ cell movement of antibody genes has been reviewed in the scientific literature (4), in a book for non-specialist readers (5), and in brief form in *HMS Beagle* (6).

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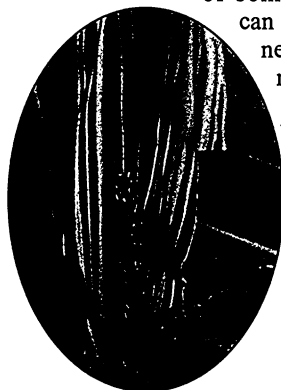
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2. E. J. Steele, *Somatic Selection and Adaptive Evolution: On the Inheritance of Acquired Characters* (Univ. of Chicago Press, Chicago, IL, ed. 2, 1981).



Jean-Baptiste Lamarck's idea that acquired traits could be passed on to progeny appears to apply in the case of antibody genes.



A corn plant grown from tissue culture with a leaf-stripping mutation.

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Database Searches for Binding Sites

In their Report "Identification of a coordinate regulator of interleukins 4, 13, and 5 by cross-species sequence comparisons" (7 Apr., p. 136), G. G. Loots and colleagues identify conserved noncoding sequences (CNSs) in orthologous regions of the interleukin (IL)-4/13/5 locus of several species (interleukins are growth and differentiation factors involved in the immune response). They demonstrated that germ line deletion of one region, CNS-1 between the genes *IL-4* and *IL-13*, reduced the frequency of *IL-4* gene activation.

Referring to their sequence analysis of CNS-1, Loots and colleagues say, "Binding sites for transcription factors known to regulate the expression of *IL-4* and *IL-13* were not found in CNS-1," on the basis of searches of the Transcription Factor Database (<http://transfac.gbfraunschweig.de/TRANSFAC/index.html>). They included searches for GATA-3, c-Maf, STAT6, and NF-AT binding sites. Their statement has specific implications for the CNS-1 mechanism, excluding actions of known T helper cell type 2 (T_H2)-specific factors, implying a need for unknown factors.

We searched the same database for these factor binding sites in CNS-1 and obtained one conserved consensus GATA-3 binding site and two NF-AT binding sites in the published sequence. The GATA-3 site resides 68 nucleotides upstream of the CNS-1 forward primer, within the region Loots *et al.* deleted for their experiments, and is conserved between mouse and human. The NF-AT sites are 5 nucleotides upstream and 31 nucleotides downstream of the CNS-1 forward primer and are conserved between mouse and human.

Although their biological significance requires study, these sites are within regions already described to exert GATA-3-dependent augmentation of the *IL-4* promoter (1). GATA-3 is a T_H2-specific transcription factor (2) shown to exert chromatin remodeling effects on the *IL-4* and *IL-13* loci (3), and NF-AT family transcription factors regulate many T cell cytokine genes (4). These GATA-3 and NF-AT binding sites in CNS-1 may or

may not be involved in its activity, but their recognition in CNS-1 is important in consideration of this study and in future work in this field.

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Response

Murphy points out an important issue concerning the use of transcription factor binding site databases, such as TRANSFAC (1), to characterize gene regulatory elements. Transcription factor binding sites are short (the "core sequence" is typically 4 base pairs in length) and highly degenerate; therefore, TRANSFAC searches invariably identify a greater number of false binding sites than functional binding sites. Because of this fact, we used relatively stringent search criteria to maximize the likelihood of discovering true binding sites in the conserved noncoding sequences, such as CNS-1, identified in our human-mouse sequence comparisons.

The criteria we used, as stated in reference 15 of our Report, included that the binding sites be conserved in multiple species in addition to humans and mice (rats, dogs, cows, and rabbits). This was based on the assumption that putative regulatory elements such as CNS-1 should have the same regulatory function in these mammals and, accordingly, should be a target for the same transcription factors. The second criterion used, which was not specifically outlined in our Report, was that the binding sites have a matrix similarity score (a quality rating) of ≥ 0.9 . The default matrix similarity threshold for MatInspector (2), the software tool that we used to search TRANSFAC, is 0.85. Neither the GATA-3 nor the two NF-AT sites pointed out by Murphy fit both of these criteria. The 3' NF-AT site, although conserved in all six species examined, had a matrix similarity score below the 0.9 cutoff value. The 5' GATA-3 and NF-AT sites, although conserved in humans and mice, were not in the region amplified and sequenced in multiple species.

Using databases such as TRANSFAC to identify putative regulatory sequences that are targets for known transcription factors is currently the only computational method available for identifying such elements. Although this is clearly a valuable approach, the results of TRANSFAC searches need to be carefully scrutinized, taking into consideration the analysis of orthologous sequences in multiple species to distinguish between real and spurious binding site matches. Although our analysis of CNS-1 did not identify binding sites that met our stringent search criteria, we do agree with Murphy that individuals studying *IL-4* and *IL-13* expression should not be deterred from examining this element for binding activity of transcription factors such as GATA-3, c-Maf, STAT6, and NF-AT.

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Conservation Conundrum

P. Daszak, A. A. Cunningham, and A. D. Hyatt present a convincing argument in their Review "Emerging infectious diseases of wildlife—threats to biodiversity and human health" (*Science's Compass*, 21 Jan., p. 443) that emerging infectious diseases (EIDs) pose a risk to wildlife, and they suggest that EIDs most often result from a change in the ecology of the pathogen or the host (or both). A situation they did not mention is that, in some cases, the protection of threatened species can increase the risk of

an EID outbreak by allowing a close association between wildlife and domestic animals where one would not have naturally occurred. An important example is northern elephant seals (*Mirounga angustirostris*) (see figure at left), which were abundant in California and Baja California, Mexico, at the beginning of the 19th century before being nearly eliminated by hunting. During the population bottleneck that resulted, there may

