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INTERFACE:

GENES AND THE ENVIRONMENT

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The Toxic Metal Cadmium "Hitchhikes" Into Our Cells by Way of Two Zinc-Bicarbonate Transporter Proteins

There are 'essential metals' and 'nonessential metals' in our environment

Most of us know that humans need certain *essential metals* for normal metabolism and biological processes in our bodies. Essential metals include zinc (**Zn**), calcium (**Ca**), magnesium (**Mg**), iron (**Fe**), copper (**Cu**), cobalt (**Co**), manganese (**Mn**), selenium (**Se**) and molybdenum (**Mo**). Chromium (**Cr**) is regarded as "essential"—to some bacteria—but there is no proof that Cr is needed in humans**;** nevertheless, one can buy "Cr tablets" as an over-the-counter supplement in most drug stores. Without enough of any of the essential metals (on a daily or weekly basis), we can develop a deficiency. With doses that are too high, they can also make us ill. In general, a normal diet should provide us with ample amounts of all nine of these essential metals.

Nonessential metals are never "needed" in our bodies**;** these include cadmium (**Cd**), lead (**Pb**), silver (**Ag**), mercury (**Hg**), nickel (**Ni**), arsenic (**As**), beryllium (**Be**), aluminum (**Al**), tin (**Sn**), barium (**Ba**), gold (**Au**), platinum (**Pt**), palladium (**Pd**), antimony (**Sb**), vanadium (**V**), tungsten (**W**), gallium (**Ga**), cesium (**Cs**), strontium (**Sr**), uranium (**U**) and many others. Whereas there can never be a "deficiency" of nonessential metals in our body, even relatively small doses of nonessential metals can cause serious damage and disease.

IN THIS ISSUE

Diseases caused by Pb and Cd

Since the 1920s, Pb in gasoline was the first nonessential metal shown to cause birth defects, mental retardation, loss of balance and other brain damage, as well as diseases of the kidney, liver or lung in humans. Despite thousands of studies between 1920 and 2005, however, no study had ever determined the precise mechanism as to why any nonessential metal causes toxicity—in humans or in any other vertebrate. On the other hand, studies in bacteria, yeast, plants and clams have demonstrated membrane transporter systems that take up various metals into the cell or that pump various metals out of the cell.

Cd is a highly toxic metal—widely distributed in contaminated soil, cigarette smoke, toxic waste dump sites, and polluted sea food. (Curiously, the

tobacco plant takes up a lot of Cd from the soil.) Acute exposure to large Cd doses can result in damage to the central nervous system, gastrointestinal tract, lung, liver, bone, ovary, placenta, and testis. Chronic exposure to low doses of Cd over decades of time results predominantly in kidney damage and bone disease**;** Cd is eliminated slowly and thus accumulates (especially in the kidney) with age.

The level of Cd in the environment has risen with the rise of industrialization, and Cd-induced human diseases are of growing concern. The increasing levels of environmental Cd, in combination with longer life expectancy, work together to enhance the body's Cd burden**:** for example, the average accumulation of Cd in the kidneys of a person who smokes at least two packs a day for 50 years is beyond the threshold sufficient for causing overt kidney failure. Furthermore, Cd is classified as a Category I human carcinogen. People who are at highest risk for Cd-associated lung cancer and other diseases include cigarette smokers, women with low body-iron stores, people with a habitual diet rich in high-fiber foods and contaminated shellfish, and malnourished populations.

Low doses of Cd cause rapid destruction of the testis in all species having testes

In 1919 it was reported that, when Cd was injected just under the skin in very small amounts, a complete vascular collapse (*toxic necrosis*) of the testis occurs within 24 to 48 hours. This "exquisite sensitivity to Cd" was found to occur in males of *every* vertebrate species tested**:** rooster, pigeon, fish, armadillo, rat, mouse, rabbit, hamster and guinea pig. Presumably, the same would happen in humans, but of course such studies would not be ethical.

In the 1960s, it was reported that some inbred mouse strains are *resistant* to this dose of Cd (whereas the vast majority of inbred strains are sensitive, just like every other wild-type species). In 1973 this genetic variant (resistance to exquisitely low doses of cadmium) was determined to be inherited as an autosomal recessive trait (*e.g.* similar to the trait for blue eyes)**;** it was named the "*Cdm* locus" and mapped to a region on mouse Chromosome 3 that spanned ~24 centiMorgans—which is ~28% of that entire chromosome. This amount of DNA was far too large for any chance of success at isolating and characterizing the single causative gene.

The Mouse Genome Project helps in identifying the correct gene

Between 1994 and 2002, the Nebert laboratory took advantage of newer molecular biology and genetic techniques—combined with the fact that both the Human Genome Project and the Mouse Genome Project were mostly completed. Using genetic "tricks," the *Cdm* locus-containing region was reduced from more than 24 million base pairs to 800,000 base pairs of DNA. Of the three genes in this 800,000-base-pair segment, one (***Slc39a8***) was a "zinc transporter gene" previously characterized only in the small mustard plant. [During evolution, if a gene is very old and therefore has arisen before animal-plant divergence ~1.2 billion years ago, then versions of that gene can exist in present-day species as diverse as human (and mouse) and plants**;** this is what has happened with this very old zinc transporter gene.]

The *Slc39a8* gene encodes the "ZIP8" transporter protein, so-called **ZIP** for "**z**inc-like, **i**ron-like **p**rotein"). The gene was then cloned and expressed in mouse cell cultures, where it was shown to be located on cell surface membranes and to pump Cd into the cell at high rates**;** this makes the cells highly susceptible to damage when exposed to Cd. Using a technique called "*in situ hybridization*" to look for cell-type-specific expression of messenger RNA (**mRNA**), Keith F Stringer at Children's Hospital helped the Dalton and Nebert laboratories to localize ZIP8 mRNA in mouse tissues. It was found that ZIP8 is highly expressed in several tissues (lung, kidney, testis, liver, brain) and at the same levels between the Cd-sensitive and Cd-resistant inbred strains of mice—with one exception. In the blood vessel cells of the testis, ZIP8 mRNA is expressed highly in the Cd-sensitive strains but negligibly in the Cd-resistant strains. This ZIP8 expression (or lack thereof) occurs specifically in the **blood/testis barrier**, which is a unique set of blood vessel cells that protect the testis from unwanted

environmental toxicants in much the same way that the **blood/brain barrier** protects the brain from unwanted drugs or other chemicals.

What is the 'normal' function of ZIP8?

By means of competition studies in mouse cultured cells and also in frog eggs (in collaboration with Manoocher Soleimani, Division of Kidney Diseases), it was then found that the normal (*endogenous*) metal substrate for ZIP8 is Zn and perhaps also Mn. Apparently very low levels of Cd are able to displace Zn or Mn from the ZIP8 transporter as an opportunistic hitch-hiker, thereby flooding the testis blood vessel cells in sensitive (but not resistant) animals. Once significant Cd levels have entered these cells, serious damage occurs, and, without proper blood supply, the testis becomes destroyed within 1 to 2 days. It was also found that bicarbonate ion (HCO_3^-) is absolutely essential for the uptake of Zn, Mn or Cd into any cell. In studies in frog eggs, it was also found that one Zn^{2+} ion travels together with two HCO_3^- ions and thus the net charge across the outer cell membrane is *electroneutral***;** therefore, ZIP8 is called a $Zn^{2+}/(HCO_3^-)$ *symporter*, or *co-transporter*, in which both one ion of positive charge and two ions of negative charge move through the membrane together.

Proof that the Slc39a8 gene is indeed the Cdm locus

The Cd-sensitive *Slc39a8* gene (which is dominant), plus more than 50,000 bases on either side of the gene, was isolated from a bacterial-artificial-chromosome (**BAC**) library and inserted into a Cd-resistant mouse (this trait is recessive). When subcutaneous Cd is given to this BAC-transgenic mouse, the trait (*phenotype*) changed from "Cd-resistance" to "Cd-sensitivity," thereby proving that the *Slc39a8* gene is indeed the *Cdm* locus that had been first identified in 1973. This is the first demonstration of the mechanism whereby genetic differences in response to a nonessential metal exist between individual vertebrate animals. What has been shown in the mouse is likely also to occur in clinical populations, *i.e.* the same dose of environmental Cd probably has different effects on specific patients—depending upon their genetic make-up.

An unanticipated side-effect: renal failure

A side effect of this BAC-transgenic mouse was that low doses of Cd actually caused kidney failure, several hours before full-blown destruction of the testis**!** This was discovered to be caused by an increased number of ZIP8 transporter molecules in special cells of the kidney**:** the *renal proximal tubule*, and on the side exposed to the *glomerular filtrate* (fluid removed from the blood and in the process toward making urine). Thus, all Cd being filtered in the kidney was unfortunately being taken up in the BAC-transgenic mice by these renal proximal tubular cells, leading to acute renal failure.

Low oral Cd doses—given to this BAC-transgenic mouse over 6 weeks or longer—cause *metabolic acidosis*, also called **renal Fanconi syndrome**, which includes bone disease. It has been known for decades that chronic exposure of humans to cadmium, lead, mercury, platinum or uranium causes renal Fanconi syndrome and associated brittle bone disease.

Discovery of Slc39a14, the gene most closely related to Slc39a8

ZIP8 expression is highest in**:** lung > kidney = testis >> liver > brain = small intestine. Of the 14 *Slc39* genes in the mouse (14 *SLC39* genes in the human), *Slc39a14* is the only one evolutionarily closely related to *Slc39a8* (*i.e.* within the last 450 million years). The gene product of *Slc39a14* is called ZIP14. ZIP14 has now been found also to be a $Zn^{2+}/(HCO_3^-)_2$ symporter. ZIP14 expression is highest in**:** liver = intestine > kidney >> brain = testis.

Clinical relevance, or "translational medicine"

The present working hypothesis in the Nebert lab is that ZIP14 is principally responsible for Cd absorption from the intestine and influx into liver cells (*hepatocytes*)**;** Cd is then transported from the liver via the bloodstream using glutathione (**GSH**) and metallothionein (**MT**) carrier molecules. ZIP8 is principally in charge of the major Cd influx into cells

of the kidney, testis and the central nervous system. ZIP8 is also responsible for transporting Cd from the air in the lung into cells lining the lung (*alveolar cells*)**;** ZIP8 (more so than ZIP14) is therefore likely to play a major role in Cd-induced lung cancer.

Welders are known to be exposed to toxic levels of Mn and to develop deafness and loss of balance. One present hypothesis in the Nebert lab is that ZIP8-mediated transport of Mn into cells of the inner ear is responsible for these environmental disorders.

In addition to the BAC-transgenic mouse line (which has five copies of the *Slc39a8* gene instead of the normal two), the Nebert lab is presently generating mouse lines having zero copies and one copy of the *Slc39a8* gene and the *Slc39a14* gene—as further models to study Cd-mediated diseases in the intact mouse. This story is an example of how basic science progresses into unexpected clinical fields. Starting with a mouse genetic model for testicular damage, the Nebert lab has now wound up with a mouse model for understanding heavy metal disease in the kidney and bone, and possibly in the inner ear, with relevance to similar diseases in humans. ***—contributed by Dan Nebert***

Evolutionarily Speaking.....

What follows is a synopsis of some of the more interesting things that have happened during the first 6 months of 2006 with the Human Genome Project (**HGP**), and **evolutionarily-related** news, provided chronologically**:**

Feb 2006 The placenta is essential for the success of *therian* (*i.e.* non-egg-laying) mammalian reproduction. A phylogenetic analysis [*PNAS* 2006; **103:** 3203] reveals that *Afrotheria* (*e.g.* elephant, golden mole) are most closely related to marsupials, *Xenartha* (*e.g.* armadillo, anteater) is the next earliest, and that *Laurasiatheria* (*e.g.* carnivores, hedgehog) and *Euarchontaglires* (*e.g.* primates, rodents) and are the latest to evolve.

Mar 2006 It was not many years ago that scientists were predicting humans would probably have at least 100,000 genes, if not 300,000—mainly because the small mustard plant and rice genomes contain ~27,000 and ~37,500 genes, respectively. But, the number of human genes keep dwindling; the latest estimate is between 20,000 and 25,000 [*Science* 2006; **311:** 1709]. Something else **has to** compensate for our "intelligence", when compared to that of a plant. Humans have >200,000 RNA transcripts and probably >300,000 different proteins. Still, much needs to be learned about why we have such a small number of genes.

The chimpanzee and human genomes differ by only **1.23%**. A new study has looked at "gene loss" instead of "gene gain" by studying pseudogenes that now exist in humans but which are still functional genes in chimpanzee; they found 80 such pseudogenes—in which the chimpanzee ortholog's function was in immune response and chemoreception [*PLoS Biol* 2006; **4:** e52].

The traditional measure of whether a protein-coding gene deviates from random genetic drift is the relative rate at which nonsynonymous (**amino-acid-changing**) and synonymous (**silent**) mutations are fixed in a population (*i.e.* the $\mathbf{K_a/K_s}$ ratio). If K_a/K_s is greater than 1.0, the gene is changing at a rate faster than would be expected under the neutral theory and is therefore subject to Darwinian selection [*PLoS Biol* 2006; **4:** e87].

Apr 2006 Within the past 60,000 to 100,000 years, *Homo sapiens sapiens* (modern human) had to adjust to a major climate change following the last ice age, as well as dramatic lifestyle changes from hunting and gathering to agriculture. Looking at ~800,000 single-nucleotide polymorphisms (**SNPs**) in the four populations studied in HapMap Phases I & II (*i.e.* African, Chinese Han, Japanese, and northern European), a recent study searched for genes that had rapidly evolved [*PLoS Biol* 2006; **4:** e72]. The genes that stood out most include those playing a role in fertility and reproduction, the immune system, morphology (*e.g.* skin pigmentation and skeletal development), and food metabolism (reflecting regional changes in diet).

May 2006 Various lines of geochemical evidence imply that life evolved on Earth roughly 3.8 billion years ago (**BYA**), yet more direct evidence (such as fossils or fossil-derived structures) is still

sparse or disputed in rocks dating several hundred million years closer to the present than 3.8 BYA. Now, a report in the journal *Geology* shows fossil microbial mats found in South African tidal sandstones that date back to ~3.2 BYA. Concentration of these layered roll-up structures suggests that microbes in these mats may have derived their energy via photosynthesis [*Science* 2006; **312:** 659].

Discovering that identical twins do not necessarily have identical DNA, due to epigenetic changes [*Nature* 2006; **441:** 143]—might contribute to the dilemma described above (*under* ***March***) as to how humans can get by with such a small number of "formal protein-coding genes".

Gene-Environment Tidbits of Interest

Tidbits on this topic from the first half of 2006**:**

Jan 2006 The protein complex called RISC is involved in some of the silencing micro-RNA pathways that participate in RNA interference. One of the RISC proteins is Armitage, which must be destroyed at particular nerve synapses in order for protein synthesis that underpins memory to occur. Armitage-null fly mutants show impaired long-term memory [*Cell* 2006; **124:** 191]. Because the RISC pathway is highly conserved, a similar mechanism may operate for long-term memory in humans.

Feb 2006 There are two clearly opposing views on the function of α-fetoprotein (**AFP**), a fetal plasma protein that binds estrogens with high affinity, in the sexual differentiation of rodent brain. AFP either prevents the entry of estrogens, or actively transports estrogens, into the developing female brain. Using the *Afp(-/-)* knockout mouse line [*Nat Neurosci* 2006; **9:** 220], investigators found that the brain and behavior of females were masculinized and defeminized. When estrogen production was blocked by embryonic treatment with an inhibitor, however, the feminine trait of *Afp(-/-)* mice was rescued. These data show that prenatal estrogens masculinize and defeminize the brain and that AFP protects the female brain from these effects of estrogens.

Mar 2006 Steroids have conclusively been shown to participate in the nematode worm, *Caenorhabditis elegans*. An endocrine-signaling pathway (which includes cholesterol, nuclear receptors and P450 metabolism) directs**:** [a] reproductive growth under favorable environmental conditions, [b] a suspended animation state (called *dauer*) under unfavorable conditions, and [c] extended lifespan via germ line signaling [*Cell* 2006; **124:** 1137].

Apr 2006 A group of Ashkenazi Jewish centenarians (N=213 people over 100 years old), their children (N=216), and an age-matched Ashkenazi control group (N=258) were studied for DNA variants in 36 candidate genes related to cardiovascular disease. A significant survival advantage (P <0.0001) was found in those having the favorable *–641C/C* homozygous genotype of their *APOC3* gene [*PLoS Biol* 2006; **4:** e113].

May 2006 Feeding genetically identical (C57BL/6J) mice a high-fat diet beginning at 8 weeks of age resulted in a 4-times greater risk of obesity, compared with mice on a regular diet [*PLoS Genet* 2006; **2:** e81]. This model is proposed as a valuable means for investigating non-Mendelian (epigenetic) mechanisms within fat cells that underlie diet-induced obesity.

Jun 2006 The *Proceedings of the National Academy of Sciences U.S.A.* [**PNAS**] has announced that their journal will begin accepting manuscripts under the category of **Sustainability Science**. This "new" field comprises "studies on interactions between human and environmental systems—as well as sustainability challenges related to agriculture, biodiversity, microbial ecology, cities, energy, health and water".

Best Typo 2008

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Latest in Genetics and Genomics.....

What follows is a synopsis of some of the more interesting things that have happened during the first 6 months of 2006 with the Human Genome Project (**HGP**), and related genetics/genomics news, provided chronologically**:**

Jan 2006 DNA sequencing and analysis of human chromosome 8, which totals 145,556,489 bases, have now been completed [*Nature* 2006; **439:** 331]. There are 793 protein-coding genes and 301 pseudogenes. The most distal 15 **Mb** (megabases; millions of bases) on chromosome 8p show the greatest amount of divergence between human and chimpanzee and the highest mutation rate in the human genome (about twice the average). This fast-evolving region contains many genes involved in innate immunity and the nervous system.

Feb 2006 The progression of **colorectal cancer** was studied. An immediate-early gene, nuclear factor *NR4A2* (Nurr1), was found to be induced by prostaglandin E2 which in turn decreases cell death, compromised immune surveillance, and stimulation of cell migration, proliferation and growth of blood vessels. Human colorectal cancers relative to matched normal mucosa show increased *NR4A2* expression, which can then stimulate progress of the neoplasm downstream from cyclooxygenase-2-derived PGE2 [*J Biol Chem* 2006; **281:** 2676].

Mar 2006 Human chromosome 11 DNA sequence has been carefully characterized [*Nature* 2006; **440:** 497], and 1,524 protein-coding genes and 765 pseudogenes were identified. Out of the 171 human disorders currently attributed to this chromosome, 86 remain for which the underlying molecular basis is not yet known.

Apr 2006 Our ideas about gene expression continue to change and evolve. Now it seems clear that different genes on several chromosomes, as long as they are located together near the surface of the nuclear membrane, can be up-regulated together [*Science* 2006; **312** 269].

May 2006 The DNA sequence and biological annotation of human chromosome 1 has been completed [*Nature* 2006; **441:** 315]. This chromosome is "gene-dense", having 3141 protein-coding genes and 991 pseudogenes, with many coding sequences overlapping. Rearrangements and mutations of chromosome 1 are known to be prevalent in certain types of cancer and many other diseases.

Human Variation, Disease, Migration and Evolution....

Tidbits on these topics from the first half of 2006**:**

Feb 2006 It has become increasingly appreciated that we all have differing numbers of our copy number variations (**CNVs**) within our genomes. As of this writing, 1,237 CNVs have been identified [*Nature* 2006; **439:** 798]. Clearly, CNVs will wreak havoc in attempts to simplify personalized medicine or individualized drug therapy, because, for example—if one happens to have several copies of a particular gene—this could alter one's response to the "recommended prescribed dosage" of a particular drug.

Mar 2006 Human earwax can be of either a dry or wet type. Dry earwax is a more frequent trait in Asians. Mutations in the transporter *ABCC11* gene have been shown to be the main determinant in earwax type [*Nat Genet* 2006; **38:** 324].

Apr 2006 The first report of human variation in taste sensitivity to the bitter chemical phenylthiocarbamide (**PTC**) was published in 1932. Less than a decade later, chimpanzees were also shown to exhibit similar genetic differences, and it has always been presumed that the mutation in some gene had occurred prior to the chimpanzee/human divergence 5-6 million years ago. A mutation in the human taste receptor gene ***TAS2R38*** was found in 2003. Now, a completely different type of mutation in the *TAS2R38* gene has been found in chimpanzees [*Nature* 2006; **440:** 930], indicating that the molecular basis of this variation has arisen twice, independently, in the two species.

PILOT PROJECT PROGRAM AWARDEES

funded from 1 Apr 2006 to 31 Mar 2007

Ana Luisa Kadekaro, MD (Dermatology) "Effect of *P* gene and MC1R interaction on the UV response of human melanocytes"

George D Leikauf, PhD (Environmental Health) "Genetic determinants of pulmonary function in mouse models of COPD"

Grace K LeMasters, PhD (Environmental Health) "*CYP2A6* polymorphism and nicotine metabolism biomarkers in children exposed to environmental tobacco smoke"

Tiina Reponen, PhD (Environmental Health) "Methodology for fungal fragments—a new exposure assessment tool for gene:mold interaction studies"

Amy M. Rohs, MD (Environmental Health) "Genetics of vermiculite-induced pleural thickening and fibrosing pleuritis"

Robert Smith, MD (Family Medicine) "Clinical, environmental and genetic factors related to response and non-response to treatment of type-2 diabetes patients with thiazolidinediones"

Peter J Stambrook, PhD (Cancer and Cell Biology) "*CHEK2*1100delC* polymorphism and susceptibility to breast cancer in childhood Hodgkin disease survivors"

Jay W Tichelaar, PhD (Environmental Health) "*Ccl17-Ccr4* signaling in lung tumorigenesis"

Ying Xia, PhD (Environmental Health) "Role of MEK kinase-1 in acute lung injury"

Jagjit S Yadav, PhD (Environmental Health) "Gene targets of trichothecene mycotoxins in indoor mold-induced lung toxicity"

In addition to the traditional Pilot Project Program Awards, the **CEG** funded four projects of a smaller scope ($2,000-$5,000 each), with the goal of bringing together new teams or collaborations, or funding individual investigators with innovative approaches to the area of environmental health and its link to genetic diversity. The following projects were funded at the discretion of the Director**:**

Erin Haynes, PhD (Environmental Health) "Community participatory research in a manganese-exposed population in Marietta, Ohio"

Christopher Mayhew, PhD (Cancer and Cell Biology) "Influence of *RB1* loss on hepatocarinogen-induced somatic mutation frequency"

David Warshawsky, PhD (Environmental Health) "Identification of endocrine-disrupting chemicals in various water sources"

Ying Xia, PhD (Environmental Health) "Mass spectrometric analysis of the MEKK1 protein"

Biotechnology....

Tidbits during the first half of 2006, concerning genetically-modified (**GM**) plants, biotechology, and related topics**:**

Jan 2006 A report from Scotland, in the Aug 2005 issue of *Environmental Science & Technology* noted that U.S.-grown rice contains an average of 1.4 to 5.0 times more arsenic than rice from Europe, India or Bangladesh [*EHP* 2006; **114:** A27]. Because of the form of arsenic in plants, the rice might not pose a threat**;** arsenic in drinking water is estimated to be 5 times more toxic. Arsenic uptake appears to occur via one or more aquaporin genes, and genetically-engineered *AQP* genes in U.S.-grown rice would be one way to offer to the world a safer food product.

Feb 2006 A full-genome transcription analysis of the *Oryza indica* rice subspecies, using high-density oligo tiling microarrays, has provided expression data support for the existence of 35,970 genes and identified 5,464 unique transcribed intergenic regions that share compositional properties with annotated exons and have significant homology to other plant proteins [*Nat Genet* 2006; **38:** 124]. This study should be useful for further understanding of the rice genome.

Mar 2006 In **issue #29** (first half of 2005) we described the first reports of mutations in the tyrosine kinase domain of the *EGFR* gene that made many non-small-cell lung cancer patients more resistant to gefitinib therapy. Recent reports noting the presence of novel mutations [*Clin Cancer Res* 2005; **11:** 1368 & *N Engl J Med* 2005; **353:** 133] have been challenged by a technical "mutation identification" problem [*N Engl J Med* 2006; **354:** 526]. It turns out that **postmortem deamination** of cytosine or adenine (resulting in uracil or hypoxanthine residues, respectively) was able to explain 45 of these "uncommon mutations in the *EGFR* gene"**..!!**

Apr 2006 Meat products are generally low in omega-3 (n–3) fatty acids, which are beneficial to human health. Cloned pigs have now been developed that express a humanized *C. elegans* gene, *fat-1*, which encodes an n–3 fatty acid desaturase enzyme. The **h***fat-1* transgenic pigs produce high levels of n–3 fatty acids from n–6 starting materials, and their tissues have a significantly reduced ratio of n–6/n–3 fatty acids, which is healthy [*Nat Biotechnol* 2006; **24:** 435]. This technology will next be applied to chicken eggs.

May 2006 GTC Biotherapeutics, Inc. based in Massachusetts, has engineered goats to express in their milk a human protein, anti thrombin, which helps stop the formation of blood clots. This bioengineered protein, marketed as ATryn, will be used to treat antithrombin-deficient patients before they give birth or undergo surgery. Using regular blood-thinning medicines in such patients usually causes serious bleeding [*Nature* 2006; **441:** 681].

Jun 2006 Scientists have been trying to determine "the origins of agriculture" by following the trail of wheat, barley and other grains at various archaeological sites throughout the Middle East, and their best guess was ~10,500 years ago. Now comes a study of a cultivated variety of wild figs**:** based on radiocarbon dating, this cultivation appears to have occurred ~11,400 years ago [*Science* 2006; **312:** 1292].

Observations by a Biologist

How do plants sense and respond to environmental stimuli?

Charles Darwin and his son Francis published a book in 1880, titled "*The Power of Movement in Plants*". They described three broad categories of plant movement**:** [a] the tropisms, [b] nastic movements, and [c] circumnutation. *Tropisms* are directed growth, in response to external stimuli (*e.g.* sunlight, gravity, water, presence of another nearby plant or an immovable object). Examples of *nastic movements* include the striking leaf movements of the Venus flytrap after the stimulus of "touch" and the less dramatic but more ubiquitous "sleep movements" in which some leaves or flowers move to a different position (*i.e.* closed) at night (*e.g.* bean plants). *Circumnutation* means oscillatory movements in which plants rotate around a central axis during their growth**;** almost all plants do this, but **vines** show exaggerated circumnutation.

Kitazawa and coworkers [*PNAS* 2005; **102:** 18472] have recently revisited these issues, studying [a] the Japanese morning glory (*Pharbitis nil*) which does not respond to gravity nor does it exhibit circumnutation, and [b] an agravitropic mutant (defective in "detecting" gravity) of the small mustard plant *Arabidopsis thaliana*. These mutant plants cannot detect gravity because of defects in their *statocytes*, which are "gravity-perceiving cells" that contain *statoliths* (starch-filled plastids). In addition, several other agravitropic *Arabidopsis* mutants also exhibited decreased or complete lack of circumnutation; one is a defective scarecrow gene (*SCR*), which determines endodermal differentiation in roots and shoots. Kitazawa and coworkers show in their 2005 publication that mutations of the *SCR* gene in either *Arabidopsis* or *Pharbitis nil* lead to defects in both circumnutation and gravitropism. Thus, it appears that the Darwins were not correct in dividing these two into different categories.

On the space shuttle Columbia, however, 93% of 4-day-old sunflower seedlings in microgravity displayed circumnutation, compared with 100% of the ground control seedlings. This finding would have made the Darwins happy. However, Kitazawa and coworkers proposed that, because these seedlings had been germinated on the ground, the plants sensed gravity before and during the launch.

The obvious experiment to do next is to **begin germination** of sunflower seedlings—and perhaps also *Pharbitis nil* and *Arabidopsis* wild-type and several mutants—not on the ground but rather in microgravity at the space station [*PNAS* 2006; **103:** 829]. This will be the defining moment as to what are the effects of genes, versus the effects of the environment (in this case, gravity), on these traits of circumnutation and gravitropism.

Treat people as if they were what they ought to be, and you help them to become what they are capable of being.

Johann Wolfgang von Goethe (1749-1832)

Ethical, Legal and Social Issues....

ELSI tidbits from the first 6 months of 2006**:**

Jan 2006 The attributes versus the liabilities of having individuals "self-report their own perceived race and genetic admixture" were reported [*N Engl J Med* 2006; **354:** 421]. All in all, self-reporting usually **does** provide an important means for identifying distinct ethnic groups.

Feb 2006 The Ohio State Board of Education has voted to remove a teaching plan from its curriculum that had been accused of "favoring intelligent design". This vote followed closely on the heels of a Dover, Pennsylvania, federal judge's ruling that the teaching of intelligent design in science class is unconstitutional [*Nature* 2006; **439:** pp 6 & 904].

Mar 2006 How to dissect racial and ethnic differences in lung cancer has been discussed [*N Engl J Med* 2006; **354:** 333 & 408]. National statistics show that African-American male smokers have an increased incidence of lung cancer, whereas Latino and Asian males and females have decreased rates, compared with their Caucasian counterparts. It is not clear, however, that genetic analysis offers a useful pathway to a largely socioeconomic problem.

Apr 2006 NIH Director Elias Zerhouni has proposed an "NIH Roadmap" for the quick translation of basic bench science to the bedside. JCI Editor Andrew R. Marks wrote an editorial, expressing concerns about decreased funding of investigator-initiated research as a consequence of these new NIH policies. Support of Marks' editorial from investigators around the country, and opposition to Marks' critical comments from directors of the 27 NIH institutes and centers [*J Clin Invest* Apr 2006; online] makes for interesting reading.

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LETTERS TO THE EDITOR

RESPONSES/COMMENTS TO VARIOUS QUESTIONS

Q The industrial revolution began in the 1870s and continues today, with more and more environmental pollution. Yet, during the past 250 years, life expectancy in Western civilization countries has doubled. Why?

A *In a recent study of four northern European countries [**PNAS** 2006; 103: 498], increasing longevity and declining mortality in the elderly occurred among the same birth cohorts that had experienced a reduction in mortality at younger ages. Also, these same people show an increasing adult height. The authors propose that both the increased longevity and greater height are promoted by the reduced burden of infections and inflammation. Therefore, early growth as well as cardiovascular diseases of old age might share infectious and inflammatory causes—rooted in our external environment. One might conclude that the inhibition of infections and inflammation plays a greater role in maintaining healthy bodies than environmental pollution and toxicants.*

Comment **Related to the Leading Article in this issue about the ZIP metal transporters, abnormal zinc (Zn) transport in the pancreatic beta cells has been postulated as the cause (or part of the cause) of diabetes mellitus type-2 [*Medical Hypotheses* 2005; 65: 887]. Ethnic differences (*i.e.* African-Americans are twice as likely to develop diabetes as European-Americans of similar age) suggest the involvement of one or more causative genes. If a direct link between Zn transport and diabetes could be established, then a specific nutritional formula, medication, or other intervention could be used to treat diabetes. Given the fact that there are several hundred single-nucleotide polymorphisms (SNPs) in the human *SLC39A8* and *SLC39A14* genes that encode ZIP8 and ZIP14, respectively (noted in the Leading Article), this hypothesis is now easily testable.**