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INTERFACE:
GENES AND THE ENVIRONMENT

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Asthma and the β2-Adrenergic Receptor (*ADRB2*) Gene Polymorphism

Millions of people are afflicted with asthma, and the disease varies in expression from mild to completely debilitating. Asthma is very costly, in terms of health care dollars and loss of productivity, as well as tremendous physical and emotional anguish. A recent survey of asthmatic patients (Allergy and Asthma Network/Mothers of Asthmatics) suggested that individuals are far more disturbed by quality-of-life issues than their physicians recognize.

Asthma is a complex disease, clearly having an underlying genetic predisposition, combined with a host of environmental risk factors (issue #6 of ***Interface***). Polymorphisms exist in many genes encoding critical membrane receptors and signal transduction proteins, which in turn regulate smooth-muscle reactivity, inflammation, secretions, repair, and lung architecture. Whereas certain of these mutations can create sensitive populations, it is the environmental exposure (*e.g.* indoor, outdoor and workplace air quality—especially house dust, animal and insect dander, molds, urban smog, and cigarette smoke) that elicits the asthmatic response. Often, however, the trigger is unknown.

IN THIS ISSUE

Properties of the β2-adrenergic receptor (ADRB2)

ADRB2 is expressed abundantly in lung—as well as in the plasma membrane of eosinophils, neutrophils, lymphocytes, macrophages and mast cells. The most obvious change in the lung after activation of ADRB2 is relaxation of bronchial smooth muscle, resulting in bronchodilation. It is this response (smooth muscle relaxation) that reflects the therapeutic effectiveness of successful pharmaceuticals (ADRB2 agonists) in the treatment of asthma or chronic obstructive pulmonary disease.

G protein-coupled receptors are abundant on the surface of cells and are critical in regulating lung function, and can be modulated by internal and exogenous agents. The ADRs are among those receptors that are coupled to G-stimulatory proteins (as opposed to G-inhibitory proteins). The ADRB2 is one of nine different adrenergic guanine-nucleotide regulatory-binding protein receptors, and one of three beta subtypes. Many mechanisms for maintaining tissue homeostasis, adaptation to environmental stressors, and pharmacodynamic agents, involve signaling from the cell surface to the cell's interior; this is accomplished in part by this large family of receptor proteins having very diverse functions. Both the agonist and antagonist domains

of the ADRB2 protein have been studied by a variety of molecular mechanisms.

The *ADRB2* gene is located on human chromosome 5q31-32 (Fig. 1). The protein is composed of 413 amino acids, with seven hydrophobic transmembrane domains, an extracellular amino terminus, three extracellular loops and three intracellular loops, with a small fourth loop before the palmitylation site, of which the latter is believed to help anchor the protein in the plasma membrane (Fig. 2). Signaling occurs when an extracellular compound binds to the extracellular portion of the G protein-coupled receptor, which, in turn, causes a conformational change that allows the receptor to bind a heterotrimeric G-protein on its intracellular side. This then, activates cyclic AMP, followed by protein kinase A, which phosphorylates multiple targets. The final effect is a change—such as opening or closing an ion channel. Most information about these receptors and their function has been, to date, derived from molecular studies, but extrapolation of this information back into the intact animal is inherently difficult.

Fig. 1

The β2-adrenergic receptor (ADRB2) in asthma and drug responses

The most effective therapy for reversal of bronchospasm, during an asthmatic attack, is to dilate the bronchi. This is accomplished by the administration of an ADRB2 agonist, which activates the receptor. The bronchodilating response to ADRB2 agonist therapy has long been known to exhibit significant variation among patients. Salmeterol and formoterol are two widely-used long-acting ADRB2 agonists.

Agonists bind at sites within the cell membrane but primarily in a pocket formed by the 3rd, 5th and 6th transmembrane domains (Fig. 2). Salmeterol has an extended binding duration, especially at the seven amino acids on the cytoplasmic side of the 4th transmembrane domain. Agonist binding causes conformational changes in the ADRB2 protein which, in turn, activate the stimulatory G proteins, and so on, down the cascade. The sites of the ADRB2 that interact with the stimulatory G proteins are in the 2nd intracellular loop, a portion of the 3rd loop, and the most proximal portion of the COOH tail (Fig. 2).

The laboratory of **Steve Liggett**, member of the CEG, is actively investigating the effect of *ADRB2* gene polymorphisms on the response of asthma patients to the administration of therapeutic drugs. Single-nucleotide polymorphisms (SNPs; described in Leading Articles in issues #4, #12 & #19 of ***Interface***) and their divergence over evolutionary time, have been studied in the *ADRB2* gene and its 5' flanking regulatory region. Thirteen SNPs were found organized into only 12 *haplotypes* (association of one SNP with another on the same chromosome), out of a possible 8,192 combinations (haplotypes). Four SNPs were found to result in amino-acid changes, of which three resulted in differences in receptor function. Among four ethnic groups, they found a 20-fold difference among the frequencies of the four predominant SNPs. They examined DNA from 23 Caucasians, 19 African-Americans, 20 Asians, and 15 Hispanic-Latinos. Also, 121 Caucasian patients with asthma were enrolled. Measures of lung function, and drug therapy (nebulized albuterol) were obtained.

The Liggett laboratory has also used Chinese hamster ovary cells, and human airway smooth muscle cells in primary culture (obtained at autopsy), in order to investigate whether the polymorphisms in the *ADRB2* gene and the 5' flanking regulatory region affect ADRB2 expression and function. In addition, transgenic mice (having the T164I variant) demonstrated a definite resistance to asthma-like symptoms. Although it might have been anticipated that a change in one SNP would alter function, Liggett and coworkers found the presence of a sole SNP, in and of itself, is not a good predictor of loss of receptor function. Rather, a

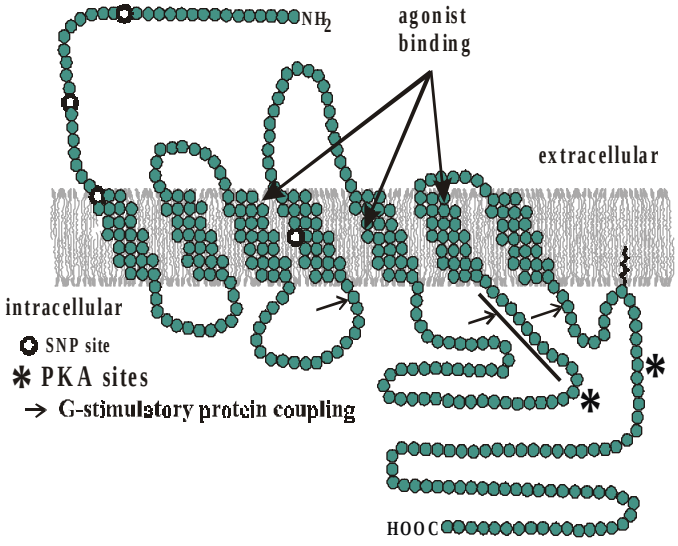

Fig.2

particular haplotype is a much better predictor of receptor function, as well as response to ADRB2 agonist therapy.

Summary

It can be concluded that the *ADRB2* haplotype patterns need to be considered when treating asthma with ADRB2 agonists in human populations. Though there may be fully functional receptors present, which are necessary for rescue of rapidly advancing airway obstruction and reduced air flow in asthma, there appear to be some molecular mechanisms likely to be involved in fatal asthma that are related to ADRB2 dysfunction.

The results of case-control and family studies to date suggest that *ADRB2* polymorphisms might act as disease modifiers in asthma and represent one of numerous genetic variables involved in such a complex disease. As the structure/functional relationships become better understood, rational treatment strategies can be formulated, which would take into account interindividual variations in response to therapeutic agents, and may highlight which particular haplotype patterns are most closely associated with the pathophysiology of asthma. Ultimately, this should allow custom therapies.

--------***Contributed by Marian Miller and Dan Nebert***

Science and Math

Who wants to know, ... secretly, ... a little more about science, health, math, and technology? Aimed at 5th- to 8th-graders, visitors to the site http://www.brainpop.com can watch as many as five cartoon movies for free.

In case you are wondering, the largest prime number to date is ($2^{6,972,593}$ - 1), as you can find out on http://www.isthe.com/chongo/tech/math/number/number.html

Future in Molecular Medicine What do experts expect in molecular medicine in 2020?

- From 500 or so prescription drugs now, at least six times this number will have been identified, tested and commercialized in another 20 years.
- Your medical record will include a copy of your complete genome—to help predict your response to certain drugs and environmental pollutants, as well as to predict susceptibility to specific diseases so that you can alter your lifestyle to prevent or delay such diseases.
- More effective drugs will be developed so that doctors will test individual genetic profiles against panels of drugs and choose the treatment with the greatest potential benefit for each patient.
- The number of mortalities due to adverse drug reactions (~100,000 per year now) will drop dramatically because of this new knowledge.
- If such information is also available to your insurer or employer, however, your risks of developing certain diseases might lead to difficulties in being insured or hired.
- Certain aberrant disease-associated alleles will be replaced with normally functioning versions, and routine neonatal genetic testing for these conditions will lead to successful gene therapy for dozens of “predominantly monogenic” diseases.
- It may be possible by 2020 to “clone” your own organs—thus cutting risks of graft rejection during organ replacement surgery virtually to zero.
- Misguided attempts to ascribe behavioral tendencies to a person’s genes will cause many problems for the courts, which must resolve such disputes when an individual’s behavior and actions conflict with laws.
- We will have a vastly greater understanding of phylogeny and evolution, as the result of more than 1,000 genomes being completely sequenced and compared.
- We will slowly gain some insights into biological complexity, and what it takes to build a functional cell having numerous interactive signaling pathways.

(http://www.tnty.com/newsletter)

Biotechnology.....

What follows is a synopsis of some of the more interesting things that have happened during the first 6 months of 2001, with regard to genetically modified (**GM**) plants, biotechology, and related topics, provided chronologically**:**

January Using a "genome array" (on average, one 25-base oligonucleotide probe per 30 base pairs over the entire genome) of the bacterium *Escherichia coli*, Selinger and coworkers [*Nature Biotechnol* **18:** 1262, 2000] found that 1,529 transcripts (out of 4,290 open reading frames) were differentially expressed under a variety of growth conditions. Sixteen websites related to *E. coli* expression are listed in [*Nature Biotechnol* **18:** 1241, 2000].

A global analysis of 2,709 published interactions between *S. cerevisiae* proteins has enabled the establishment of a single large network of 2,358 interactions among 1,548 yeast proteins [*Nature Biotechnol* **18:** 1257, 2000].

A German group has isolated a bacterium, named CBDB1, similar to *Dehalococcoides ethenogenes*, that is capable of dechlorinating chlorobenzenes [*Nature* **408:** 580, 2000]. Their long-range goal is to enable more effective bioremediation of waste sites contaminated with halogenated hydrocarbons.

There is no evidence that the estimated 3.5 trillion GM plants grown in the U.S. since 1994 have had any measurable ill effects [*Biotechniques* **29:** 832, 2000; *Nature Biotechnol* **19:** 3, 2001].

Since 1994, free radicals generated by β-amyloid have been implicated in Alzheimer disease. Although most of the 42 amino acids making up β-amyloid are fairly unreactive, Met-35 is regarded a thioether and, as such, a good free radical generator. A research team (University of Kentucky) compared wild-type Met-35 β-amyloid with a mutant Norleucine-35 (having a C in place of a S) β-amyloid by transfecting one or the other protein into the worm *C. elegans***;** the nematodes making the normal β-amyloid generated free radicals that damaged their proteins, whereas those making the mutant β-amyloid did not [*Science* **291:** 427, 2001].

February Depending on the environmental stimulus (diet), honeybee larvae have the potential to develop into either queens or workers (http://www.genomebiology.com/2000/2/1/research/0001/). Using microarray technology, Evans & Wheeler [Jan 01 issue of *BioEssays*] examined the differing patterns of gene expression between the worker and queen developmental caste programs. Genes regulating steroid hormone levels, nutrient storage and metabolic rates were most strikingly different [*Nature Genet* **27:** 139, 2001].

When the Human Genome Project was begun (Oct 90), the cost of sequencing a single DNA base was about $10 due to the lack of vital automation tools and high-throughput sequencing technologies. Today, these costs have fallen to 7 to 10 cents per base and are still dropping rapidly.

March Researchers in Mexico City and San Diego have genetically converted the leaves of a plant into petals [*Curr Biol* **11:** 182, 2001]. Well, commercially, florists might like such exotic-looking plants. Mutations in *ABC* genes were previously known to make leaves in place of flower organs. The combined action of the *SEP1,2,3* genes with *ABC* genes of the A and B classes was shown to be sufficient to convert leaves into petals.

April A protocol in which single-base changes can be visualized in single cells has been developed [*Proc Natl Acad Sci USA* **98:** 3940, 2001]. Rolling-circle DNA amplification (RCA) and specific oligonucleotide probes revealed two green nuclear signals representing the wild-type cystic fibrosis *CFTR* gene, two red signals in the homozygous mutant, and one green + one red in heterozygous cells. Similar changes were also shown in the human *TP53, BRCA1* and patched (*PTCH*) genes in single cells.

The tree philodendron (*Philodendron selloum*) generates as much metabolic heat as a 3-kg cat**;** the plant has the ability to maintain its blooms at ~35 ºC, regardless of the ambient air temperature [*Science* **292:** 186, 2001]. The warm flowers release a pleasant scent that attracts the large scarab beetle—the only species that pollinates *P. selloum*. After the beetles arrive, the outer leaf envelopes them, creating what Roger Seymour (University of Adelaide, Australia) calls a "nightclub for beetles." The insects "enjoy themselves" overnight and depart the following morning after being dusted with new pollen**..!**

Taste cells on the tongue recognize five distinct tastes**:** sweet, sour, bitter, salty and umami (or, monosodium glutamate). Last year the genes for receptors that detect bitter and umami were identified. Now, four research groups have isolated a gene believed to code for the sweet receptor [*Science* **292:** 619, 2001], a member of the large G-protein-coupled receptor (*GPR*) superfamily. The gene is defective in "nontaster" mice and normal in "taster" mice**;** studies in human populations are underway to prove this is indeed the human receptor for recognizing "sweet."

When did the malaria parasite become such a

ubiquitous killer in humans? By tracing two genetic mutations [*A-* variant and the *Med* variant of the glucose-6-phosphate dehydrogenase (*G6PD*) gene] that give people anemia but also confer resistance to malaria, a research team concludes that the disease first began to cause a severe effect on humans about 11,000 years ago [*Science* **292:** 628, 2001]. Other groups had looked for clues to the origins of malaria by tracing the evolution of the parasites (*Plasmodium falciparum* and *P. vivax*), or the *Anopheles* mosquitoes that transmit them, but without success.

May RNA interference (RNAi) has been described in issues #16 and #19 of ***Interface*** as a means to "shut off" gene expression in *C. elegans, Drosophila*, plants, and now mammalian cells in culture. A higher success rate at "knocking down" genes in mammalian cell cultures has been achieved [*Nature* **411:** 428, 494, 2001; *Science* **292:** 1470, 2001] using an RNAi intermediate called "small interfering RNA" (siRNA).

Solexa, a biotech start-up company in Cambridge (England), is developing a new technology that it claims—within 2 years—will have "the sequencing capacity of 20 Sanger Centres" and able to sequence an individual's entire genome within a few days [*Nature* **411:** 402, 2001].

Through joint collaborations with the electronics firm Hitachi (Tokyo), software provider Oracle (Redwood Shores, California) and Friedl Corporate Finance (Zürich), Myriad Genetics has formed Myriad Proteomics (Salt Lake City) in an ambitious plan to map and produce a database of the interactions of all human proteins by 2004 [*Nature Biotechnol* **19:** 399, 2001].

The excrement of livestock animals is becoming a major environmental problem. So, transgenic pigs and cattle have been proposed [*Nature Biotechnol* **19:** 415, 2001], which would express a bacterial enzyme that liberates phosphate from animal feed. The result is environmental-friendly, biodegradable farm animal poo**..!**

June The dragline silk of the spider's web has high tensile strength comparable to that of the synthetic superfiber Kevlar but shows high elasticity as well (perfect for bulletproof vests). Production of spider silk proteins (spidroins) in bacteria has shown limited success, whereas the transgenic synthesis in tobacco and potato leaves and potato tubers has resulted in spidroins being at least 2% of total protein in these plants [*Nature Biotechnol* **19:** 573, 2001]**..!**

The U.S. Food & Drug Administration (FDA) has received several reports of alleged allergic reactions to foods such as taco shells containing "StarLink corn," a GM food engineered with the insecticidal protein Cry9C. The U.S. Centers for Disease Control and Prevention (CDC) took blood samples from 17 persons having such complaints but found no evidence for antibodies against Cry9C in anyone [*Nature* **411:** 878, 2001].

Potato blight currently destroys more than 10% of Russia's crop each year. A new variety of GM potato (*New York-121*) that is resistant to blight is entering field trials in the St. Petersburg and Moscow regions this summer [*Nature* **411:** 879, 2001].

The combined impacts (losses of corn, soybean and cotton) in the U.S. of the four major indigenous rootworm species (western, northern southern, Mexican) exceeds $1 billion per year [*Nature Biotechnol* **19:** 624, 668, 2001]. The latest GM corn and cotton, made by two complementary endotoxins introduced by *Bt* technology (http://www.epa.gov/pesticides/biopesticides/biop_fr_date.htm) are making great inroads into controlling these rootworms, caterpillars and cotton boll feeders and benefitting these farmers.

Pilot Projects 2001

LaVerne Mayfield, Greater Cincinnati Occupational Health Center
"Community-based education on environmental health."

Susan Pinney, Department of Environmental Health
"Gene-environment interactions in Parkinson disease."

Sudha Khurana, Department of Cell Biology, Neurobiology and Anatomy
"Effects of xenoestrogens on cell signaling and mitogenesis in prostate cancer cells."

Satish Nair, Department of Cell Biology, Neurobiology and Anatomy
"Determination of the mechanism of action of environmental estrogens in cells harboring a hypersensitive estrogen receptor-alpha mutation."

M. Kathryn Brown, Department of Environmental Health
"Assessing the public's perception of genetic risks and environmental issues: An on-line survey."

Ranjan Deka, Department of Environmental Health
"Linkage disequilibrium in an isolated population."

John Greinwald, Children's Hospital Medical Center,
"Understanding the genetic susceptibility to noise-induced hearing loss in humans."

Greg Oakley, Department of Environmental Health
"Functional characterization of the replication protein A (*RPA*) genetic polymorphism."

15th Anniversary of Chernobyl

On 26 April 1986, the world's worst nuclear accident began at 1:23 am when Unit Four exploded at the Chernobyl Nuclear Power Plant (just south of Pripyat, Ukraine), sending a plume of radioactive material 2 kilometers into the air. As the cloud moved northwest, radioactive particles rained on a swath across Belarus, Poland, the Baltic nations, and Scandinavia. During the next 10 days, the reactor pit emitted 100-200 million curies (about 100 times the amount released by the bombs that hit both Hiroshima and Nagasaki). Two power plant personnel were killed in the blast, and 28 firefighters and plant workers died horrible deaths days later from acute radiation poisoning. Subsequently, many more illnesses, deaths and psychological problems have been blamed on this accident.

Fifteen years later, scientists fear the worst is yet to come. In Belarus more than 700 children under age 14 have thyroid cancer (the usual spontaneous rate is 1 in a million children). Among the 600,000 workers who cleaned up the heaviest contamination in the plant's vicinity and entombed Unit Four's remnants in concrete, a disproportionate rise in endocrine gland disorders and stroke is occurring. The rates for cancer are expected to increase dramatically in the next 1-2 decades. Many studies of plants and animals heavily exposed to radiation in the "Exclusion Zone" have found chromosome breaks and high rates of mutations; some nearby regions designated as "control areas" had to be changed because they were found to have plants and animals with increased mutation rates and therefore were in fact contaminated far above background. The cost of cleaning up all the radiation is expected ultimately to exceed $20 billion. Many believe that the Chernobyl disaster was "one of the final nails in the coffin" that helped to destroy the former Soviet Union.

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be nothing.

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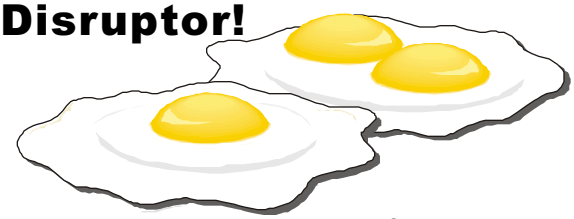
Environmental Endocrine Disruptor!



Are chickens that are fed hormones to increase egg laying more likely to produce eggs with double yolks than chickens not being fed hormones? Is this analogous to the way people taking fertility drugs produce quintuplets, quadruplets, etc? The eggs I buy from Cub Foods have at least two double-yolked eggs in each dozen, while the eggs I buy from Kroger virtually have none.

"Q"uote of the Month.....

If you eat a huge roast chicken,
And I nothing at all,
According to statistical theory,
We have each eaten half a chicken.

--from "La statistica" by Carlo Alberto Salustri (1871-1950), a famous dialectal Roman poet better known as 'Trilussa'.

Observations by a Biologist

One in 16,000 babies is born with severe **birth defects** called *cyclopia* (having a single eye, like Cyclops) and *holoprosencephaly* (HPE, fusion of front brain/face). The incidence might be as high as one in 250 pregnancies, but most might abort spontaneously during the first trimester. Babies with the severest form die within a few days after birth, whereas people with the mildest form survive, having a single upper front incisor. An epidemic in sheep of cyclopia with associated HPE [*Am J Vet Res* **24:** 1164, 1963] was found to be caused by their eating the subalpine lily *Veratrum californicum*, and the chemical structures of the active teratogenic agents (cyclopamine, veratosine) were noted to be very similar to that of cholesterol [*Teratology* **1:** 5, 1968]. It was then found that mouse embryos lacking a normal sonic hedgehog (*Shh*) gene show cyclopia and HPE-like defects [*Nature* **383:** 407, 1996]. Furthermore, HPE associated with the Smith-Lemli-Opitz syndrome was found to be due to a defect in Δ7-dehydrocholesterol reductase activity [*N Engl J Med* **330:** 107, 1994]. Although all the details have yet to be understood [*Bioessays* **23:** 888, 2001], it is now clear that these plant teratogens derail the normal SHH-mediated trafficking of cholesterol within cells during the crucial development of the embryonic anterior brain of mammals—including humans**..!**

The larvae of **jewel beetles** (genus *Melanophila*) can develop only in the wood of trees freshly killed by fire. To be successful, the beetles need to approach forest fires from distances as far away as 50 km**..!** It was found [*Nature* **398:** 298, 1999] that the *Melanophila* use two sensory systems to identify fire**:** [a] thoracic infrared receptors that detect heat, and [b] olfactory receptors on their antennae that can sense guaiacol derivatives (volatile phenolic compounds from smoldering wood) at levels as low as a few parts per billion. This is sufficient for the beetle to detect "a single pine tree 30 cm in diameter that has smoldering bark to a height of 2 m, a bark depth of 1 cm, releasing about 7 g of guaiacol per hour under light wind conditions from a distance of more than 1 km." Beetles not of the *Melanophila* species have neither kind of sensory system.

The pedigree of a number of the world's most renowned grapevine varieties, or cultivars, in making the most outstanding **wines**—has been fiercely disputed for decades, if not centuries. Finally, using DNA fingerprinting techniques, similar to those used by criminologists or to settle paternity suits, scientists at the University of California (Davis) tested 322 French grape varieties**;** they found that 18 varieties that have long been grown in northeastern France (including the best of the whites, Chardonnay, as well as reds such as Pinot and Gamay Noir) are remarkably close relatives [*Science* **285:** 1470, 1562, 1999]. In fact, 16 of the varieties might be the offspring of one highly-prolific pair of parents, Pinot and Gouais Blanc.

Perhaps 50 million years ago, a lone species of South American **leaf-cutter ants** (*Atta* species) changed from its primitive hunter-gatherer lifestyle to adopt an agrarian way of living. These agricultural pioneers learned to weed, manure and propagate their fungal crops, ensuring a reliable source of food. From these innovative ancestors have now arisen at least 210 Attini species—largely concentrated in wet South American rain forests. Colonies of some Atta species can number 8 million, with the collective biomass of an adult cow. Although the ants cut leaves and bring them home, they do not eat the leaves but, rather, chew them into a pulp substrate on which their fungal crops are grown. The fungus, in turn, produces gongylidia which serve as food for the ants. This symbiosis combines the ants' ability to circumvent the various

plant antifungal defenses (*e.g.* waxy coatings of leaves, which the ants scape away) with the ability of the fungus to subvert plant anti-insect defenses (*e.g.* chemical insecticides and phytolexins, which are discarded by the fungus and so are absent from the fungal tissue actually consumed by the ants). This symbiosis is now realized to involve the evolution together of three partners from three separate kingdoms—ant, fungus and an antibiotic-producing bacterium—as well as a parasite fungal weed that infects the attine gardens [*Nature* **398:** 701, 747, 1999].

Colonization of the human stomach by the bacterium *Heliobacter pylori* is now known to be a predisposing factor for gastrointestinal disease such as gastritis (stomach infection) and **peptic ulcers**. Since most infections are asymptomatic, however, could it be that *H. pylori* may actually provide beneficial effects to infected carriers who are heavily exposed to other gastrointestinal pathogens? It turns out that *H. pylori* possesses antibacterial activity to which it is itself resistant [*Nature* **398:** 671, 1999], and the activity is due to cecropin-like amino-terminal peptides derived from the ribosomal protein L1 (RpL1). When its host is invaded by other nasty bugs, *H. pylori* undergoes an "altruistic lysis," which appears to release cecropin-like peptides that are active against faster-growing microorganisms found there**..!** The data suggest that cecropins might have evolved from an early *RpL1* gene in a prokaryote that passed from being an intracellular parasite, to a symbiont, ending up as an organelle.

A signaling pathway that promotes in the worm (*C. elegans*) a long **life-span** also operates in the fruit fly (*Drosophila*)[*Science* **292:** 41, 104, 107, 2001]. At the heart of the pathway lies the *daf2* gene, which encodes a member of the insulin family of cell-surface receptors.

Example of Finding a Gene in the Sequenced Genome

If DNA were really made up of letters, and if a gene makes a word, then the sequence in the Human Genome Project might "evolve" as follows.

The 3.165 billion bases of the haploid human genome was sequenced in thousands of segments, and these overlapping fragments were resolved (or still are being resolved) into the correct orientations and the proven creation of a functional gene that gives a gene product (protein).

Original fragments found (some out of order, some inverted):

wluy**i** shig**n** junkk c**r**ce lrr ccs**a** rhizq mk**f**h eeq qanb v**c**w **e**dt lke**t** ty**e**gn sagfd desllv

More sequence added, contaminating sequences deleted, and proper orientation of all real DNA fragments figured out**:**

vllsed wluy**i** shig**n** **t**ekl ng**e**yt c**r**ce lrr ccrhizq mk**f**h**a**s eeq qanb dfgas v**c**w dt**e** vlls

Transcribed region of gene delineated**:**

luy**i** shig**n** **t**ekl ng**e**yt c**r**ce mk**f**h**a**s v**c**w dt**e**

Final DNA sequence of the gene**:**

i n t e r f a c e

Of course, in the real world, DNA comprises just 4 bases (A, C, G, & T), instead of 26 letters. The DNA is transcribed into a large transcript, which then gets spliced to form a messenger RNA (still having just 4 bases, A, C, G, & U), which then gets translated into proteins (containing any of the 20 amino acids).

Genomics and Clinical Medicine.....

What follows is a synopsis of some of the more interesting things that have happened during the first 6 months of 2001, with regard to genomics and clinical medicine, and related topics, provided chronologically**:**

January The olfactory receptor (*OR*) superfamily represents the largest known gene family in mammalian genomes [*Genome Biol* **2:** 18, 2001], numbering about 1,000 members, the majority of which appear to be pseudogenes in humans—presumably because we don't use the sense of smell for nearly as many things as rodents, etc. The inability to taste phenylthiourea is an autosomal recessive trait described almost 70 years ago [*Ohio J Sci* **32:** 436, 1932] as one of the first human pharmacogenetic differences. It is very likely that the "nontaster" phenotype reflects a defect in one or more of the "bitter-sensing" *OR* genes [*Science* **291:** 557, 2001].

February Moderate drinkers of alcohol, whose alcohol dehydrogenase-3 (ADH3) enzyme is encoded by the homozygous slow-oxidizing *ADH3*G2* allele, exhibit higher HDL levels and a substantially decreased risk of myocardial infarction [*N Engl J Med* **344:** 549, 2001].

April Millions of liters of Agent Orange and other dioxin-containing herbicides were sprayed on Vietnam and Cambodia between 1962 and 1971. Two studies suggest that parents of children with acute myelogenous leukemia (CML), which represents 8% of childhood cancers, were more likely to have served in the Vietnam War or used pesticides in the workplace. Another study found a high prevalence of disease among children of Australian veterans of the Vietnam War. Earlier studies had been successful in showing such an association [*Nature* **410:** 1016, 2001].

"What is the heart of conservatism if it does not include leadership in conservation? And why have conservative thinkers needlessly, and against all logic and their own self-interest, surrendered the moral high ground on this issue to the liberals?" —Harvard biologist E. O. Wilson in a talk about species extinction at the American Enterprise Institute, a prominent conservative think tank, Washington, D.C.

June Some say that there are enough publicly available SNPs to create a genome-wide SNP marker map**;** the obstacle to disease gene identification now lies in genotyping. Linkage disequilibrium (**LD**) is the association between neighboring SNPs and alleles in the genome. A short LD (short region occupied by a particular group of SNPs) would be seen in populations that have undergone long-term uninterrupted expansion. A long LD would suggest a relatively new population that has arisen from what was once only a few people. Analyzing 19 randomly selected genomic regions in a population of Northern European descent and a Nigerian population, researchers (from Cambridge, Massachusetts, and Oxford, England) found that Northern Europeans but not the Nigerians have a long LD—with SNP groups occupying an area 8 times larger than the previous estimate of 3,000 bases. The researchers speculate that there must have been some bottleneck event to cause the long LD, and they suggest that as few as 50 individuals between 800 and 1600 generations ago may have founded the population from which most people of Northern European descent have arisen [*Nature* **411:** 199, 2001].

A language instructor was explaining to her class that in French, nouns unlike their English counterparts, are grammatically designated as masculine or feminine. " House," in French, is feminine-- "la maison." "Pencil," in French, is masculine-- "le crayon."

One puzzled student asked, "What gender is computer?"

The teacher did not know, and the word wasn't in her French dictionary. So.....for fun she split the class into two groups appropriately enough, by gender and asked them to decide whether "computer" should be a masculine or feminine noun. Both groups were required to give four reasons for their recommendation.

The men's group decided that computers should definitely be of the feminine gender (la computer) because:

1. No one but their creator understands their internal logic;
2. The native language they use to communicate with other computers is incomprehensible to everyone else;
3. Even the smallest mistakes are stored in long-term memory for possible later retrieval; and
4. As soon as you make a commitment to one, you find yourself spending half your pay check on accessories for it.

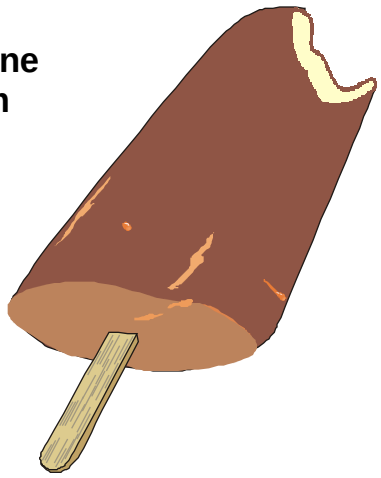
The women's group, however, concluded that computers should be masculine (le computer) because:

1. In order to get their attention, you have to turn them on;
2. They have a lot of data, but are still clueless;
3. They are supposed to help you solve problems, but half the time they ARE the problem; and
4. As soon as you commit to one, you realize that if you'd waited a little longer, you could have gotten a better model.....(**the women won**).

SCIENCE LITE

Handy Engineering Conversions

- 2.4 statute miles of intravenous tubing at Yale University Hospital: 1 IV League
- Time between slipping on a peel and smacking the pavement: 1 bananosecond
- Time it takes to sail 220 yards at 1 nautical mile per hour: Knot-fur long
- Ratio of an igloo's circumference to its diameter: Eskimo Pi
- 16.5 feet in the Twilight Zone: 1 Rod Serling
- Shortest distance between two jokes: A straight line
- Weight an evangelist carries with God: 1 billigram
- 1 millionth of a mouthwash: 1 Microscope
- 1 kilogram of falling figs: 1 fig Newton
- 2000 pounds of Chinese soup: Won ton
- 453.6 graham crackers: 1 pound cake
- 1000 grams of wet socks: 1 literhosen
- Half of a large intestine: 1 semicolon
- Basic unit of laryngitis: 1 hoarsepower
- 1000 aches and pains: 1 megahurtz
- 1 million bicycles: 1 megacycle
- 365.25 days: 1 unicycle



The Washington Post's Style Invitational asked readers to take any word from the dictionary, alter it by adding, subtracting or changing just one letter, and supply a new definition. Here are some recent winners:

Reintarnation: Coming back to life as a hillbilly.

Foreploy: Any misrepresentation about yourself for the purpose of obtaining sex.

Giraffiti: Vandalism spray-painted—very, very high

Tatyr: A lecherous Mr. Potato Head.

Sarchasm: The gulf between the author of sarcastic wit and the recipient who doesn't get it.

Inoculatte: To take coffee intravenously when you are running late.

Hipatitis: Terminal coolness.

Osteopornosis: A degenerate disease.

Burglesque: A poorly planned break-in. (See: Watergate)

Karmageddon: It's like, when everybody is sending off all these really bad vibes, right? And then, like, the Earth explodes and it's like a serious bummer.

Glibido: All talk and no action.

Dopeler effect: The tendency of stupid ideas to seem smarter when they come at you rapidly.

Intaxication: Euphoria at getting a refund from the IRS, which lasts until you realize it was your money to start with.

If one is not part of the solution, one is part of the precipitate.

Fax News, cincinnati, Downtown/Clifton Edition, 11-20-01

The problem with the gene pool is that there is no lifeguard.

Fax News, 11-5-2001

CEG Members in the News

Tatiana Foroud gave invited presentations/lectures/ workshops at the First International Workshop on the Genetics of Bone Disease, Davos, Switzerland entitled ***"Genome search for QTLs contributing to normal variation in bone density"*** (March 2001) and at the Inherited Disease Research Branch, National Human Genome Research Institute, Center for Inherited Disease Research, National Institutes of Health, Baltimore MD which was about ***"Mapping of genes contributing to peak bone density"*** (May 2001).

George Leikauf presented a keynote lecture at the NIEHS Center at University of Arizona, Tucson,AZ entitled ***"Genomic wide approaches in toxicology"*** (January 2001) and at the Into the New Millennium: The AstraZeneca COPD Symposium , Lund, Sweden, entitled ***"Mucin apoprotein in models of chronic obstructive pulmonary disease"*** (April 2001). He delivered an invited lecture on ***"Utilization of cDNA Microarray In Environmental Genetics"*** at the National Academy of Science, Irvine, CA (May 2001) and entitled ***"Differential gene expression systems in lung injury"*** to the American Thoracic Society, San Francisco, CA (May 2001). The keynote address was presented by him at the Mickey Leland Center, Houston, TX entitled ***"Hazardous air pollutants in susceptible populations"*** (May 2001) and an invited presentation entitled ***"Strategic transgenesis"*** was delivered at the Eighth International Inhalation Symposium, Hanover Medical School, Hanover, Germany (June 2001). An invited lecture on ***"Acute lung injury: functional genomics and genetic susceptibility"*** was delivered at the Thomas Petty Aspen Lung Conference, Aspen, CO (June 2001).

Grace Lemasters received a training grant in Molecular Epidemiology of Children's Environmental Health and is about to begin a 5-year study of the environmental exposure to diesel exhaust and indoor aeroallergens and childhood atopy.

Dan Nebert was an invited speaker in the symposium on "Pharmacogenetics and Drug Metabolism" at the 21st Annual Meeting of the Society of Toxicology in San Francisco (March 2001)**;** an invited speaker at the National Institute of Child Health and Human Development/Food and Drug Administration (NICHD/FDA) Workshop on "Adverse Drug Reactions in Pediatrics" in Bethesda, MD (April 2001)**;** the keynote speaker at the Penn State University Life Sciences Consortium's Colloquium in Hershey, PA (April 2001)**;** and an invited speaker at a symposium on "Transcription Factors and Cancer" at the 9th International Union of Toxicology (IUTOX) Congress of Toxicology in Brisbane, Australia (July 2001).

Susan Pinney presented a poster entitled ***"Cancer incidence in a population living near a nuclear materials processing plant at Fernald, Ohio,"*** at the Congress of Epidemiology, Toronto, Ontario, Canada (June 2001).

Alvaro Puga (Professor; Department of Environmental Health), accepted the invitation by College of Medicine Dean **John Hutton** to set up the University of Cincinnati's ***Genomics & Microarray Laboratory***, one of several new Cores at the Medical Center that play a crucial role in boosting biomedical research. This facility is part of a broader effort at UC that already involves hundreds of scientists, hundreds of millions of dollars in grants, and infinite possibilities for advancements in genetic research. "Cincinnati could become one of the 10 to 12 major centers for biomedical research nationwide," said **Donald Harrison** (Senior Vice President and Provost for Health Affairs at the UC Medical Center). "This is the growth industry of the future, for the next two decades, for the entire country," Dr. Harrison said. "Every medical center in the country is trying to expand in these areas." UC hopes to double its biomedical research funding to about $286 million-per-year by 2006, while adding hundreds of researchers and support staff.

"The hardware was purchased in late 1999," said Puga, "After several months of testing and troubleshooting, the lab started producing data in February 2000 that have been published." The Genomics & Microarray Facility includes a $25,000 DNA amplifier (which creates samples of genetic material), two $80,000 robots (that process the samples and prepare microwell racks of as many as 384 test tubes), a $65,000 microarrayer (which takes only 12 h to "print" up to 10,000 genes at a time onto a single glass microscope slide). Before 1999, it took experts days to perform such work on just a single gene.

A $50,000 piece of hardware contains a dual set of lasers that scan all those genes, so they can appear on the computer as a grid of multicolored dots. These dots—each no bigger than 1/250th of an inch—show which genes get turned on and which ones get turned off, when a sample of healthy tissue is exposed to a drug, chemical, or disease-causing virus, bacteria or toxin. The dots also can show how diseased tissue responds to a medication under development (*e.g.* it can determine which therapy is best for a particular patient with leukemia or cancer). Once the data are scanned, the microarray lab staff burns a CD of the data and ships it off to the researcher who needs it.

"As scientists gain understanding of the complex genetic circuitry revealed by the dots, entire realms of treatment options emerge," **Puga** said. "This is the kind of work that has allowed researchers nationwide

to discover genes linked to many kinds of disease, from breast cancer to Alzheimer's. This is the technology that helped develop the breakthrough chemotherapeutic drug, Gleevec, recently approved by the Food & Drug Administration (FDA) for wide-scale use."

"The newer, faster technology and completed gene maps mean the work is coming at scientists faster each year, each month," said **Nebert** (Professor, Department of Environmental Health). "The rate of research accomplishments has leaped yet again this summer, with the installation of a $2.3-million supercomputer at Children's Hospital. This computer allows faster interpretation of data from the microarray lab. In the 1980s, the average graduate student or postdoctoral fellow in a university lab could isolate and analyze between 15 and 100 bases of DNA a week, he said. But now, in summer of 2001, we are able to analyze more than 1 million bases a week."

Researchers are using UC's microarray lab to delve into the causes and possible treatments of cystic fibrosis and asthma, better treatments for head-and-neck cancers, analyses of rare types of hemophilia, genetic links to lung cancer, and a new understanding of how people respond differently to the same dose of a drug or environmental chemical or how people may be affected by mixtures of toxicants found in Superfund toxic waste dump sites. **Nebert** envisions a day "when people might have their genetic code scanned like a bar code, similar to what is now in grocery stores, so that doctors could detect health problems long before serious symptoms appear. Such data could also help doctors know exactly which drugs may best treat health problems, and which ones might cause harmful side effects. None of this would be possible without the advances in computers and robotic technology at work in the new Microarray Laboratory." Yet, as powerful as that equipment may be, it has limitations. "These machines speed up work, but they cannot replace good, sound science," he said. "You still have to interpret the results. The scientist still has to have vision to develop solutions to what the computer is telling him."

Puga was also a member of the Program Committee, chaired a symposium and gave a presentation on the ***"Role of the aryl hydrocarbon receptor,"*** at the meeting of the Society of Toxicology, San Francisco, CA (March 2001). He gave an invited talk on ***"Role of the aryl hydrocarbon receptor in cell cycle regulation"*** at the 9th International Union of Toxicology Congress in Brisbane, Australia (July 2001). He is now a permanent member of the ALTox-1 Study Section.

Daniel Prows started a new faculty position as Assistant Professor in the Division of Human Genetics, Children's Hospital Research Foundation. He presented invited seminars at the American Thoracic Society (ATS) International Conference, Postgraduate Course-10, San Francisco, CA (May 2001) ***"Genetic analysis of lung development and injury using mouse models: use of recombinant inbred mice in the genetic analysis of acute lung injury"*** and ***"Combining cDNA microarray and quantitative trait locus analysis to identify candidate genes for nickel-induced acute lung injury."*** At the National Institute of Environmental Health Services, Seventh International Congress on "Combustion By-Products: Origins, Fate, and Health Effects," he presented an invited seminar entitled ***"Genetic susceptibility to nickel-induced acute lung injury"*** (June 2001) and also at the Children's Hospital Medical Center, Cincinnati, OH, Summer Genetics Institute he gave a talk on ***"Pharmacogenetics: tailoring genes to suit your needs"*** (June 2001).

Nancy Steinberg-Warren was elected to the Accreditation Chair, American Board of Genetic Counseling and received funding from Speaking of Women's Health and Southwestern Ohio Chapter of the March of Dimes for support of the Greater Cincinnati/Northern Kentucky Start Healthy Project. She has partnered with the Every Child Succeeds Program of Children's Hospital Medical Center to provide folic acid education to in-home visitors (nurses and social workers) who, in turn, teach about 1500 first time mothers, and mothers-to-be about the benefits of folic acid to babies (prevents birth defects) and to adults (decreases risks for heart disease and cancer).

Yolanda Sanchez (Assistant Professor; Department of Molecular Genetics, Biochemistry & Microbiology), (MGBM) has been named "One of the 20 most promising biomedical researchers in America" by The Pew Charitable Trusts (June 2001). Sanchez received $240,000 from the Trusts to help support her research over a 4-year period. The Pew Scholar Awards are granted to researchers and investigators that show outstanding promise in the basic and clinical sciences, and are intended to encourage scholarly innovation. The awards provide flexible support to scholars as they establish their laboratories and continue their research—in areas from AIDS to cancer, and from childhood infectious diseases to diseases affecting the elderly. Sanchez received the award for her research in understanding how cells respond to DNA damage. "This area of research may lead to better cancer treatment in the future," said Jerry Lingrel, MGBM Chair. Sanchez is the third UC researcher to receive a Pew Scholar Award during the last decade.

LETTERS TO THE EDITOR

RESPONSES/COMMENTS TO VARIOUS QUESTIONS

Q **This morning in the newspaper was an article about a new pill as a potential cure for cancer. It's called STI-571, or "Gleevec." What is your opinion of this new drug? And how does it work?**

A *Taken orally as a pill, Gleevec is a small chemical designed to slip into, and jam, the cancer cell's signaling proteins. In the 30,000 to 40,000 genes in the human genome, possibly one-fifth make proteins that are involved in "signaling" (cell division, tissue growth, migration, contact with one another, etc.). The astonishing feature of Gleevec's success is that cancerous cells pack up and die—even though the drug's role is to shut down only one of their signaling proteins; it seems that cancer cells have become so dependent on this one hyperactive signaling pathway that its loss is a lethal blow. On the contrary, normal cells appear to be totally unaffected by having just this one signaling pathway shut down. Gleevec seems to be a smash hit, although it has been proved so far to work successfully on only two relatively rare cancers, chronic myelogenous leukemia (one of four kinds of leukemia) and gastrointestinal stromal tumor (a deadly type of relatively rare stomach cancer).*

COMMENT **I was pleased to read issue #20 of *Interface*. A nice job. However, the item (from the Internet(!) about "drinking sufficient amounts of water" is, as far as I can see, one of those "urban legends" that we are warned about. The National Academy of Sciences says about 8 glasses of water are needed daily but we get most of this in the prepared foods we eat. I don't think the Internet is a source of reliable items unless there is strong additional attribution. Nice publication, otherwise.**

While some might not feel the internet is a good source of information on water, its message is still valid. We all know that caffeinated and highly concentrated electrolyte fluids, as well as alcoholic beverages, do not count as "glasses of water." Check out the article "Water: an essential but overlooked nutrient (*J Am Diet Assoc* **2**: 200-206, 1999), which states that "dehydration of as little as 2% loss of body weight results in impaired physiological and performance responses...." The article goes on to list disease risks which relate to fluid consumption. In addition, the American Dietetic Association states in response to the question "Do you know how much water you need every day to keep your body health?" The pat answer, eight--8 ounce glasses, is generally correct. The real answer depends upon your body size, activity level, and the air temperature. Fluid needs are higher when the temperature is very high or very low, higher when performing strenuous activity and higher for men because they have more muscle mass. Get the appropriate amount of fluid you need by starting with the minimum eight--8 ounce glasses per day and add 1 to 3 glasses per hour of activity. The ADA also states that by the time you are thirsty, you have lost about 1% of your body fluid; between 2-5% - dry mouth, flushed skin, fatigue, headache, impaired physical performance, you are half way to the 11% where kidney function begins to fail. The bottom line.....being fat at the computer requires less water than being a lean mean active machine.

Q **In your Leading Article "Racial and ethnic differences in our genes" (issue #19), I take serious exception to the 3rd and 4th lines that state "... every gene has evolved because of a function that is useful to the organism." Could this really be true?**

A *Absolutely. New genes "appear" all the time with each new generation; this happens because of gene duplications, unequal crossing-over, mutations, genetic drift, etc. Someone in Cincinnati, for example, might have two copies of the GHR gene encoding the receptor for growth hormone. In his/her children or grandchildren, if there is no need for two genes doing the same thing, one of the genes will start to "fall apart." If, during the process of "disappearing," the mutated gene encodes a protein having a beneficial effect on the person, this new mutated gene will become "fixed" and be passed on to future generations. And "beneficial" here means better health, more resistance to some infection (or plant metabolite in the food we eat), more viability during pregnancy or childhood—anything that promotes reproduction of a healthier offspring (but there is no need to promote genes that lead to increased healthiness later on in life, after the reproductive period!). It has been estimated [Nature Genet* ***27:*** *234, 2001] that, based on 6 billion people on this planet and a mutation rate of 2 x 10^{-8} per base per generation, every site at which mutations are compatible with life has been mutated, on average, 240 times in just the most recent generation. And, of course, the same has*

happened to every previous generation on Earth. And this is how new genes appear and become fixed: because the animal (or plant, bacterium or virus) can use the new gene to benefit its survival/ reproduction in its environment. If there is no useful function, the gene in that species will no longer be needed and disappear.

Ethical, Legal and Social Issues.....

What follows is a synopsis of some of the more interesting things that have happened during the first 6 months of 2001 with ethical, legal and social issues (**ELSI**) related to the Human Genome Project, provided chronologically**:**

January The U.S. and Vietnam are planning joint research on dioxin—which has contaminated southeast Asia as a by-product of the defoliant Agent Orange during the Vietnam War. Suggested topics include the possible effects of dioxin on the rates of cancer, birth defects, developmental and neurological diseases, as well as new methods for assaying and analyzing soil and water residual contamination (http://www.niehs.nih.gov/oc/factscheets/dioxin.htm).

In a great disservice to anthropology, journalist Patrick Tierney wrote a controversial book about the Yanomami Indians of South America, *Darkness in El Dorado: How Scientists and Journalists Devastated the Amazon* (W.W. Norton) that was discussed at the annual meeting of the American Anthropological Association [*Science* **291:** 416, 2001]. Several major concerns are described in the book**:** [a] how a 1968 measles epidemic that killed "hundreds, perhaps thousands" of Yanomami might have been exacerbated or even unintentionally caused by James V. Neel's group giving a "questionable" measles vaccine**;** [b] how the researchers studying this tribe (especially Neel of University of Michigan, Napoleon A. Chagnon of University of California Santa Barbara, and documentary filmmaker Timothy Asch) "conducted unethical genetics studies"**;** [c] how Chagnon's characterization of the Yanomami as "fierce" and violent was erroneous and actually promoted the Indians to fight**;** and [d] how the anthropologists directly disturbed the Yanomami economy by "providing goods that actually upended traditional political balances, debased wealth-seeking villagers and even led to murderous conflict." These charges by Tierney are vehemently attacked as "lies" by many of the 3 dozen anthropologists who have studied this tribe, the least westernized peoples of the Americas. Dr. Neel tried to prevent the measles epidemic, for example, by going out of his way to vaccinate thousands**;** experts argue that the vaccine could not have touched off the epidemic. Genetics studies could hardly be regarded in the 1960s as "unethical" because standards for informed consent forms did not prompt federal regulations until 1974, following the 1971 revelation of the Tuskegee experiment (Alabama) in which syphilis patients had been deliberately left untreated.

For the first time, a for-profit group—the First Genetic Trust, Inc. of Deerfield Park, Illinois—announced a plan to act as an intermediary between patients and researchers [*Science* **291:** 575, 2001]. Because many people fear their genetic data won't be kept confidential, this company hopes to alleviate the patients' concerns. First Genetic Trust has joined with the Memorial Sloan-Kettering Cancer Center (New York City) to test the scheme. CEO Arthur Holden says he hopes to create a "structure with Swiss bank-grade security" to hold confidential deposits of genetic information about research subjects and other patients. Holden also chairs the SNP Consortium, a non-profit collection financed in part by pharmaceutical companies to collect data on variations in the human genome that might be used to track down disease genes (discussed in previous issues of ***Interface***). Robert Gellman, a consultant on patient privacy issues, sees "nothing but problems," noting that the trust's policies are undefined. "This could turn out to be more complex than the present system of controlling access to medical data, which does not require researchers who are using anonymous data to seek the consent of each individual."

The final report of the U.S. Secretary's Advisory Committee on Genetic Testing (SACGT) on the overseeing of genetic tests is available at http://www4.od.nih.gov/oba/. One interesting point is that "predictive tests, or those for low-penetrance conditions, present a greater ethical challenge than do diagnostic ones and therefore will require a higher level of scrutiny."

February Patenting and licensing of inventions are usually necessary steps in converting scientific discoveries into useful products. The Howard Hughes Medical Institute has developed policies [*Science* **291:** 989, 2001] to strike a balance between "enabling the scientist to interact productively with companies" and "minimizing potential distractions and conflicts from commercial entanglements." First is a 5% limit on equity that a scientist can hold in a company. Second, scientists cannot both consult for and collaborate with the same company. Third, the company must ensure that these arrangements do not intrude on the scientist's research autonomy.

March Prior to books and libraries, the passing down of stories from one generation to the next was the way humans have always done to preserve

their culture. This interesting perspective is described by Jared Diamond [*Nature* **410:** 521, 2001]. This phenomenon has been and is at least as valuable to these tribes as DNA banks are to modern researchers today.

The U.S. Equal Employment Opportunity Commission (EEOC) went to court, alleging that Burlington Northern Santa Fe Railroad (BNSFR) had required workers with work-related carpal-tunnel syndrome (a disorder causing pain in the wrist) to provide blood samples for genetic testing. At best, the validity of a cytogenetic aberration on chromosome 17 (which affects 2-5 people of every 10,000) is questionable as a valid predictor for the risk of developing wrist injuries**..!** BNSFR argued that the genetic test could "prove" that the injury was genetic rather than work-related. The EEOC contended that such genetic tests violate the "Americans with Disabilities" Act. After 3 days of court hearings, BNSFR announced it would drop these genetic tests [*Nature Genet* **27:** 243, 2001].

April Jerry A. Coyne (University of Chicago) was asked to review the book *Icons of Evolution: Science or Myth? Why Much of What We Teach about Evolution Is Wrong* [*Nature* **410:** 745, 2001] by Jonathan Wells. It makes for fun reading! Coyne concludes with "Wells has it backwards. It is creationists like him who are conspiring to purge evolution from American education."

More recommended reading**:** Daniel Fox's review of Sally Satel's book *PC, M.D.: How Political Correctness Is Corrupting Medicine* (2000 publication date) [*N Engl J Med* **344:** 462, 2001]. And her challenge of his review, and his compelling response [*ibid* **344:** 1170-71].

A United Kingdom parliamentary committee has called for a 2-year moratorium on the use of genetic testing by insurance companies [*Nature* **410:** 733, 2001]. They concluded that insurers are "more interested in establishing their future rights to use genetic test results in assessing premiums, than in whether they are reliable or relevant."

May This subject [*Science* **292:** 1315, 2001] was touched upon in issue #20 of ***Interface***. Is there any relationship between the number of genes in an organism's genome and its complexity? If the tiny mustard plant has almost as many genes as humans—and it looks like rice and lily might have more than humans—where does that place us? Perhaps the "connectivity of gene-regulation networks" correlates better with complexity.

Laws and policy discussions about cloning human beings appear to be "an ill-informed response to media hype and are supportive of the current trend toward accepting genetic determinism" [*Nature Biotechnol* **19:** 403, 2001].

If inheritable genetic modifications (IGMs) become accepted by the public, it would likely be through infertility clinics. Yet, this industry is virtually unregulated, it has been averse to public scrutiny, and its practices have been subject to severe criticism and law suits [*Science* **292:** 1303, 2001].

A report by the National Bioethics Advisory Commission (NBAC) adds to the growing criticism of the way in which research involving humans is regulated in the U.S. See http://www.bioethics.gov.

The U.S. Supreme Court decision could compromise biotech patents [*Nature Biotechnol* **19:** 394, 2001]. This decision narrows the patentee's protection against infringement to only those products that "literally" infringe upon the patent claims.

For more fun reading [*Nature Genet* **28:** 15, 2001], see the book report by James Gould (Princeton), who assessed Avital and Jablonka's book *Animal Traditions: Behavioral Inheritance in Evolution* (publication date 2000).

June Standing on two feet (bipedality) is the key evolutionary event that allowed the redirection of breathing in the service of making sounds, concluding that humans obviously walked before they began to converse with one another. This is called "the Walkie-Talkie Theory" [*Science* **292:** 2429, 2001].

Is it possible that the choice of which car one prefers to buy [*Nature Genet* **28:** 109, 2001] is genetically determined?

President Bush announced plans to ban genetic discrimination by employers and insurers [*Nature* **411:** 985, 2001]. A new bill is expected to pass through Congress and be signed before year's end. More on consent and privacy issues is discussed [in *Nature Genet* **28:** 195, 207, 2001].

What expressed sequence tags (ESTs), single-nucleotide polymorphsism (SNPs) and open-reading frames (ORFs) can be patented? Patent specifications should disclose functional and structural details sufficient to satisfy the utility, written description, and enablement requirements of the patent statute [*Nature Biotechnol* **19:** 683, 2001].

If you can't be big, don't belittle.

Genomically Speaking.....

What follows is a synopsis of some of the more interesting things that have happened during the first 6 months of 2001 with the Human Genome Project (**HGP**), and related genomics news, provided chronologically**:**

January In a public statement, *Science* announced it was parting from the tradition of depositing published sequences in GenBank/EMBL/DNA Database of Japan and allowing a company (**Celera**) to retain control of access to the sequence described in a published paper. Many researchers are crying "foul play" [*Nature Biotechol* **19:** 1, 2001].

Jean-Michel Claverie wrote a very insightful letter [*Nature* **403:** 12, 2000], proposing that a new large international center is needed to coordinate the annotation of the human genome in a highly organized and orderly fashion.

The demand for more sophisticated Bio-Informatics solutions is expanding opportunities for biotechnology start-ups, but traditional information technology (IT) companies are also moving into the market [*Nature Biotechnol* **18:** 1247, 2000].

Functional assignments for newly discovered proteins have been made by partnering them with proteins of "known" function via analysis of large experimental data sets for co-regulation of mRNA expression (DNA microarrays, SAGE) or protein-protein interactions (2-hybrid analysis, mass spectrometry). Now, computational biology-driven approaches, combined with these experimental data sets, is offering the best success rates [*Nature Biotechnol* 2000; **18:** 1242]. Protein network databases in progress include**:** BIND at http://binddb.org and DIP at http://dip.doe-mbi.ucla.edu.

Using microarray of about 25-30% of all 26,000 genes of the tiny mustard plant *Arabidopsis thaliana*, scientists have determined an "expression profile" in response to systemic acquired resistance [*Nature Genet* **26:** 403, 2000].

To determine gene function, it is best to develop mutants of that gene. Large-scale projects, using ethylnitrosourea (ENU) mutagenesis, are being developed for both the zebrafish [*Mamm Genome* **11:** 511, 2000] and the mouse [ibid, p 471; *Nature* **406:** 559, 2000].

February The initial sequencing and analysis of the human genome by the government-funded International Human Genome Sequencing Consortium appeared [*Nature* **409:** 860, 2001], and the entire Feb 15th issue was devoted to this subject. The sequence and analysis of the human genome was also reported by the for-profit company Celera [*Science* **291:** 1304, 2001], and the entire Feb 16th issue was devoted to this. By far the biggest surprise was the total number of estimated genes: 30,000 to 35,000—much lower than previous estimates of 80,000 to more than 140,000 that had been based on extrapolations from gene-rich areas of the human and other genomes. Other facts**:** there are 3164.7 million nucleotide bases (A, C, T, & G)**;** the average gene is 30,000 bases long, while the largest known gene, dystrophin (*DMD*), is 2.4 million bases**;** about 99.9% of all bases are the same in all people**;** more than 50% of the discovered genes currently have unknown functions**;** less than 2% of the genome encodes proteins**;** "junk DNA" (repeated sequences that do not code for proteins) make up at least 50% of the human genome**;** during the past 50 million years of human expansion on the planet, a dramatic decrease has apparently occurred in the rate of accumulation of repeats in the human genome**;** genes are concentrated in random stretches along the genome, with vast expanses of noncoding DNA in between**;** vast areas of as many as 30,000 C and G bases repeating (called "CpG islands") appear to form barriers between the genes and junk DNA and are believed to help regulate gene activity**;** chromosome 1 has the most (2,968) and the male Y chromosome the fewest (231) genes. Reasons for the differences in gene density (**number of genes per million sequenced DNA bases**) between species are not known but intriguing to some of us**:**

Escherichia coli (bacteria)	932
Saccharomyces cerevisiae yeast	483
Arabidopsis thaliana (tiny mustard plant)	221
Caenorhabditis elegans (roundworm)	197
Drosophila melanogaster (fruit fly)	117
Homo sapiens (human)	12

Various metaphors have been used to describe the importance of sequencing the human genome ("finding the Rosetta Stone," "landing on the moon," "discovery of nuclear fission"), but William Schrader suggests this be compared to the discovery of the use of fire [*Nature* **409:** 856, 2001]. "Using fire set us humans on the path toward control of

energy and, subsequently, control of the environment outside our bodies. Sequencing the human genome sets us on the path toward control of the environment inside our bodies."–

The Japanese company RIKEN has systematically characterized gene expression patterns in 49 adult and embryonic mouse tissues by using microarrays containing their 18,816 mouse cDNA clones [*Proc Natl Acad Sci USA* **98:** 2199, 2001]. Quite a few U.S. university and public sector labs have already acquired many of these mouse cDNA clones from RIKEN [*Science* **291:** 963, 2001].

After a genome has been sequenced, how can we be certain of the number of expressible genes? A two-stage approach that combines the merits of stringent genome annotation with the benefits of over-prediction has been described [*Nature Genet* **27:** 337, 2001], leading to 1,042 new candidate genes in the fruit fly genome**;** this is above-and-beyond the original 13,601 annotated genes. Open-reading-frame sequence tags (OSTs) now support the existence of at least 17,300 genes in the nematode *C. elegans* [*Nature Genet* **27:** 332, 2001]**;** a range of 13,600 to 25,000 genes has been reported in earlier issues of ***Interface***.

April The Human Proteome Organization (HUPO) has been officially launched [*Nature Biotechnol* **19:** 297, 2001]. This is a worldwide consortium to efficiently elucidate the complex interactions of human proteins. The first project, expected to take 4 to 7 years, is to create an antibody for each and every human gene product.

In the new facility in Tokyo, RIKEN's technology allows researchers to read as many as 384,000 DNA samples per day [*Nature* **410:** 1013, 2001]. Their plans include genotyping 120,000 single-nucleotide polymorphisms (SNPs) in each of 768 Japanese patients—especially focusing on asthma and arthritis.

May A set of 15,247 unique cDNA clones from mouse has been developed from 52,374 3'-expressed sequence tags (3'-ESTs) and has been distributed and is in use at more than 100 sites [*Nature Genet* **28:** 17, 2001].

The international Mouse Sequencing Consortium (MSC) announced this month "the completion of a draft map (3x coverage) representing at least 95%" of the C57BL/6J mouse genome. Celera has completed a similar amount of sequencing of genomes derived from three other inbred strains (129X1/SvJ, A/J, and DBA/2J) [*Nature* **411:** 8, 2001; *Science* **292:** 822, 2001].

A zoology student is compiling tables of the number of chromosome pairs and haploid genome size in more than 2,900 vertebrate and invertebrate species, from *Homo sapiens* to sponges (http://www.genomesize.com).

June "Ensemble" consists of genes and genomic features automatically identified using Genscan and Genewise software. Genscan evidence is generated from protein predictions that are compared against protein databases. The genomic sequence used is the "Golden Path" assembled at Santa Cruz (http://genome.cse.ucsc.edu/). Genscan over-predicts by 30% to 40% but does hit ~95% of genes. Genewise is used to predict exons directly from genomic sequence. Pseudogenes are not currently well represented, because the prediction systems are not accurate enough and they "find multiple exons" having 1-base introns. One in 10 pseudogenes might actually be due to sequencing errors.

Targeted genes can be disrupted in large animals, but so far falls short of producing viable offspring [*Nature Biotechnol* **19:** 529, 2001]. It won't be long before we have knockout lines of sheeps and cows.

In order to help scientists capitalize on the genome sequence of the 26,000+ genes of the tiny mustard plant *Arabidopsis thaliana*, the National Science Foundation (NSF) has begun the "2010 Project," which plans to determine the function of all these plant genes. Thus far, the function of only about 1,000 genes have been experimentally determined, and another ~14,000 are projected to have a particular function based on similarities to genes with known functions.

With DNA-sequencing of the human and mouse genomes in the Clade III group nearing completion, what other mammalian genomes should be of highest priority? Beyond agricultural animals and pets (cow, pig, sheep, horse, dog, cat) in Clade IV (most closely related to those of Clade III), Steve O'Brien et al. [*Science* **292:** 2264, 2001] suggest that, at a minimum, we need to know about at least one genome from Clade II (sloth, anteater, armadillo) and Clade I (elephant, manatee, aardvark).

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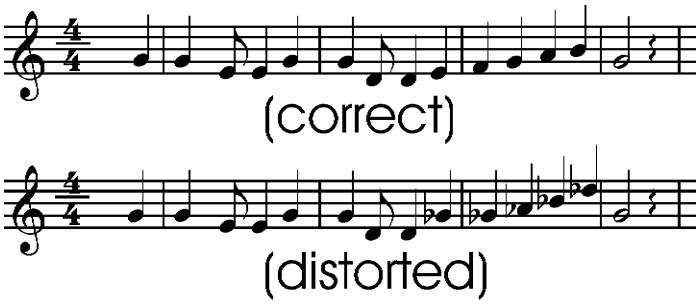
Music and Our Genes

Absolute pitch (AP), commonly referred to as "perfect pitch," is the ability to identify a muscial tone without having an external reference source. Virtually all individuals with AP have had muscial training from an early age (before 6 years old), implicating a strong environmental component to the trait. There is growing evidence, however, that the AP trait has a genetic component [*Am J Hum Genet* **67:** 755, 2000].

In a more recent study in identical versus fraternal twins [*Science* **291:** 1969, 2001], it was demonstrated with the Distorted Tunes Test (DTT) that perfect pitch has a large genetic component. This is great for cocktail parties. Below is a segment of "America the Beautiful," written in the normal way and in a corrupted way. Play this on your piano and test your friends**..!!**



http://www.ucsf.edu/its/listserv/ucsfnews/0302.html
http://www.cnn.com/HEALTH/9605/19/perfect.pitch/

CEG Sponsored Speakers

Larry Thompson, Ph.D.
Biochemistry and Biophysics Research Program,
Lawrence Livermore National Laboratory,
California
May 17, 2001 ***"Homologous recombinational repair of DNA ensures mammalian chromosome stability."***