

New Director at the Biotechnology Center

Jonathan Sweedler, Lycan Professor of Chemistry, has accepted the position of Director of the Biotechnology Center. Sweedler is broad-based in his University affiliations and training. He plans to carefully access the needs and capabilities of the campus in nanotechnology, biological sensors and other technologies being developed by the engineering and physical sciences and to integrate this development with the needs of the life sciences. "The Biotechnology Center is well-positioned to be the bridge between these disciplines," says Sweedler. His goals include expanding the Protein Sciences Facility and its capacity to characterize large numbers of proteins. "I feel it is important that our campus have a strong facility in proteomics," says Sweedler. He plans to pursue external funding opportunities to further develop and broaden the Biotechnology Center capabilities to better serve the faculty needs.

Sweedler obtained his Ph.D. in Chemistry from the University of Arizona in 1988 and subsequently spent three years at Stanford on a NSF Postdoctoral Fellowship in Chemistry and Neuroscience. He joined the University of Illinois faculty in Chemistry in 1991. Currently he is affiliated with the campus-wide Neuroscience program, the Department of Integrative Physiology, the Beckman Institute, and the Biotechnology Center. In 1994, he co-founded a small biotechnology start-up company, Magnetic Resonance Microsensors. Sweedler has received many awards and fellowships, most recently the American Chemical Society's Analytical Instrumentation Award and the Merck Prize from Europe. He is currently on the editorial advisory boards of six journals and advises a large research group consisting of twenty-five graduate students and postdoctoral associates. His research interests are given in the following excerpt.

K. Brinkmann

My research interests are in analytical neurochemistry, with a research program emphasizing two major interlocking areas. The first research area is the development of analytical methods that allow trace components to be measured while in complex biological microenvironments, and the second is the application of these methods to improve our understanding of cellular communication and neurotransmitter distribution and release. These two efforts, of course, are synergistic; new measurement tools allow the probing of details of learning and memory, while new understanding leads to more questions, which often require advances in measurement strategies.

The development of new biotechnology

Much of our work involves scaling a variety of measurement technologies to work with the nanoliter or smaller volume range. This allows the identification and quantitation of neuroactive compounds from the environment around a living cell. We have developed several unique detection systems for capillary electrophoresis that enable low concentration measurement of several classes of signaling molecules. As one example, we developed a unique wavelength-resolved fluorescence detector that provides <100 molecule detection limits with the acquisition of complete fluorescence spectra; using this system, tyrosine- and tryptophan-containing peptides, the catecholamines, indolamines and nitric oxide synthase cofactors can be detected from a small fraction of a single cell. We are now characterizing the interaction of serotonin and NO in neurons that produce both compounds.

We have a large research effort to develop new mass spectrometry protocols to profile the peptides in individual neurons and cellular processes. Currently, we can obtain high quality mass spectra from samples ranging from single cells and nerves, and

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Strategies human poxviruses utilize to evade the immune response

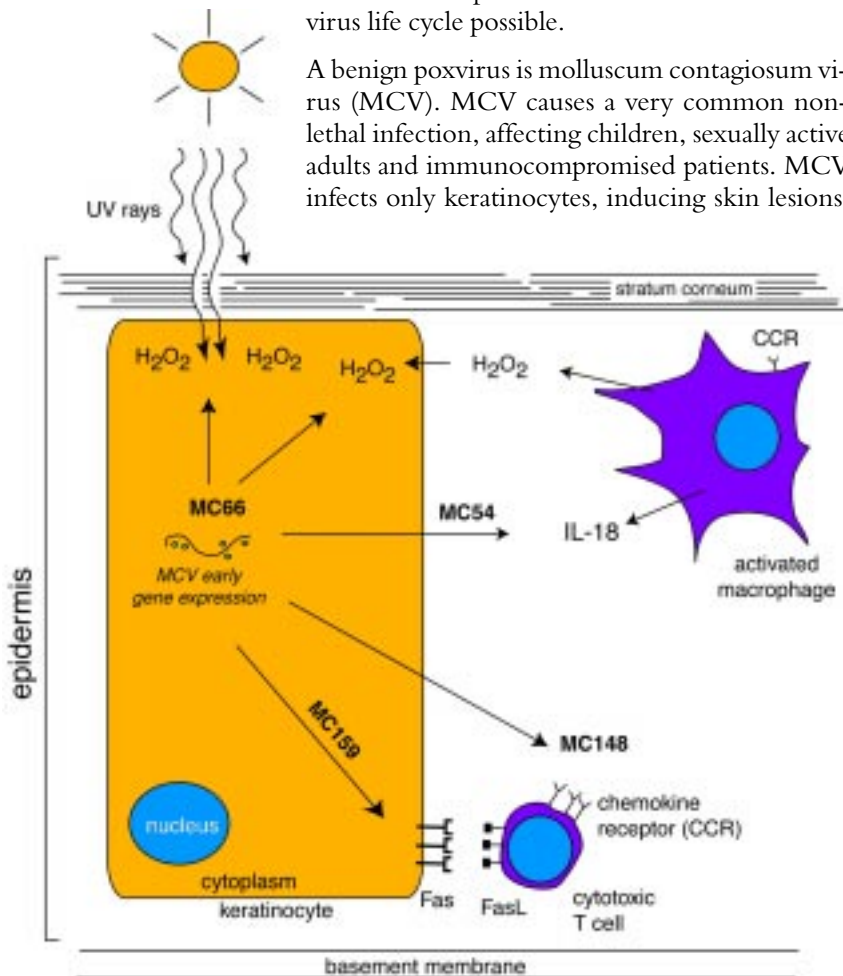


Joanna Shisler

Dr. Joanna Shisler joined the University of Illinois Department of Microbiology in November 2001, following her post-doctoral studies at the National Institutes of Health in Bethesda, Maryland. Her research focuses on the delineation of mechanisms that viruses use to evade anti-viral immune responses, using poxviruses as a model system.

My laboratory delineates mechanisms viruses use to evade anti-viral immune responses, using poxviruses as a model system. Poxviruses are large, double stranded DNA viruses that are important to the medical community because of disease they cause in humans. Recombinant and mutant poxviruses are easy to engineer, making functional studies of viral proteins within the context of the virus life cycle possible.

A benign poxvirus is molluscum contagiosum virus (MCV). MCV causes a very common non-lethal infection, affecting children, sexually active adults and immunocompromised patients. MCV infects only keratinocytes, inducing skin lesions,



Immunomodulatory proteins produced by MCV. MCV replicates exclusively in keratinocytes within the human epidermis. An MCV-infected keratinocyte that is expressing virus-encoded immune-defense molecules is depicted. The expression of selected antiviral molecules by a macrophage and T lymphocyte that have entered the epidermis in response to tissue injury is also shown. UV rays from the sun, as well as inflammatory cells, can generate hydrogen peroxide.

or “pocks” that are small, but persist for months and sometimes even years before spontaneously resolving. It is of great interest to determine the mechanisms MCV utilizes to persist for such lengthy periods of time, specifically focusing on the immunomodulatory proteins MCV produces to fight anti-viral immune responses. These products are synthesized early during infection. For example, MCV produces a secreted MC54 protein that binds to and inhibits the IL-18 cytokine, dampening macrophage activation. MC148 is secreted into the extracellular milieu to competitively bind to the CCR8 chemokine receptor on lymphocytes. MC148 prevents cellular chemokines from binding to CCR8, inhibiting migration of other immune cells to areas of infection.

Using a diverse range of genetic and molecular techniques, I am studying three MCV gene products predicted to inhibit apoptosis, a programmed form of cell death utilized by effectors of the immune response to eliminate virus-infected cells.

Apoptosis is induced by myriad insults—cytokines, UV irradiation, other cells—and each insult triggers a unique signal transduction pathway resulting in apoptosis. Of course, as we learn about the function of anti-apoptosis MCV proteins, we also learn about the signal transduction events of apoptosis. For example, my laboratory has shown MC66 inhibits apoptosis induced by hydrogen peroxide produced by the immune system (macrophages) or by extracellular insults (UV irradiation). MC66 is a glutathione peroxidase homolog, converting toxic hydrogen peroxide into water and oxygen. MC159 protects against Fas-induced apoptosis. Fas is a cellular receptor that cytotoxic T cells bind to via Fas Ligand (FasL) to kill virus-infected cells. To gain a better understanding of MC159, we are currently investigating other apoptosis pathways in which MC159 functions to learn more about the biological role of this protein. MC66 cannot protect against Fas cytolysis, indicating that the signal transduction pathways of Fas and hydrogen peroxide are distinct.

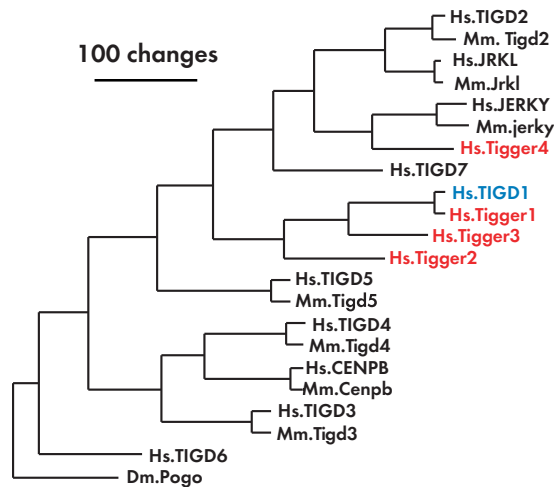
Other dermatotropic viruses produce similar immune-evasion proteins, notably herpesviruses. Thus, work delineating MCV persistence mechanisms will lead to a greater understanding of persistent infections of the skin and facilitate subsequent design for intervention or prevention treatments.

Domesticated Jumping Genes in our Genome

With the completion of the human genome sequence the assertion of those who work on transposons (jumping genes) that they constitute a major part of our genome has been overwhelmingly confirmed. Approximately half of the 3.2 billion base pairs in our genome is clearly attributable to transposons that have expanded their number in our genome lineage in the past 200 million years, with perhaps another 25% so ancient they can no longer be recognized as deriving from transposons. Indeed just 3–5% of our genome codes directly for our $\pm 50,000$ proteins. Many find it hard to accept that the vast majority of these transposon sequences are simply junk DNA resulting from the selfish spreading behavior of these transposons. It is clear that this level of genomic baggage is not necessary for vertebrate function, e.g. the Fugu pufferfish genomes are mostly devoid of this baggage and hence are about 1/7 the size of ours, while at the other extreme, lungfish genomes reach 30X the size of ours, most of which is surely also transposon baggage. Nevertheless, there are many interesting examples of particular copies of certain transposons that have come to be useful to their hosts, which we then call “domesticated” transposons.

In vertebrates the most spectacular example of a domesticated transposon are the **RAG** genes that encode the proteins responsible for the rearrangement of the constant, joint, and variable regions of the immunoglobulin genes encoding antibodies and T-cell receptors in our immune system. These genes appear to have been derived from a transposon approximately 450 million years ago, when the sophisticated immune system of vertebrates was evolving. They have even been reconstituted into an artificial jumping gene as partial confirmation of their transposon origins.

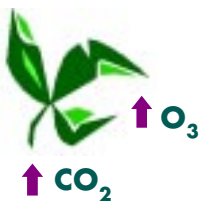
A few years ago we described the youngest example of a domesticated transposon in our genome: the in-frame splicing of two endogenous exons encoding a **SET** domain protein to the mariner transposase gene of a particular copy of **Hsmar1**. **Hsmar1**, the first of two mariner family transposons found in the human genome, invaded our genome lineage about 50 million years ago, and generated about 200 copies. All but this copy are now inactivated by a variety of mutations and hence have joined our junk DNA. We recently showed that in tarsiers, the basal lineage of anthropoid primates (our lineage), this **Hsmar1** copy is



not present downstream of this **SET** domain gene. We have yet to figure out what the **SETMAR** protein does for us and other anthropoid apes. **SET** domains have recently been shown to catalyze methylation of lysines in histones, indicating that it might be involved in chromatin modification and hence gene regulation.

In the past two years, we (postdocs Barry Williams and David Witherspoon and technician Kim Walden) have focused our attention on a set of ten domesticated jumping genes derived from transposons belonging to the pogo family, which in turn is related to the mariner and other families of DNA-mediated transposons that are widespread in animal genomes. One of these is **CENPB**, a well-known centromere binding protein found throughout mammals. Another called **JERKY** was discovered as a mutation that somehow caused epilepsy in mice, and the orthologous gene in humans is also implicated in epilepsy. Several more were identified by various investigators and seven of the ten were listed amongst 40 examples of domesticated transposons in the public Human Genome Draft paper in Nature last spring. We have identified a very interesting example, which like **SETMAR** is relatively young, and was derived from a particular copy of the **TIGGER1** transposon shortly after it invaded an early primate genome, which happened approximately 80 million years ago. Unlike the other ± 3000 copies of **TIGGER1** in our genome that are all now junk

*A phylogenetic tree relating all ten of the domesticated genes derived from Tigger transposons. The tree is rooted with the pogo transposase from *Drosophila melanogaster*. The four Tigger transposases are shown in red, and Eeyore (*Hs.TIGD1* in the formal human gene nomenclature) is in blue type. Note that the human and mouse versions of each gene are relatively closely related (the mouse version of **TIGD6** has been lost while the mouse *Tigd7* is a highly degenerate pseudogene). The divergence of Eeyore (**TIGD1**) from the *Tigger1* consensus is slightly lower than most human/mouse gene divergences, in keeping with it being approximately 80 Myr old.*



SoyFACE

Carbon dioxide (CO_2) in the world's atmosphere is on the rise. In the last century it rose 20% and this geologically unprecedented pace will continue resulting in an atmosphere in 2050 containing 50% more CO_2 than it did in 1900. While this enrichment of the atmosphere portends certain hazards of which global warming is the most publicized, it also offers the potential to increase in plant production. This will be a result of increased photosynthetic efficiency at elevated CO_2 in important crops such as soybean, wheat and oats as well as making it possible for all crop plants to be more water efficient. Carbon dioxide is in effect a fertilizer, and is in fact used as such in greenhouse production of horticultural crops. But just as germplasm had to be specially selected to achieve the potential increases in yields provided by nitrogen fertilization, so selection will be necessary to maximize response to the rise in CO_2 . However, there is little

evidence that plant breeding has, to date, selected lines capable of fully realizing this potential.

Perhaps less well known is the fact that the pollutant ozone is also on the rise in the portion of the atmosphere closest to the Earth's surface. In fact, in agricultural areas of the industrialized regions of the world, such as the Midwest, ozone is increasing more rapidly than CO_2 . The effects of ozone pollution on crop production are already costing US Agriculture an estimated \$2 billion in lost production per year. In Central Illinois ozone reaches, on average, 60 parts per billion (ppb) each day during the growing season. On occasional days, levels exceed 100 ppb. Levels above 40 ppb decrease soybean production (Fig. 1) but the transient and unpredictable nature of ozone pollution levels has prevented successful selection for ozone tolerance in sensitive crop species.

SoyFACE is an innovative facility for growing crops under production field conditions anticipated for the middle of this century, namely an atmosphere with higher levels of carbon dioxide and ozone. SoyFACE is designed to discover the effects of atmospheric change on the agronomy and productivity of Midwest crops as well as to

find solutions that will lead to crops better adapted to this future. This unique facility and the highly interdisciplinary research teams that it serves seek answers to important questions including:

- What yield and quality change will result with current major cultivars?
- What genotypes and genes may be exploited to increase yield and maintain quality under the changed atmosphere?
- What system changes will increase yields and maintain quality under the changed atmosphere?
- To what extent will soil water use be decreased, and what implications will this have for drainage, soil moisture and nitrogen transport?
- Will crop canopy temperature increase and what effects will this have on development and seed-set? Is there genetic variation that may overcome this problem?
- How will nitrogen fixation and rates of extraction of soil nutrients be affected?
- Will soil quality be degraded and how can this be alleviated?
- Will rates of soil carbon deposition be increased and what value might this have in terms of carbon credits?

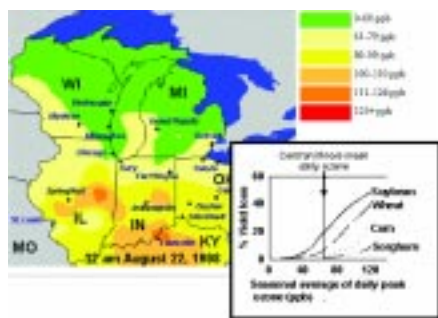


Figure 1. Surface ozone in the Midwest on a sunny August day in 1999. Inset is a summary from USDA chamber studies yield loss at different levels of ozone pollution.



Figure 2. One of eight FACE rings that were operated in 2001. Within the ring soybean is grown from sowing to harvest at 550 ppm carbon dioxide, mimicking the level anticipated for the year 2050. The right side of this ring is planted to a single cultivar—that used throughout the field, the left side to 26 different lines. Currently corn is growing at the same location, also under elevated carbon dioxide.

What is SoyFACE?

As the name suggests, the primary research focus is on soybean, but the impacts of atmospheric change on corn are also under investigation. SoyFACE is situated on 80 acres at the southern edge of the new South Farms of the University of Illinois making it the largest “open-air laboratory” worldwide for investigating the impacts of the changing composition of the atmosphere on crops. It exploits a new technology: Free Air Concentration Enrichment (FACE), now being implemented at four other international sites. It consists of rings (ca. 70’ diameter) of pipes that release carbon dioxide or ozone into the wind as it flows across the crop (Fig. 2). A computer continuously measures wind speed and direction, and the gas concentration within the ring to determine which pipes release the gas and how much they release. The carbon dioxide or ozone is released into the naturally moving air so that the concentration within the ring is elevated to a preset level. The rings use about a ton of carbon dioxide or about a pound of ozone a day. Downwind of the ring the added gas is quickly diluted in the flowing air so that concentrations are close to background within about 300 feet.

A standard corn-soybean rotation is practiced, so that each year 40 acres are in each crop. In 2001, the western half was in soybean and the eastern in corn, switching in 2002. In 2001, four elevated and four ambient CO₂ rings were established within the 40 acres of soybean. In 2002, corn is being grown in these eight rings. Twelve additional rings have been added to the 40 acres now in soybean, four ambient, four elevated CO₂ and four elevated ozone. In 2003, four further rings will be added to study the interactive effects of elevated ozone and CO₂. One half of the ring is sown to a single commercial variety; the other half is used to test a range of germplasm (Fig. 2). With the development of funding and appropriate technology, there are plans to add free air temperature increase treatments to simulate the global warming that is anticipated for the Midwest by the middle of the century. How well does the present system work? The fast computer feedback results in surprisingly good control, the concentration achieved is within 20% of the target for 97% of the time. Increased CO₂ decreases evaporation of water and therefore cooling of leaves in sunlight. As a result leaves are

warming. In Fig. 3, an increase of temperature of about 5°F within the ring illustrated can be seen.

What has been learned?

The SoyFACE project, while just getting underway, has already resulted in important discoveries. In 2001 growth of soybean in elevated CO₂ showed significant increases in photosynthetic carbon uptake and decreases in water loss from emergence to final senescence of the crop. Total soybean seed production was increased from 59 to 69 bushels per acre. Although large, this 17% increase is less than half of the 40% increase in photosynthesis documenting a large unrealized potential of modern cultivars in responding to carbon dioxide fertilization. The yield increase was in the number rather than size of seeds. There was no significant change in oil and protein content, but isoflavone content rose significantly. Great variation was observed within the germplasm trials, the largest yield increase being 30% compared to a minimum of 10%. The most striking effect of elevated CO₂, was a delay in crop senescence of almost two weeks, this was apparent in all germplasm (Fig. 4).

Acknowledgements. The SoyFACE facility and project were launched with enabling funding from the Sentinel program of C-FAR (Council for Food and Agricultural Research). With this key funding in place ADM, Argonne National Laboratory (ANL), Pioneer Hi-Bred, USDA-ARS and USDA-NRI have provided funding and further support that has greatly expanded and enhanced the facility.

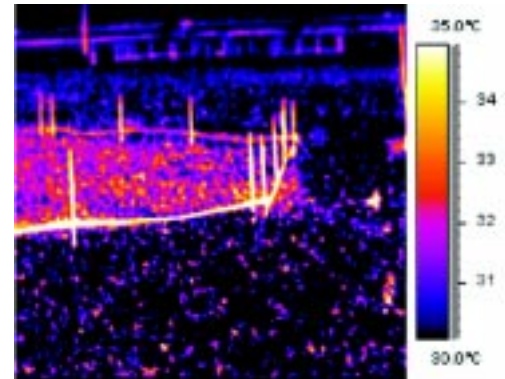


Figure 3. False color photography showing that soybean plants within the ring in elevated carbon dioxide are 5°F (3°C) warmer than the plants outside of the ring at the current carbon dioxide concentration. The increase in temperature within the ring in response to increased carbon dioxide illustrates the reduction in water vapor loss by the leaf canopy. (Photo credit: Andrew Leakey, Visiting Fulbright Scholar)



Figure 4. Soybean “Pana” in elevated carbon dioxide remains green while the same crop outside senescences and dries down.

Donald Ort

Tools for Manipulating cDNA libraries

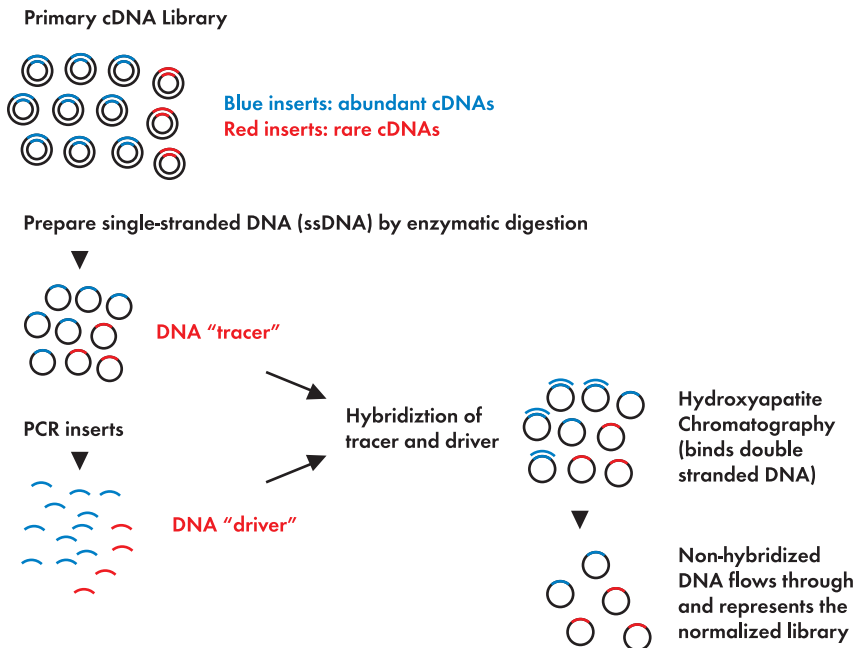


Figure 1. Normalization of cDNA Library. cDNA reannealing follows second order kinetics. The most abundant cDNAs anneal rapidly, whereas the rarer species remain single stranded. cDNAs that remain single-stranded after hybridization represents the normalized library (decreased frequency of most abundant cDNA clones).

The initial step in many functional genomic projects involves the creation of a cDNA library and mass sequencing of resulting cloned cDNA fragments. A cDNA library is derived from the reverse transcription of all the mRNAs in a specific cell type or tissue at a given time. As researchers are focusing more on studying large groups of interrelated genes, mass sequencing of cDNA libraries has become widely used in gene discovery projects. Though isolation of mRNA, synthesis of cDNA and cloning are relatively standard techniques, there are several advanced tools available to the researcher that can enhance a successful sequencing project, and lead to a more cost-effective strategy for the identification of rare expressed genes. Currently in the High Throughput Sequencing and Genotyping Laboratory in the W.M. Keck Center two of these strategies, library normalization and subtraction, are routinely used in the generation and manipulation of cDNA libraries.

The most prevalent mRNAs in a typical somatic cell make up to 65% of the total message, yet represent at most only 2,000 distinct species. As a general rule, the frequency of a cDNA clone in a library is equivalent to that of the mRNA from which it originated. Therefore, the power of large-scale sequencing of standard libraries is hindered by repetitive identification of the most abundant cDNA clones. **Normalization** of a cDNA library equalizes the representation of all distinct species in the cDNA population, significantly reducing the abundance of the most redundant cDNAs. Thus, identification of novel and rare cDNAs is enhanced in normalized cDNA libraries (see figure 1). However, after sequencing several thousands of clones, discovery of low copy cDNAs becomes progressively less cost-effective. If further

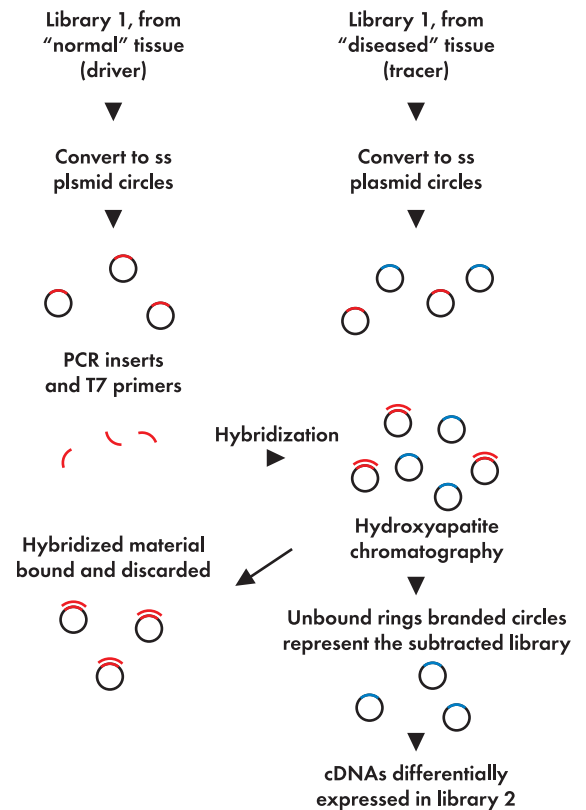


Figure 2. Subtracted libraries. Differentially expressed genes in library 1 or 2 can be obtained depending on which library is used as a driver (driver library = cDNAs one wishes to eliminate).

Domesticated Jumping Genes in our Genome, continued

sequencing is planned, the already sequenced clones can be subtracted from the library from which they originated. The new **subtracted library** is therefore enriched for rarer transcripts that are likely to be missed in a random sequencing approach. This technique has also been successfully used in the W. M. Keck Center to subtract redundant clones that are commonly expressed in multiple tissues, from previously constructed libraries, allowing researchers to sequence “deeper” into their library in a cost-effective manner.

When isolation of differentially expressed genes is preferred, sequencing of genes common to both states lowers the efficiency of discovery for uniquely expressed genes. Differential gene expression as a result of physiological changes, development of disease or treatment with drugs or toxics may be more efficiently addressed using subtracted libraries. For this, two cDNA libraries are hybridized and cDNAs common to both populations are removed. The resulting **subtracted cDNA library** is enriched for uniquely expressed genes (see figure 2).

In summary, the use of normalized and subtracted cDNA libraries greatly improves the identification of rare and/or differentially expressed genes. Normalized and subtracted libraries can also be used for construction of “targeted” microarrays in gene discovery programs, pharmacogenomics, toxicogenomics and disease diagnosis.

H. Hernandez and P. Schweitzer

Reference: Bonaldo, M.F., G. Lennon, and M.B. Soares. 1996. Normalization and subtraction: Two approaches to facilitate gene discovery. *Genome Res.* 6: 791-806.

DNA, this copy at 3p26 near the left telomere of our third chromosome, which we call **EEYORE**, encodes a full-length **TIGGER1** transposase protein just 7% diverged from that of the **TIGGER1** consensus transposon. Amplification and sequencing of **EEYORE** from all major lineages of primates including multiple prosimians (lemurs, bushbabies and other strange little arboreal primates that make up the other major basal lineage of primates) reveals its conserved presence. It is clearly doing something useful for all primates. Molecular evolution analysis indicates that most of the crucial amino acid changes that made it useful occurred shortly after **TIGGER1** invaded. Again we do not know what **EEYORE** and most of the other *domesticated* transposons are doing for us, but they clearly have contributed a small part to our existence.

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New Director at the Biotechnology Center, continued

use these new methods to discover multiple new neuropeptides in simpler nervous systems.

Cotransmission in simpler nervous systems. A subset of our research is designed to understand the molecular (chemical) nature of learning and memory. Specifically, the new instrumental methods we are developing allow trace-levels of the chemical signaling molecules present in and released from neurons to be identified and quantified. Using the methods we and our collaborators have: discovered and characterized new neuroactive peptides such as *Aplysia* insulin, the enterins and cerebrin; measured NO production and NO interactions with classical transmitters and studied neurotransmitter cotransmission, all at single cell levels.

J. Sweedler

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Heavy Metals, Human Sewage May Contribute to Killing Coral Reefs



*A head of *Diploria strigosa* (the common brain coral) exhibiting classic symptoms of black band disease (starting at the top and killing the coral in a downward trajectory).*

Global warming may not be the only culprit killing the coral reefs. Human sewage and metals from shipyard discharge may be involved in the development and spread of deadly black band disease in corals, say researchers in LAS.

“Black band disease is characterized by a ring-shaped bacterial mat that migrates across a coral colony, leaving dead tissue in its wake,” says geologist Bruce Fouke.

“Like a rainforest, a coral reef system is a cradle of biodiversity. If we destroy the reefs, we destroy a major portion of the ocean’s ability to reproduce.”

To better understand the disease, Fouke and his colleagues—microbiologist Abigail Salyers and postdoctoral researchers George Bonheyo and Jorge Frias-Lopez—studied corals off the island of Curacao in the Netherlands Antilles, near the

Venezuelan coast. They mapped outbreaks of the disease along the reef, then looked for metals such as aluminum, cadmium and zinc that are common pollutants from shipyards and oil refineries.

They discovered that the highest number of infected corals, as well as the highest concentration of dissolved metals, occurred near the city of St. Annabaai, which has a major harbor and a large oil refinery. This suggests that diseased coral may be experiencing increased environmental stress due to pollution, which in turn decreases the coral’s resistance to bacterial infection.

Healthy corals contain a natural population of bacteria within a mucous-rich biofilm that provides protection from light, exposure, and sedimentation, Fouke says. “Environmental stresses cause corals to secrete more of this mucous to coat their outer tissues. This leads to elevated levels of natural microbial populations as well as the introduction of new, potentially harmful bacterial populations.” Among the organisms the researchers found inhabiting the black band biomat were *Arcobacter* and *Campylobacter*, which are human pathogens and could be a direct link to raw sewage.

J. Kloeppe and B. Fouke



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