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INTERFACE:
GENES AND THE ENVIRONMENT

CENTER FOR ENVIRONMENTAL GENETICS UNIVERSITY OF CINCINNATI Spring 1997

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# Differences in Patient Response to Psychotropic Drugs

Any drug, food, physical agent, heavy metal, or other environmental chemical is expected to produce some variously-shaped curve of toxicity at increasing doses of exposure. The theme of our Center for Environmental Genetics (CEG) is to pay particular attention to ***outliers***. Within any species (human, mouse, rat, fly, worm, yeast, bacterium), the individual who exhibits little response at high doses is considered to be genetically "resistant," and the individual who shows exaggerated response at low dose is genetically "sensitive." We can take advantage of these interesting variants, within any species, in order to gain a better understanding of the basic molecular and genetic mechanisms underlying any particular toxic response to an environmental signal.

Today ***Prozac***$^{(R)}$ (fluoxetine, a selective serotonin re-uptake inhibitor) is one of the most popular, widely prescribed antidepressant drugs in the United States. ***Halcion***$^{(R)}$ (triazolam, a benzodiazepine) is a commonly prescribed psychotropic sleep medication. In the vast majority of the population, these drugs are ***efficacious***, *i.e.* most patients taking these drugs feel less depressed and are able to get a good night's sleep with little or no residual effects. The last several years have seen a lot of publicity, however, about the occasional individual taking either of these drugs who has been seriously frightened with hallucinations, amnesia, rebound insomnia, and suicidal or violent tendencies. Also, a recent study in the ***New England Journal of Medicine*** suggests a possible relationship between pregnant women taking Prozac and minor birth anomalies in some of their children. Can these differences in patient response be due to underlying genetic predisposition? Could the ***phenotype*** (undesirable drug reaction) be correlated with ***genotype*** (alteration in DNA)? Might a DNA test then be developed--so that patients prone to such "idiosyncratic drug reactions" can be given alternative medication, and physicians prescribing these drugs can be spared the grief of having a drug reaction happen to one of their patients?

### *The Therapeutic Index (TI)*

In clinical pharmacology the TI might be defined as the average toxic dose observed in 50% of a population, divided by the average effective dose seen in 50% of the population ($\mathbf{TD_{50}/ED_{50}}$). If the TI is 1000 (*e.g.* as it is with aspirin), this means that the drug is very safe and interindividual genetic differences of 50- or 500-fold--in the drug eliciting an unwanted response--would not usually be detected clinically. If the TI is 2 or 5, however, the drug would be described as having a "very narrow therapeutic window," and interindividual genetic differences of even 10-fold in a toxic drug response would readily be detected.

### *What Is a "Genetic Polymorphism?"*

Spontaneous mutations in DNA are known to occur at frequencies ranging between 1 in 10 million and 1 in 100 million. Recall that a "gene" comprises two alleles, one on each of a pair of chromosomes in all eukaryotes (*i.e.* all animals and plants, everything except bacteria). In the simplest case, if ***p*** and ***q*** represent two alleles of a given

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gene, and $p + q = 1.0$, then the Hardy-Weinberg distribution $(p + q)^2$ predicts the frequencies of the three possible genotypes to be $p^2 + 2pq + q^2$. If an allele is present in the human population at frequencies of 1 in 2, or 1 in 100, this would lead to an autosomal recessive trait ($q^2$) occurring in 25% (1 in 4) and 0.01% (1 in 10,000) of the population, respectively.

The usual reason for a second (variant) allele persisting in a population--at frequencies much, much higher than those of spontaneous mutations--is that the variant allele confers some sort of selective (reproductive) advantage to the species as a whole (so-called "***balanced polymorphism***"). A timely example is the mutant allele for the idiopathic hemochromatosis (***HFE***) gene**;** an allelic frequency of 0.05-0.08 in certain Caucasian populations (meaning that 25 to 64 people per 10,000 will have the autosomal recessively-transmitted disease) is believed to be maintained due to the benefits of iron-overloading in women during menstruation and pregnancy.

Although any frequency greater than that of a spontaneous mutation might be called a polymorphism, "human genetic polymorphisms" are generally regarded as the reflection of two or more groups of individuals in which the variant group represents at least 1 in 10,000 people, *i.e.* frequency of the variant allele is ≥0.01.

### *The* CYP2D6 *Polymorphism*

Whenever a drug, usually recently introduced on the market, exhibits a broad spectrum of responses, a genetic polymorphism is likely to exist. This happened in the case of ***debrisoquine***, an antihypertensive medication introduced in the United Kingdom in the mid 'seventies. Robert L. Smith (St. Mary's Hospital, London) noticed that debrisoquine caused an unexpectedly high incidence of side effects, or "idiosyncratic drug reactions." Smith reasoned that an underlying genetic difference in the way individual patients metabolize the drug (the difference occurring at a significant frequency in the population) might be responsible for this high incidence of undesirable responses. Smith and three laboratory colleagues took the prescribed dose and measured the levels of metabolites in their urine. Smith became hypotensive--from ingesting the "recommended" dosage of debrisoquine, and he found that his urinary 4-hydroxy metabolite was about 20 times lower than that of his three colleagues.

More unrelated people were screened. Poor metabolizers (PMs) of debrisoquine were found to represent 6% to 10% of Caucasian populations, as compared with extensive metabolizers (EMs) who handle the drug 10 to 200 times more efficiently. PM frequencies are about 5% in Black populations and <1% in Asians. Similar independent studies with the oxidation of ***sparteine*** (an antiarrhythmic agent) were carried out in the laboratory of Michel Eichelbaum (University of Bönn, Germany) in the late 'seventies, leading to the realization that both debrisoquine and sparteine were metabolized by the same enzyme. These studies have led to the discovery of a "panel" which currently includes more than three dozen commonly prescribed drugs--including antihypertensives, antiarrhythmics, **ß**-blockers, monoamine oxidase inhibitors, morphine derivatives, antipsychotics and tricyclic antidepressants--that are metabolized by the same cytochrome P450, now called CYP2D6.

The ***CYP2D6*** gene was first cloned in 1988, and PM alleles were shown to encode a defective protein and/or incorrect splicing of the gene transcript--resulting in lowered, or completely absent, enzyme activity. Duplication of ***CYP2D6*** genes--up to 13 copies--have been found in some families (especially in Ethiopia and Saudi Arabia), meaning that these individuals require much larger doses of a "debrisoquine panel drug" to be effective. The ***CYP2D6*** polymorphism has therefore become one of the most important pharmacogenetic differences involved in clinical drug efficacy versus undesirable drug reactions. A unified nomenclature system for the human ***CYP2D6*** alleles (***CYP2D6*1, *2, ... *17***) was proposed in early 1996.

### *Fluoxetine is a member of the "CYP2D6 Panel"*

***Figure 1*** shows the chemical structures of the two psychotropic drugs discussed in this article. Recent studies with human P450 cDNA-expressed cells in culture [ *e.g.* ***Clin Pharmacokinet*** **29:** 1-19 (1995)] have confirmed that CYP2D6 is the best enzyme for detoxifying Prozac(R) (fluoxetine). This would suggest that individuals of the CYP2D6 PM (poor metabolizer) phenotype, when taking the commonly prescribed dose of Prozac(R), might be more prone to unwanted side effects.

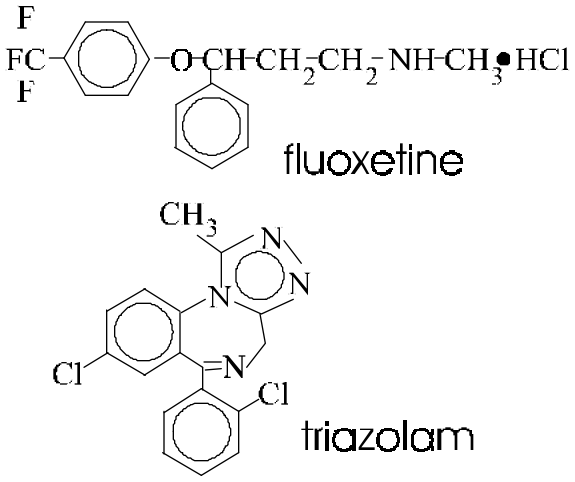


***Figure 1***. Chemical structures of the two psychotropic prescribed drugs being discussed.

It is increasingly common for patients, especially the elderly, to be taking two or more prescribed drugs for their maladies. Other members of the "CYP2D6 panel" include

phenothiazines, codeine, dextromethorphan (related to "uppers" and "speed"), and the secondary amine tricyclic antidepressants desipramine and nortriptyline. There is a growing appreciation that, when given the recommended prescribed dose of these drugs, CYP2D6 PM individuals not only have prolonged serum levels due to their genetic predisposition (compared with that in EM individuals) but are more prone to drug-drug interactions--especially with other CYP2D6 substrates. Hence, the potent inhibition by fluoxetine of the O-demethylation of codeine to morphine might lead to a decrease in the abuse and dependence potential of codeine**;** conversely, perhaps CYP2D6 EM individuals might have an increased potential for codeine abuse and dependence. The same might be said about the efficacy, versus the adverse drug reactions, for phenothiazines, dextromethorphan, desipramine, nortriptyline and all other CYP2D6 substrates.

Most evidence suggests that many depressions are caused by a relative deficiency in neurotransmitters such as norepinephrine and serotonin. Norepinephrine deficiency can be associated with relatively low urinary 3-methoxy-4-hydroxyphenol glycol (MHPG) levels, whereas serotonin deficiency may be associated with low spinal fluid concentrations of 5-hydoxyindole acetic acid. While the precise mechanism of action of tricyclic antidepressants is unknown, a leading theory suggests that these drugs restore normal levels of neurotransmitters by blocking the re-uptake of norepinephrine or serotonin from the synapses in the central nervous system. Secondary-amine tricyclics, such as desipramine and nortriptyline, appear to have greater activity in blocking norepinephrine re-uptake, whereas tertiary-amine tricyclics such as amitriptyline have greater activity in blocking serotonin re-uptake.

***Triazolam appears to be metabolized best by CYP3A4***

Triazolam, a psychotropic sleep medication on the market in The Netherlands as a 5-mg tablet, was removed in 1979 due to excessive numbers of complaints about undesirable side effects. The same thing happened in the United Kingdom in 1990 with a 2.5-mg tablet. Triazolam is currently sold in the U.S. as 0.25- and 0.50-mg tablets. Still, with these recommended prescribed small doses, there are the infrequent side effects of hallucinations, amnesia, rebound insomnia, and suicidal tendencies. Psychiatrists are easily able to score a range of three or five different graded responses to triazolam. Among the (relatively small) intolerant subpopulation, are physicians ethically able to give the patients another small dose of this drug in order to identify any genetic polymorphism? Can we extrapolate a patient's reaction to triazolam to his/her reactions to other benzodiazepines?

Instead of (or in addition to) human clinical trials, pharmaceutical companies are turning more and more to human P450 cDNA-expressed enzyme activities in cell cultures, in addition to antibody inhibition of human liver P450 activities. A recent study [***J Pharmacol Exp Ther* 280:** 927-33 (1997)] showed that citalopram N-demethylation was strongly correlated with triazolam methyl-hydroxylase activities, suggesting that CYP3A4 is the major P450 involved in this metabolic pathway. CYP3A4 is the most abundant P450 in human liver and, although large differences in human CYP3A4 levels/activities are known to exist, there are as yet no known DNA marker(s) associated with this polymorphism.

The fact that the tablet size of triazolam has been decreased from 5 mg in 1979, to 0.50 and 0.25 mg currently, would suggest that this particular drug has a narrow therapeutic window (TI < 5), indicating that this drug would probably not be the best probe in trying to elucidate the CYP3A4 polymorphism.

***"Classes" of Psychotropic Drugs Need Not Be Metabolized the Same Way***

If one looks at the various chemical structures of the benzodiazepines, clearly different enzyme polymorphisms can be expected from different benzodiazepines. The availability of an amino group, for example, might make that particular drug an excellent candidate for the N-acetylation polymorphism, whereas another benzodiazepine without an available amino group would make it not a candidate for the N-acetylation polymorphism. The same can be said for the selective serotonin re-uptake inhibitors, the norepinephrine re-uptake inhibitors, and the opiates. This realization should allow physicians in the future to "shop around" for the appropriate prescribed drug, when patients in need of--for example--a selective serotonin re-uptake inhibitor might have a genetic deficiency in a particular metabolic pathway (*e.g.* having the CYP2D6 PM trait).

***The Research Focus of the CEG***

CYP2D6 is believed to handle as much as 20% of all commonly prescribed drugs. It is worth noting that--although humans probably have about 60 unique P450 genes--only about a half dozen (***CYP1A2, CYP2C9, CYP2D6, CYP2E1, CYP3A4, CYP4A11***) appear to be responsible for metabolism of the vast majority of prescribed and over-the-counter drugs. The day might not be in the too-distant future that selection of drugs, as well as dosage of drugs, could be adjusted principally by the physician knowing the individual's genotype--instead of by monitoring plasma drug concentrations or by waiting for favorable therapeutic responses versus idiosyncratic reactions to occur. Another small group of unique human P450 genes (***CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2B6, CYP2E1, CYP3A4, CYP3A5***) seem to be responsible for the metabolism of virtually all environmental chemicals known or likely to be human carcinogens.

Instead of challenging a patient (having a sensitive phenotype) with a potentially dangerous drug, it would

certainly be advantageous to develop a DNA assay--which is noninvasive, sensitive, unequivocal, and inexpensive--for aiding the physician in identifying the genotype, thereby being able to predict (and thus prevent) unwanted idiosyncratic drug reactions before they occur. The same philosophy/approach holds true for the CEG with any environmental agent and the Center's goals of "preventive toxicology." From the standpoint of our NIEHS-sponsored Center, although emphasis cannot be placed on pharmaceutical research ***per se***, it is acceptable to use a specific drug as a prototypic probe for attempting to identify, and ultimately understand, the underlying mechanisms of genetic variability in toxic response to environmental chemicals or physical agents. Hence, the discussion here of clinical differences in response to fluoxetine and triazolam, as a way to introduce the human ***CYP2D6*** and ***CYP3A4*** polymorphisms.

**---Contributed by Daniel W. Nebert**

# When Is *This* Transgenic Mouse Not the Same as *That* Transgenic Mouse?

Targeted mutagenesis and gene knockouts in mice have become a powerful tool for the analysis of gene function and human disease. Genetically manipulated DNA, at the laboratory bench, is traditionally put into embryonic stem (ES) cells derived from the mouse "***129***" inbred strain. Interestingly, numerous phenotypes, or a large range of responses (traits), have been described when different laboratories have knocked out the same gene and presumably used the "same" mouse ***129***-derived ES cells.

A recent report [Simpson et al., ***Nature Genet* 16:** 19-27 (1997) ] documents that the outcrossing of ***129*** substrains, both deliberate and accidental, has led to extensive genetic variability among the mouse substrains and ES cells derived from them. These coauthors compared the genomes of fifteen mouse ***129*** substrains and ten ES cell lines, by examining allelic variants of **86** simple-sequence-length-polymorphism (SSLP) markers--selected to provide an average 20-centiMorgan genomic spacing throughout the entire genome. Incredibly, they found that 37 of the 86 markers, or 43%, differed among the ***129*** substrains! These data are part of our growing awareness of how very significantly different the genetic backgrounds can be, even between substrains derived from the same mouse inbred strain.

# Evolution of Antifreeze Glycoprotein (*AFGP*) Genes

Almost 30 years ago it was known that "resistance to freezing" in Antarctic fish was due to blood serum glycoproteins that lowered the fish's freezing temperature below that of the subzero sea water surrounding them. Later it was shown that not all antifreeze proteins are glycoproteins. Now it has been reported that an ***AFGP*** gene in an **Antarctic** fish has arisen, in part, from noncoding DNA and that a very similar ***AFGP*** gene from an **Arctic** fish is the product of some completely unrelated molecular processes [*reviewed by* Logsdon Jr & Doolittle, ***Proc Natl Acad Sci* USA 94:** 3485-87 (1997) ].

The ***AFGP*** gene from the Antarctic notothenioid *Dissostichus mawsoni* is derived from a pancreatic trypsinogen gene, wherein the "ice-binding function" of the ***AFGP*** gene has originated from the recruitment and iteration of a small region spanning the boundary between the first intron and second exon of the trypsinogen gene--to produce 41 tandemly repeated segments! Retention of the 5' end of the trypsinogen gene might be significant, because this region codes for a signal peptide needed for secretion from the pancreas into the digestive tract. Therefore, this ***AFGP*** gene is one of a very few genes "recently invented by Mother Nature" that can be said with confidence to have arisen by processes other than duplication or exon-shuffling.

The researchers isolated the ***AFGP*** gene from the Arctic cod, *Boreogadus saida*, by using the Antarctic AFGP repeat "Thr-Ala-Ala" as the probe. The Arctic ***AFGP*** gene was found not to be identifiably similar to any known sequence in the database. There is a very strong case for independent origins of the Antarctic and Arctic ***AFGP*** genes (different numbers and locations of introns, different coding regions flanking the ***AFGP*** repeats, different usage of codons within these repeats, and unrelated spacer regions between these repeats).

Using the rate calculated from salmon mitochondrial DNA, it was estimated that the origin of the *Dissostichus* ***AFGP*** gene arose between 5 and 14 million years ago (MYA), which correlates very well with the presumed date of freezing of the Antarctic Ocean about 10-14 MYA. In contrast, the *Boreogadus* ***AFGP*** gene probably originated about 2.5 MYA and was most likely induced by Arctic glaciation at that time. This is a great example of a new function that has arisen out of strong selective pressure and an abrupt change in environmental conditions--or, so-called "adaptive molecular evolution!"

# LETTERS TO THE EDITOR

## RESPONSES/COMMENTS TO VARIOUS QUESTIONS

**COMMENT** **We found your article "Ethical issues and genetic research," as well as your article "Intriguing ethical questions," in *Interface* issue #10 (Winter, 1996-97) to be extremely interesting, exciting and timely. We are using these articles in our Ethics course this spring.**
***---A reader from the State of Washington***

**COMMENT** **In our *Interface* issue #1, we proposed that genetic differences in metabolism of the nerve gas *sarin* among exposed soldiers might account for some of the variability in response seen (Gulf War Syndrome illnesses). In June 1996 the Department of Defense admitted that nerve agents might have drifted over the desert when U.S. soldiers blew up an Iraqi weapons depot at Khamisiyah. The latest theory is that very low-level exposures to these nerve agents may explain some of these illnesses in which the symptoms are not the same as those expected from doses "large enough to cause a full-blown nerve agent poisoning." Researchers from the University of Texas at Dallas reported [15 Jan 1997 issue of the *J Am Med Assn*] that 25% of a group of 249 Persian Gulf War veterans had memory deficiencies, muscle pain, and other symptoms that appeared to correlate with possible exposure to specific combinations of chemicals, and that 9% of the soldiers had abnormal brain and nerve tests, when compared with healthy veterans.**

**What about the remaining 75%, or the remaining 91%? These differences in response remain unexplained, as to whether there were differences in exposure to the nerve agents or in underlying genetic predisposition.**

**Q** **A U.S. presidential advisory commission on bioethics, reporting the end of May, recommends the continuation of a ban on the use of federal funds "for cloning of human beings." What effect will this have on other cloning research?**

**A** *The National Bioethics Advisory Commission (NBAC) appears unanimous in its opposition to any use of newly discovered cloning technology for the production of living human beings. Some NBAC members argue that backing the presidential ban is "meaningless," because no one has any plans to experiment with human cloning in the near future. Other NBAC members suggest that such a law could be detrimental to research. For example, legislation could easily be drafted inadvertently to include the kind of simple DNA technology that many are doing every day in laboratories around the country.*

*A working group on cloning, set up by the World Health Organization (WHO) has argued that much of the opposition to human cloning stems from "science fiction accounts" which have resulted in "fear and ignorance on the part of the public." The report points out that a ban, or moratorium, may be "most incautious," because a hasty prohibition might result in loss of actual and potential benefits." Thirty members of the International Academy of Humanism (Amherst NY), in a "Defense of Cloning and the Integrity of Scientific Research," say that--contrary to President Clinton's bioethics panel--the cloning of any higher animals, including humans, would not raise "moral issues any more profound than those faced in connection with any previous scientific or technological development."*

*Everyone agrees that some guidelines will need to be developed in order to prevent abuses of higher mammal cloning technology. Stay tuned! The story is not over yet!*

# Observations by a Biologist

## Sea gulls on the west coast of the United States

If you've ever been fortunate enough, as I have recently, to stroll along a Pacific Ocean beach in California, Oregon or Washington, you no doubt have seen the various sea gulls that inhabit this coastline. They all look the same, but there are actually six species of these white gulls**:** the Western gull, the California gull, the herring gull, Thayer's gull, the glaucous-winged gull, and the ring-billed gull. Except for the ring-billed gull (which has a black stripe, or ring, around its beak), the other five species have a distinct 5- to 10-mm ***bright red-orange circular spot*** on each side of their lower beak; the rest of the beak is light yellow and the adult birds are white. What selective pressure might have encouraged this spot to appear and what keeps this spot from disappearing?

Well, some clever ornithologists (or gullologists?) developed the hypothesis that this spot had to do with feeding of the young. Indeed, if the scientists placed comparably-sized spots of red-orange, green, blue, yellow, purple, brown or black on wooden tongue depressors and passed them near a nest of newborn sea gulls, the baby birds would only strike out (pecking the colored spot) at the red-orange-colored signal! Observing what happens in the nest, the scientists saw that when either parent flies in with their gullet full of food, the baby gulls peck at the red-orange spot on the parent's beak in order to cause regurgitation of the food into the nest--whereupon the newborns quickly eat it up! If another species of bird arrived in the nest, of course, without any red-orange spot this behavior would not occur.

So, the next time you are walking with someone on the beach and see one of these gulls, you might tell your companion that the red-orange spot has arisen and remains in these five species because of the evolutionary selective pressure of the young, receiving regurgitated food from their parents. Here is yet another example of an interaction between genes and the environment.

# Genotyping of Cat Hair Leads to Murder Conviction

Simple tandem repeat (STR) loci are increasingly being used to compare genotypes in forensic medicine. A 32-year-old woman disappeared 3 October 1994 from her home on Prince Edward Island, Canada. Her abandoned automobile was discovered several days later, and blood found inside the car matched that of the victim. Three weeks later, a man's leather jacket stained with the victim's blood was discovered in the forest 8 km from her home. In the lining were found several hairs from what appeared to be a white domestic cat. The victim's body was uncovered in a shallow grave on 6 May 1995, and the victim's estranged common-law husband (who allegedly owned the bloody leather jacket) was arrested and charged.

The suspect lived with his parents and their pet cat--a white American shorthair named 'Snowball.' ***Steve O'Brien***'s laboratory (National Cancer Institute, Frederick, Maryland) was asked to determine whether genomic DNA from the cat hairs found in the jacket matched Snowball's DNA profile. Composite STR genotypes were judged to match at all seven heterozygous and three homozygous loci tested. Using allelic frequencies predicted from two STR populations (nineteen unrelated cats from Prince Edward Island and nine cats from varying parts of the United States), the researchers estimated the incidence of the composite hair genotype to be ***one in 220 million*** and ***one in 69 million*** for cats on Prince Edward Island and in the United States, respectively. These data convinced the jury, who convicted the defendant of second-degree murder on 19 July 1996. This case [Menotti-Raymond et al., ***Nature*** **386: 774** (1997) ] represents a legal precedent for STR genotyping of pet animal hairs in forensic medicine!

# Cancer Genome Anatomy Project (CGAP)

There is a burgeoning array of online databases to help cancer researchers and other biologists search for new genes and understand what they do. Now the CGAP, starting this June, is an ambitious effort by the National Cancer Institute (NCI) that plans to provide a complete catalogue of all genes expressed in cancerous cells. Starting with various types of ***pure*** cancer cells--CGAP will be able to classify tumor genes by the type of cancer cell from which they are derived and the degree of the cell's malignancy [***Science* 276:** 1023-24 (1997)].

The biggest problem in the past has been the mixture of cell types in a typical tumor. Researchers cannot get an accurate picture of gene expression in cancer cells--if RNA extracted from the whole tumor represents a mixture of molecules from all the different cell types. With CGAP, one starts with a thin slice of tumor tissue, placed on a glass microscope slide and covered with a transparent cap from a tiny vial, the underside of which is lined with a thin layer of plastic. The scientist simply scans the tumor to find a uniform group of malignant cells, then zaps them with a weak laser--which heats the plastic so that it adheres to the cells just underneath. When the cap is lifted from the sample, it pulls off the targeted cells, leaving the rest of the sample intact. The tiny amounts of RNA are then able to be amplified by polymerase chain reaction (PCR) and reverse-transcribed to make complementary DNA (cDNA) libraries. Similar cDNA libraries are currently being made for normal cell types and cancers of the breast, colon, lung, ovary and prostate. These bits of cDNA, called expressed sequenced tags (ESTs), will be sequenced because they are unique fragments useful for identifying transcriptionally active genes in that particular tissue under study. And the sequences will be entered on the CGAP home page.

There is now a Website with this information available, so that any new gene sequence entered can immediately be matched up to a particular tumor cell type and even the degree of malignancy! These data will be important in determining the progression of cancer or to serve as markers of the disease--a catalogue of cancer cell types, shared immediately on the Internet, the same day that the data are determined!

# SCIENCE LITE

## Computer Nerd Describes Revolutionary Breakthrough!!

In the midst of this computer revolution, there has been the recent announcement by Günther Fingleqvist (Copenhagen) of the new **B**uilt-in **O**rderly **O**rganized **K**nowledge device (BOOK). It's a revolutionary breakthrough in technology**:** no wires, no electric circuits, no batteries, nothing to be connected or switched on. It's so easy to use that even a child can operate it! Just lift its cover! Compact and portable, the BOOK can be used anywhere—even sitting in an armchair by the fire—yet, it is powerful enough to hold as much information as a CD-ROM disk.

Here's how it works**:** Each BOOK is constructed of sequentially numbered sheets of paper (recyclable), each "page" capable of holding thousands of bits of information. These pages are locked together with a custom-fit device called a "binder" which keeps the sheets in their correct sequence. By using both sides of each sheet, the manufacturers have discovered that they are able to cut costs in half. Each sheet is scanned optically by the human eye, registering information directly into your brain. A flick of the finger takes you to the next sheet! And the bound pages can be protected from food and drink by a covering device called a "BOOKcover."

The BOOK may be picked up at any time and used, by merely opening the cover. The "browse" feature allows you to move instantly to any sheet, and move forward or backward as you wish. Most BOOK devices come with an "index" feature, which pinpoints the exact location of any selected information for instant retrieval. An optional "BOOKmark" accessory allows you to open the BOOK to the exact place you left it during a previous session—even if the BOOK has been closed! BOOKmarks are being designed to fit universal design standards; thus a single BOOKmark can be used in BOOKs by various manufacturers.

"Portable, durable and affordable, the BOOK appears to be the entertainment wave of the future," says Günther Fingleqvist, and many new titles are expected soon--due to the very recent surge in popularity of its programming tool, the **P**ortable **E**rasable-**N**ib **C**ryptic **I**ntercommunication **L**anguage **S**tylus**.....**

# CEG Members in the News

**Zalfa Abdel-Malek** organized and participated in a mini-symposium entitled: *"Signaling Pathways in Pigment Regulation"* during the 7th meeting of the Pan American Society for Pigment Cell Research in June 1997 (Providence, Rhode Island). During the same meeting she was invited to speak on ***"The role of melanocyte death during development and adaptive responses of skin to damaging agents."***

**Grace Lemasters** presented a talk entitled ***"Incidence of genotoxic changes following low-level solvent and fuel exposure"*** at the 30th Annual Meeting of the Society for Epidemiologic Research, June 1997 (Edmonton, Alberta, Canada). She has also become editor of a new newsletter, called ***"The Outlier,"*** which has been established at the University of Cincinnati, as an outreach of genetic epidemiology.

**Dan Nebert** was an invited speaker at the Second International Meeting on "Redox Processes and Cancer," in April 1997 (Banff, Alberta, Canada), and an invited speaker at the First International Nomenclature Workshop, sponsored by the International Committee on Standardized Nomenclature in Mice and the HUGO Human Gene Nomenclature Committee, in May 1997 (Bar Harbor, Maine). He also accepted an invitation in May 1997 to join the **Committee on Developmental Toxicology** (Chair: Ellen M Faustman), established under the aegis of the Board of Toxicology and Environmental Health Hazards, Assembly of Life Sciences, National Research Council, National Academy of Sciences (Washington, D.C.) and an invitation in June 1997 to join the National Institute of Environmental Health Sciences (NIEHS) Environmental Genome Working Group (Chairman: Samuel Wilson), established to define the scientific problems and the ethical, legal and social issues in development of the **Environmental Genome Project** (Research Triangle Park, North Carolina).

**Alvaro Puga** was invited to give a "magistral lecture" in April 1997 on ***"Interaction between genes and the environment"*** during the Gallaecia Fulgit celebration of the 500th anniversary of the Foundation of the University of Santiago de Compostela (Spain).

**Rakesh Shukla** has become the primary programmer for a weekly show, 10:00 am-12:00 noon Sundays, on the volunteer radio station WAIF, in Cincinnati, Ohio. This program has the potential for communicating the goals of the COEP Core for outreach education. He also received a contract for statistical research on "Innovative Statistical Approach for Regulative Toxicology."

**Nancy Steinberg-Warren** has been appointed Co-Chair of the Practice-Based Symposium for the National Society of Genetic Counselors Annual Education Meeting for 1998 (Denver, Colorado). She has also been appointed to an ad-hoc committee of the American Board of Genetic Counseling regarding recertification of genetic counselors. A Preconceptional Health Newsletter was begun by her division, entitled ***"Start Healthy Times."*** It is proving to be an enormously effective outreach activity, which hopefully will be on every high school biology class reading list. Their Start Healthy Hotline is (513) 636-5839. The newsletter identifies useful internet sites:

http://babynet.ddwi.com/tlc/pregnancy/pregnancy.html#PRECONCEPTION
http://www.medicinenet.com/mainmenu/encyclop/article/art_p/pregplan.htm
http://www.noah.cuny.edu/pregnancy/pregnancy.htm#TESTING
http://www.diabetes.org/DiabetesCare/Supplement/s25.htm
http://ehpnet1.niehs.hin.gov/docs/1995/103(11)/review/html
http://www.opc.on.ca/beststart/bodyimg/bodyimg4.html
http://www.dilcom.com/~paladin.peri.html

**Glenn Talaska** published a paper entitled ***"Monitoring of aromatic amine exposures in workers at a chemical plant with known bladder cancer excess"*** in the ***J Natl Cancer Inst*** **88**:1046-1052, 1996, which has been nominated for the **Sheppard Science Award**.

**Jeffrey Whitsett** was presented with the **Distinguished Research Professor Award** from the University of Cincinnati, at Commencement Exercises in Shoemaker Center, June 1997. A committee composed of Professor Emeriti select a recipient each year from a list of outstanding individuals compiled by the various Deans.

# Novel Study on Use of the Word 'Novel'

Having trouble getting your papers accepted by first-rate journals? Are your papers not receiving the frequency of citation that you'd like to see them get? The solution for many coauthors during this past decade appears to be insertion of the word 'novel' into the title of their publication. A statistical analysis of the MedLine database by Friedman and Karlsson [***Nature* 385:** 480 (1997)] has revealed an exponential increase in the use of the word 'novel' in the titles and abstracts since 1985. In contrast, use of the word 'new' in the MedLine database has increased about 3-fold between 1975 and the present (Figure 2). A possible explanation, offered by Batchelor et al. [***Nature* 387:** 337 (1997) ] is that, what used to be regarded as 'new' in 1970, is now regarded as both 'new' and 'novel' by the molecular biologists and geneticists.

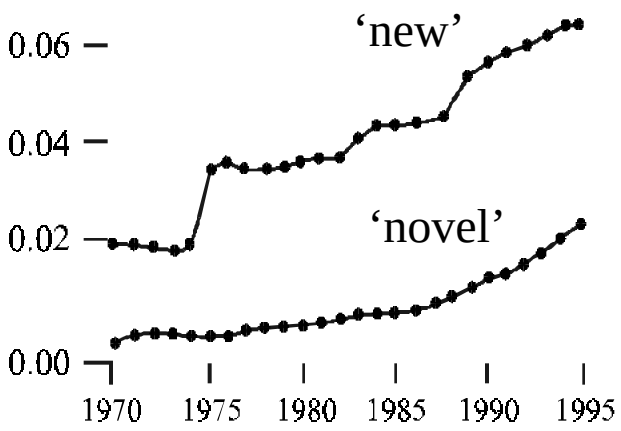


***Figure 2.*** Proportion of papers per year in MedLine that contain the words 'novel' (***lower line***) and 'new'(***upper line***) in the title or abstract.

# CEG Leadership:
## A Changing of the Guard

As of 4 June 1997, the day before our site visit, ***Daniel W. Nebert MD*** handed over the baton to ***Marshall W. Anderson PhD***, who became the new Director of the Center for Environmental Genetics (CEG). ***Grace Lemasters PhD*** also handed over the baton to ***Kathleen Dixon PhD***, who became the new Associate Director of the CEG. Marshall Anderson has been the new Chair of the Department of Environmental Health since July 1996. Dan Nebert has accepted the invitation of Marshall Anderson to remain, however, as the person in charge of the Distinguished Seminar Series and Editor of the CEG NewsLetter, ***Interface***.

# Is Pollution Causing Global Warming?

A Russian-American and French international effort for drilling in ice has achieved both technical and scientific advances by reaching a depth of 3,350 m at the Russian Vostok station. In addition to being the deepest ice core, the material gathered represents the past four glacial-interglacial cycles of this planet (about 420,000 years of climatology data). Studies of the deuterium dust, the radioisotope $^{18}O$ records, and electrical conductivity measurements of the ice core are consistent with global warming phenomena peaking at approximately 400,000, 325,000, 210,00 and 115,000 years ago (Figure 3). Interestingly, the data are consistent with a fifth major warming peak that is just about maximal--right now!

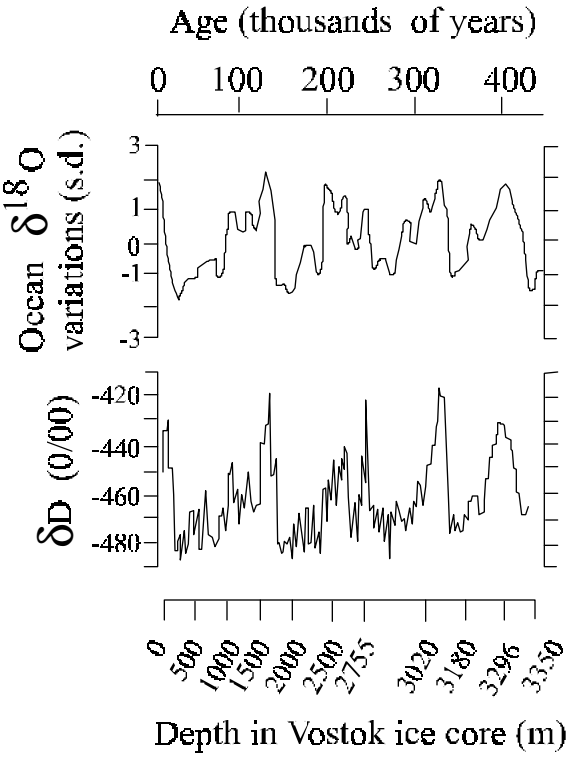


***Figure 3.*** Vostok and marine climate records during the past 420,000 years. ***Top***, ocean $^{18}O$ variations. ***Bottom,*** Vostok deuterium profile [*modified from* Petit et al., *Nature* **387:** 359 (1997) ]. The reasons for an increased abundance of $^{18}O$ and deuterium, at times of global warming, are unknown.

More experiments will be needed to corroborate this ice core study, but one interpretation of this finding is that we might be in the midst of a global warming period based not on industrial pollution or overpopulation or thinning of the ozone layer--but simply based on planetary changes over hundreds of thousands of years that we currently do not yet understand.

# Human Chromosome Fragment in Mice

For more than 5 years, human genes have been inserted at random in tandem arrays to make human gene-carrying transgenic mouse lines. Recently, it has become technically possible to target a single human gene to the same DNA site which houses the mouse ***orthologous*** gene, *i.e.* replacement of the mouse gene with the equivalent human gene.

Now it has been demonstrated [Tomizuka et al., ***Nature Genet*** **16:** 133-143 (1997)] that human chromosomes, or large chromosomal fragments, derived from fibroblasts can be introduced into mouse embryonic stem (ES) cells by way of microcell-mediated chromosome transfer (MMCT). Viable chimeric mice were produced, and then the human chromosome 2-derived fragment was shown to be transmitted to the offspring through the germline. This new technique should allow the study of functional relationships of numerous human genes arranged in their normal order on the chromosome, or human genes on an entire chromosome, in the intact mouse!

# CEG - SPONSORED SPEAKERS

**Clem E. Furlong, PhD**
Research Associate Professor, Department of Genetics,
University of Washington, Seattle, WA
May 7, 1997 ***"Human paraoxonase polymorphosm: Role in insecticide and lipid metabolism."***

**Sten Orrenius, MD, PhD**
Professor and Chair, Department of Toxicology,
Karolinska Institute, Stockholm, Sweden
April 2, 1997 ***"Activation and modulation of proteases during apoptosis."***
April 3, 1997 ***"Molecular mechanisms and biomedical implications of apoptotic cell death."***

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