The body is a big sagacity, a plurality with one sense, a war and a peace, a flock and a shepherd.
Friedrich Nietzsche (1844–1900)

INNOVATIVE TECHNOLOGIES

Peptide Arrays Break the Species Barrier

Phosphorylation, in which phosphate groups attach to proteins or small molecules, is among the cell's primary means of controlling protein activity. It regulates nearly every aspect of cell life, including cell metabolism, proliferation, viability, differentiation, apoptosis, and transmission of hormone and growth factor signals. Protein kinases are the enzymes that catalyze this myriad of molecular transactions. With more than 500 unique kinases and one-third of mammalian protein expression known to be associated with phosphorylation events, the study of kinase activity via high-throughput analysis represents a high priority for pharmaceutical researchers seeking drug candidates and environmental researchers seeking harmful agents.

Phosphorylation events and characteristics of the kinome (the set of protein kinases in an organism's genome) are well defined for mice, but mouse models are available only for a subset of human disease states. Other species may offer more accurate models for specific disease or physical conditions in humans, but the phosphorylation profiles of many animals are largely unknown. What is known, however, is the unique composition of the genomes of an ever-expanding number of species. And that turns out to be enough to assemble a peptide array to profile a cell's kinase activity in a quick and cost-effective fashion, as investigators at the University of Saskatchewan have demonstrated.

"It turns out the protein sequences can be predicted," explains Scott Napper, a program manager and scientist with the university's Vaccine and Infectious Disease Organization and International Vaccine Centre (VIDO/InterVac). "You don't have to rely on existing phosphorylation databases. You can extrapolate from these into other species."

In a paper published in the 20 January 2009 issue of *Science Signaling*, Napper and his colleagues offer what amounts to a recipe for this sequence-predicting process. In a nutshell, the process relies on the fact that amino acid sequences surrounding phosphorylation sites within specific proteins—along with the biologic function of those phosphorylation events—are often conserved from one species to another.

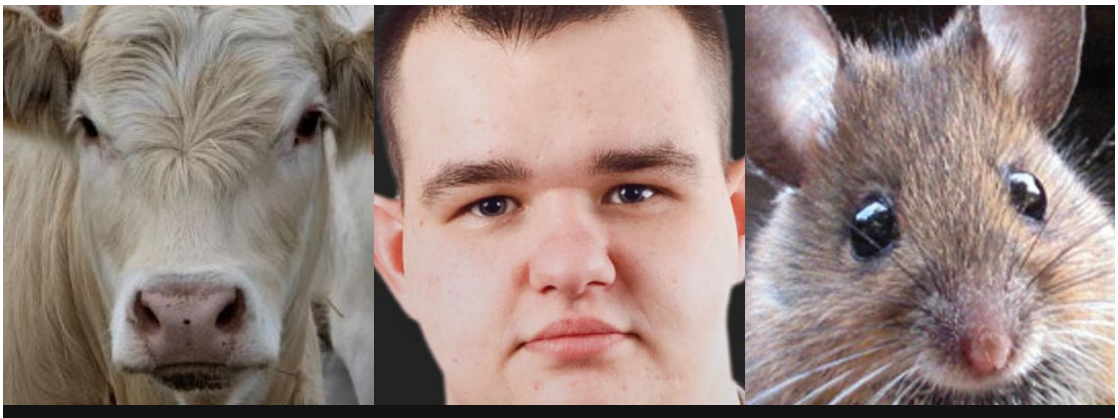
As a proof-of-principle demonstration focusing on specific proteins previously shown to undergo phosphorylation in human and mouse cells, Napper's team created a custom array of peptides representing the corresponding proteins in cow cells. Specifically, they identified phosphorylation events relevant to pathways of interest, used publicly available databases to identify human peptide sequences for the phosphorylation sites, searched for bovine peptides that matched the human sequences, and confirmed that the human and bovine peptides were from functionally equivalent proteins. The phosphorylation sites were then arrayed onto slides and exposed to protein kinases from cow cells to profile phosphorylation activity.

Napper describes the approach as remarkably cost effective. An array revealing 300 different phosphorylation events takes about two days and $100 to produce, says Napper. Obtaining these data using a more conventional approach would take much longer, and the price tag could run into tens of thousands of dollars. Moreover, in many cases, species-specific antibodies simply may not be available. "The peptide arrays optimize the amount of data collected yet minimize the amount of laboratory waste generated, compared with traditional approaches," says Napper. "In addition, we have adjusted the experimental protocol so that radioactivity is no longer required for signal detection."

The motivation for developing the original peptide array stemmed from Napper's ongoing work on Johne ("yo-knee") disease, an intestinal infection that leads to fatal wasting in cattle, sheep, and other ruminants. The new array may provide a quick, practical way to solve the long-standing problem of this disease, Napper says. "By providing insight into the mechanisms of various pathogens of livestock animals, these arrays have the potential to facilitate new treatments that will reduce the prevalence and shedding of these pathogens into the environment," he says.

Napper adds that the peptide array has made it possible to obtain preliminary insights into mechanisms by which bacteria appear to shut down the host's immune response. Implications of treating Johne disease could extend beyond veterinary circles: the bacterium responsible for this condition may be associated with Crohn disease in humans, as reported in a review in the January 2008 *Current Opinion in Gastroenterology*. An understanding of the phosphorylation events responsible for Johne disease could point the way to treatments for Crohn disease. Moreover, says B. Alex Merrick, a staff scientist in the NIEHS Laboratory of Respiratory Biology, testing for inflammatory signaling with peptide arrays like this might be useful for screening everything from investigational new drugs to environmental pathogens.

"What is key, and the reason we are excited about it, is the biological information that can come out of it," says Andrew Potter, director and CEO of VIDO/InterVac. "If we can generate new insights to solve some of the problems we're working on, that'll be a huge step forward."
–Tim Lougheed



The nuts and bolts of phosphorylation, which regulates nearly every aspect of cell life, are largely conserved across species.

Left to right: Scott Napper; Marfot/Shutterstock; Creative Commons

CHILDREN'S HEALTH

Methylation Links Prenatal PAH Exposure to Asthma

Research suggests that a mother's exposure to pollution during pregnancy may predispose her child to asthma, and there is preliminary evidence implicating transplacental exposure to polycyclic aromatic hydrocarbons (PAHs)—generated mainly by the burning of fossil fuels and abundant in high-traffic areas. Until recently, progress in the study of prenatal exposures to PAHs and other pollutants has been hampered by a paucity of biomarkers for predicting asthmatic risk. Researchers from the University of Cincinnati and Columbia University Mailman School of Public Health now report that methylation of *ACSL3*, a gene expressed in lung and thymus tissue, may provide a possible biomarker linking prenatal exposure to PAHs to childhood asthma.

The researchers hypothesize that transplacental exposure to PAHs might lead to aberrant DNA methylation changes, which in turn alter the expression of genes in the fetal lung or immune system, perhaps setting the stage for childhood asthma. When toxicant-induced aberrations in DNA methylation occur during critical developmental periods, there is evidence they can lead to inappropriate gene expression and disease later on.

"It's thought that pollutants that can cross the placenta may alter the function of some systems in later life," explains study coleader Shuk-mei Ho, director of the Center for Environmental Genetics at the University of Cincinnati. These prenatal exposures can predispose the fetus to develop compensatory—and sometimes detrimental—responses in anticipation of continued exposure after birth. "During fetal life, anticipatory responses to the future external environment may come about via changes in the course of tissue or cell differentiation, often via epigenetic reprogramming of genes by DNA methylation," she says. "Our data support the concept that environmental exposures can interact with genes during key developmental periods to trigger disease onset later in life, and that tissues are being reprogrammed to become abnormal later."

The researchers initially examined PAH exposure data for 729 female participants in a longitudinal cohort study, all of whom were living in high-traffic areas of Northern Manhattan and the South Bronx, where childhood asthma rates hover around 25%. Next they identified 10 women with PAH exposure above the group median PAH level of 2.3 ng/m^3 (as determined by personal air monitoring) and 10 women with exposure below the median. Using methylation-sensitive restriction fingerprinting to examine the DNA of umbilical cord white blood cells collected from the women's children at birth, the researchers identified gene sequences whose methylation status differed between the high and low maternal exposure groups. These included sequences in or near the promoter region of six genes expressed in the lung and/or lymphoid tissue that are involved in inflammation or other immune response functions.

A study of pregnant black and Dominican women has revealed a potential biomarker predicting childhood asthma.

When the researchers compared the methylation percentages of these six gene sequences with their transcript expression levels in matched fetal placental tissue, the strongest inverse relationship (due to methylation's silencing of the gene) was seen for *ACSL3*. "The methylation of this gene is likely to regulate its expression, so it was therefore a good candidate for assessing a biological response to PAH exposure," explains study coleader Frederica Perera, director of the Columbia Center for Children's Environmental Health.

The researchers then confirmed their findings in a group of 56 women and their fetuses from the same cohort study. Of the children born to mothers with high PAH exposure, 81% had a methylated *ACSL3* gene promoter, compared with 23% of children born to mothers with low PAH exposure. An association was also found between the fetal methylation of *ACSL3* and parental reporting of asthma before the child's fifth birthday, with 73% of asthmatic children showing *ACSL3* methylation compared with 41% of children without asthma. These findings were reported in the 16 February 2009 issue of *PLoS ONE*.

"This study is interesting but still preliminary," remarks Manel Esteller, director of the Cancer Epigenetics and Biology Program at the Bellvitge Institute for Biomedical Research, Barcelona, Spain. "Results now need to be obtained with more subjects, the results duplicated in cell culture and animal models, and a mechanistic connection between PAH and the methylation of this gene demonstrated."

Peter Helms, a professor of child health at the University of Aberdeen, United Kingdom, adds that the *ACSL3* link with asthma needs to be more convincingly demonstrated, with better control of possible confounding factors (such as maternal smoking), rigorous diagnostic criteria, and long-term follow-up. "Asthma is notoriously difficult to diagnose in children under age five, and if the study sample were larger and the children followed up for longer, I think we'd be closer to knowing whether methylation of *ACSL3* has potential in the early identification of childhood asthma," he says.

Perera says the study authors plan to further test the association as the children mature and reach ages where they can be clinically diagnosed with asthma. If substantiated by future research, the methylation of *ACSL3* might serve as a tool to identify children at increased risk of developing asthma, thus opening up new avenues for prevention. –Adrian Burton

DIET AND NUTRITION

Vitamin D Regulates MS Gene

Multiple sclerosis (MS), an autoimmune disease affecting some 2.5 million people worldwide, is thought to arise from a confluence of genetic and environmental factors. Dietary vitamin D intake has been associated with lower MS risk, and vitamin D deficiency has been associated with increased risk, but direct links between vitamin D and MS have not been identified. A team of Canadian and British researchers has now reported evidence that vitamin D interacts with a variant form of the *HLA-DRB1* gene, which has been associated with MS.

As reported in the 6 February 2009 issue of the online journal *PLoS Genetics*, study leader George Ebers, a clinical neurologist at the University of Oxford, United Kingdom, and colleagues examined cells with two copies of the *HLA-DRB*15* form of *HLA-DRB1*. They identified a vitamin D response element (VDRE)—a short stretch of DNA that is a signature of genes regulated by vitamin D—next to the gene. When they examined DNA from study participants they found the same VDRE sequence in each of 322 individuals with two copies of *HLA-DRB1*15* (including people with and without MS), but found different VDRE sequences in DNA samples from 168 study participants without *HLA-DRB1*15*. The researchers also showed that the VDRE sequence found in people with *HLA-DRB1*15* could bind to the vitamin D receptor, and that the *HLA-DRB1* gene responded more strongly to vitamin D in cells with the *HLA-DRB1*15* VDRE sequence than in cells without it.

"This is the first direct evidence that vitamin D regulates the gene," says Ebers. He says that whereas the general public has a 1 in 1,000 chance of developing MS, the estimated risk of MS is 1 in 300 for people with one copy of the *HLA-DRB1*1501* gene variant, and 1 in 100 for those with two copies of *HLA-DRB1*1501*.

The next step is determining how vitamin D and *HLA-DRB1*15* might interact to modulate the autoimmune nature of MS. Epidemiologic and animal studies of MS point to involvement of the thymus gland early in development. The thymus produces T cells, a type of white blood cell involved in immune responses to foreign proteins (antigens). Each T cell is made with a unique antigen receptor that allows the immune system to react quickly to any foreign protein a person might encounter. However, T cells with antigen receptors that could respond to normal "self" proteins must be destroyed to prevent an autoimmune response. The researchers reason that a lack of vitamin D in the thymus in early life could limit this process, thus enabling "self-directed" T cells to survive and trigger an autoimmune response to the myelin sheath on nerve fibers—a classic feature of MS.

The authors propose that vitamin D supplementation at critical periods during pregnancy and early childhood might reduce the risk for developing MS—a view endorsed by Bruce Hollis, a nutritional biochemist and professor of pediatrics at the Medical University of South Carolina. Whereas the Institute of Medicine recommends 200 international units of vitamin D per day (IU/day) for people under age 50, including pregnant and lactating women and infants, Hollis wrote in the December 2007 issue of the *Journal of Bone and Mineral Research* that as much as 6,000 IU/day might be needed to maintain adequate blood levels of the vitamin in these populations. He is currently wrapping up NIH-funded research that suggests at least 2,000 IU/day may be an optimal intake. [For more information on vitamin D deficiency, see "Benefits of Sunlight: A Bright Spot for Human Health," *EHP* 116:A160–A167 (2008)].

"Many obstetricians are oblivious to their patients' vitamin D status and falsely assume that the miniscule amount of vitamin D contained in prenatal vitamins will meet the needs of their patients," Hollis says. "In fact that amount is not even close." Considering that the body makes up to 20,000 IU of vitamin D when exposed to sunlight for 30 minutes on a summer day, Ebers says 2,000 IU/day "is not a dangerous amount." –Carol Potera

The Beat | by Erin E. Dooley

Europe Puts EMFs on Alert

In April 2009 the European Parliament adopted a report urging the European Commission to set limits on how close mobile phone masts, antennas, high-voltage power lines, and other electromagnetic field (EMF)–transmitting devices can be to neighborhoods, schools, and health care facilities. The report asks the commission to review the scientific basis and accuracy of current EMF limits and recommends the annual publication of a map showing areas of EMF exposure, an annual report on EMF levels in Europe, labeling requirements to state EMF transmission levels of wireless devices, and increased education about the safest use and potential dangers of mobile phones, particularly for children and teenagers.

First Bloom of Sister Study Findings

Early findings from the NIEHS-sponsored Sister Study, published in the February and March 2009 issues of *Cancer Epidemiology Biomarkers & Prevention*, showed that women who maintained a healthy weight and perceived themselves to have less stress were less likely to have shorter telomeres, the repeating DNA sequences that protect the ends of chromosomes and help maintain genetic integrity during cell replication. Shortened telomeres are associated with a heightened disease risk and increased mortality rates for cancer and heart disease. These reports add to previous research showing that positive lifestyle changes can aid telomere activity.

The Sister Study includes women whose sister had breast cancer.

Claudiad/istockphoto

Water Certification in the Works

At the March 2009 World Water Forum, the Alliance for Water Stewardship announced a new labeling program that would state whether the water used to make a product came from a sustainable source. The initiative is similar to the Forest Stewardship Certification, which labels wood products as sustainably harvested from certified lands. Alliance members hope to establish universal "core standards" for sustainable water management by mid 2009; from there, local agencies could add their own criteria to address regional needs. Within the next two decades, an estimated 3.9 billion people could live in drought-stricken areas, according to *OECD Environmental Outlook to 2030*, a 2008 report from the Organisation for Economic Co-operation and Development.

Diacetyl Deadlock Broken?

Diacetyl, a chemical compound used to impart a buttery flavor to foods like microwave popcorn, may become hazardous when it is heated and inhaled over a long period. In exposed workers, it has been linked with bronchiolitis obliterans, a

NATURAL RESOURCES

Reversing Human Impacts on Fish Evolution

Next time you catch a "big one," throw it back. This practice might seem counterintuitive, given that minimum size regulations favor keeping the larger fish, but it could reap long-range dividends for fish and humans alike, according to a study published online 4 March 2009 ahead of print in the *Proceedings of the Royal Society B*. Since 1990, scientists have observed that fish are getting smaller and growing more slowly as humans have continued to harvest the largest fish in wild stocks. Selecting out the large fish from a population sets the stage for earlier adult maturation, which means smaller fish are producing fewer eggs and offspring. This, in turn, could shrink many wild harvests. But the news is not all bad: The new study by David O. Conover, dean and director of the School of Marine and Atmospheric Sciences at Stony Brook University in New York, indicates fishery-induced genetic change can be slowly reversed.

Conover and his colleagues report on an empirical simulation experiment wherein captive populations of Atlantic silverside (*Menidia menidia*), a bait fish, were caught off the coast of the Great South Bay, New York. Groups of fish were selectively culled for the largest fish, mimicking the practice of most fisheries. The populations evolved smaller body size during the first five generations, and the smaller fish became less fertile because they produced fewer eggs.

"In our experiment," says Conover, "the females were getting so small and producing eggs that were so small that the survival of those eggs and larvae were reduced dramatically. We would have eventually driven our own study population into extinction if we hadn't stopped the large harvesting after five generations. We were struggling to produce enough fish for the sixth generation."

From the sixth through tenth (and final) generation, fish were harvested at random. The fish populations showed a slow but significant increase in size, although they did not reach full recovery. The researchers estimate that it would take about 12 generations of random harvests for the body size of this fish to return to normal. Harvested fish species typically have generation times of 3–7 years, so recovery of some overfished populations could take 3–8 decades, given a size decline of the magnitude induced in this study.

Chris Darimont, a postdoctoral fellow at the University of California, Santa Cruz, says, "Scientists have almost exclusively studied how exploited populations have undergone undesirable changes such as smaller sizes or less productive breeding schedules. But this study looks at what happens when a population is released from size-selective predation." Darimont wrote that human predation is rapidly accelerating the rate of observable trait changes in commercially harvested species in the 20 January 2009 issue of the *Proceedings of the National Academy of Sciences*.

Fish provides more than 2.9 billion people with at least 15% of their average per-capita animal protein intake, according to the *World Fisheries and Aquaculture 2008* report by the Food and Agriculture Organization of the United Nations. The report says that 80% of all marine fish stocks for which assessment information is available are fully exploited or overexploited.

To help recover an overexploited population, fishery managers could establish "slot limits" that allow harvesting only of mid-size fish, according to Conover. Individual fish above a maximum size or below a minimum size would have to be returned. Slot limits apply evolutionary pressure on young fish to grow more quickly so they become larger and mature earlier. "The individual fish that survive the window would be allowed to grow large, and fecundity goes up exponentially with length," says Conover. Maine's lobster fishery has a slot limit.

Slot limits for some fisheries could have direct human health benefits, Conover says. In some fish such as tuna, the largest and oldest accumulate the highest levels of pollutants such as mercury. By not commercially harvesting the largest and most contaminated fish, people would be less likely to eat them. –John Tibbetts

Some popcorn makers have stopped using diacetyl.

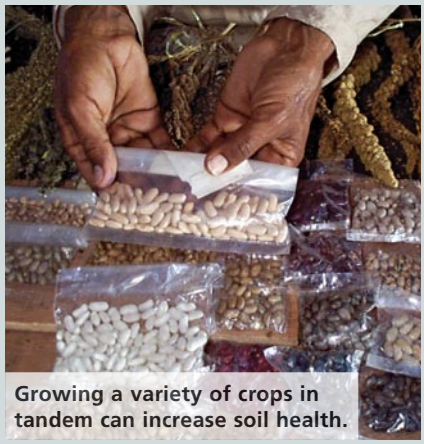
degenerative and potentially fatal lung disease. Regulation of worker exposure to diacetyl had been delayed by a Bush-era Advance Notice of Proposed Rulemaking, but in March 2009, Secretary of Labor Hilda Solis announced the withdrawal of the notice, which could allow OSHA to move forward more quickly with new regulations. The flavoring is deemed "generally regarded as safe" by the FDA; one case of bronchiolitis obliterans was identified in a consumer who ate two bags of microwave popcorn each day for several years.

New Gases on the Radar

At March's Greenhouse 2009 meeting in Perth, Australia, researchers presented new information on two gases that have been linked with global warming for over a decade. Atmospheric levels of nitrogen trifluoride (which replaced perfluorocarbons in circuit board manufacturing) and sulfuryl fluoride (which replaced methyl bromide in pest control) are still low, but they are increasing rapidly—which is not unexpected, given their relatively recent introduction to the atmosphere. Controlling nitrogen trifluoride could be particularly key because it persists for hundreds of years in the atmosphere. The team called for the two gases to be added to future versions of the Kyoto Protocol.

Climate Change Concerns Farmers

Across the globe, agricultural experts are calling on farmers to take climate change into account as they go about the business of feeding the world. In March 2009, the European Commission released a draft report warning that farmers in some regions of Europe may face disparities in crop production because of uneven effects of global warming across the region. To reduce emissions and prepare for climatic changes, the report endorses renewable energy and biotechnology, and also advocates a variety of organic soil management practices that help store carbon and are more resilient to climate fluctuations. In southern India, a women's collective is already planting novel crop combinations, using fewer chemicals, and embracing other sustainable practices.



Growing a variety of crops in tandem can increase soil health.

Left to right: Creative Commons; Daniel Buckles/IDRC CRDI