ACS chemical biology

Spotlight

Pheromone Phenomena

Pheromones are chemical signals that influence various behaviors, including mating. These chemicals dictate female reproductive behavior, such as a preference for dominant males, but the mechanisms that govern these responses are not well understood. Two sites in the adult mammalian brain, the olfactory bulb, which is the part of the brain responsible for detecting pheromones, and the hippocampus, a part of the brain implicated in memory, are also the sites where new neurons are produced. Mak *et al.* (*Nat. Neurosci.* 2007, *10*, 1003–1011) now report that neuron formation in the olfactory bulb and the hippocampus in female mice is stimulated by male pheromones, linking the processes of neurogenesis and mating.

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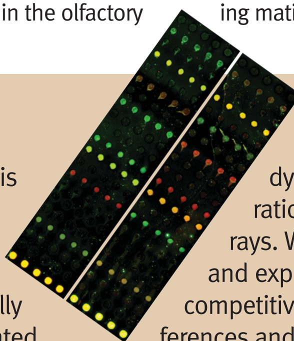
When female mice were exposed to odors from male mice, an increase in proliferating cells in the olfactory bulb and the hippocampus was detected, as evidenced by bromodeoxyuridine incorporation. It is known that exposure to male hormones elicits increases in two hormones, luteinizing hormone (LH) and prolactin (PRL). Experiments in which wild-type female mice and female mice lacking the LH receptor were exposed to LH suggested that this hormone specifically mediates neurogenesis in the hippocampus. In contrast, analogous experiments with PRL indicated that this hormone mediates neurogenesis in the olfactory bulb. Additional experiments where female mice were exposed to pheromones from dominant males, which caused the observed increase in neurogenesis, revealed a preference for the dominant male. In contrast, females exposed to subordinate-male pheromones, which do not produce increased numbers of neurons, showed no preference for dominant *versus* subordinate males. These findings implicate male pheromones in the process of neurogenesis and point to mechanisms underlying mating behavior. **Eva J. Gordon, Ph.D.**

Comparing Carbohydrates



Pilobello, K. T., *et al. Proc. Natl. Acad. Sci., U.S.A.*, *104*, 11,534–11,539. Copyright 2007 National Academy of Sciences, U.S.A.

Microarray technology has revolutionized the analysis of DNA expression patterns, and its use is rapidly expanding to the evaluation of other biological constituents, such as proteins and even cells and tissues. However, many proteins and lipids, especially those involved in cell–cell interactions, are glycosylated, and their glycosylation patterns are often critical for their activity. Although microarray technology has been applied to carbohydrate analysis, its development has lagged far behind that of DNA, in part because of poor quality-control methods. Now, Pilobello *et al.* (*Proc. Natl. Acad. Sci. U.S.A.* 2007, *104*, 11,534–11,539) present the development of a ratiometric lectin microarray system that will help carbohydrate analysis catch up to that of its fellow biomolecules.

Microarrays consisting of up to 58 different carbohydrate-binding proteins, or lectins, were constructed to provide a measure of different oligosaccharide substructures present in a given sample. Samples derived from cell membranes, and thus likely consisting primarily of cell surface glycoproteins and glycolipids, were fluorescently labeled with orthogonal dyes; this is analogous to the two-color ratiometric approach used in DNA microarrays. When the samples were mixed together and exposed to the lectin-based microarray, competitive binding revealed relative binding differences and enabled reproducible, semiquantitative elucidation of the distinct carbohydrate substructures present in the samples. This approach was further applied to the evaluation of glycosylation changes upon differentiation of a leukemia cell line into neutrophils. The authors determined that compared with undifferentiated cells, differentiated neutrophils displayed increased levels of high-mannose N-linked oligosaccharides and N-linked structures containing β-1,6-*N*-aceytlglucosamine and α- and β-terminal *N*-acetylgalactosamine residues but decreased amounts of structures containing α-2,6 sialic acid epitopes. This compelling approach will facilitate systematic analysis of the mammalian glycome and enable correlation between glycomic and genomic changes in cells. **Eva J. Gordon, Ph.D.**

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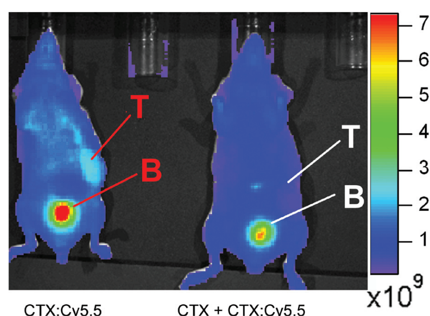
Painting the Tumor Red

Surgical removal of tumors remains one of the most effective strategies for combating cancer. However, the ability to eliminate all cancerous tissue while leaving healthy tissue intact is a significant challenge. Optical imaging contrast agents have the potential to greatly facilitate visualization of cancerous tissue during surgery, but poor cancer specificity, unfavorable biodistribution and pharmacokinetic properties, or the need for enzymatic activation of probes has prevented most previous agents from advancing to human clinical trials. Particularly well suited for intraoperative imaging are agents that emit in the near-infrared spectrum, such as the commonly used fluorescent dye Cy5.5. In a new study, Veiseh *et al.* (*Cancer Res.* 2007, *67*, 6882–6888) develop an imaging agent that effectively "paints" cancerous tissue, enabling more precise visualization during surgical procedures, by attaching Cy5.5 to the exquisite tumor-targeting agent chlorotoxin (CTX).



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CTX is a peptide with 36 amino acids that is thought to bind to a macromolecular complex containing several proteins, including matrix metalloproteinase-2 (MMP-2), involved in extracellular matrix degradation during cancer metastasis. The CTX:Cy5.5 conjugate was examined for its ability to target cancer cells and illuminate cancerous tissue. Initial testing revealed that cancer cells exposed to CTX:Cy5.5 emitted fluorescence, whereas primary fibroblasts did not. In mouse models of brain cancer, injection of CTX:Cy5.5 resulted in substantially higher fluorescence emission in cancerous tissue than in normal tissue and enabled noninvasive visualization of cancerous tissue through intact skull. In addition to brain cancers, CTX:Cy5.5 was capable of imaging prostate, intestinal, and soft tissue cancers. It is important that toxicity studies did not reveal any adverse effects of CTX:Cy5.5 in mice. Though MMP-2 does not appear to be the direct target of CTX:Cy5.5, evidence suggests that the imaging agent targets a component of the MMP-2 complex. Perhaps continued development of "tumor paint" such as CTX:Cy5.5 will enable more and more cancer patients to go out and paint the town! **Eva J. Gordon, Ph.D.**

Methyl Methodology

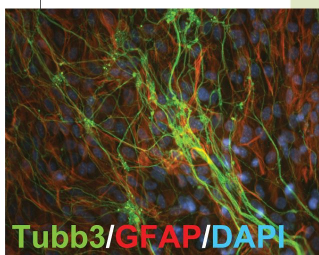
Though all cells in a multicellular eukaryote have the same DNA, epigenetic regulation tunes the transcriptional program of each cell type or in response to a developmental cue. Often, the chemical status of the DNA itself impacts the expression of the nearby genes. Regulatory DNA sequences near a gene can be modified by cytosine methylation, and these methyl groups usually signal silencing of the downstream gene. The business of putting on and taking off these methyl groups has become a hot topic for researchers in recent years, because this is both a central mechanism for gene control and a potential route to gene therapy. Changing the on or off status of a particular gene by modulating promoter methylation could be quite valuable, but as with other forms of gene therapy, a targeted strike on a single gene with high potency and exquisite specificity is challenging. A drug to inhibit the methyltransferases that make methyl Cs could have catastrophic consequences, but what if the methyltransferase could be sent to just one site? Now, a split enzyme strategy tackles this feat in bacteria, and the authors postulate that it could work in eukaryotes as well. Nomura and Barbas (*J. Am. Chem. Soc.* 2007, *129*, 8676–8677) take apart a well-characterized bacterial methyltransferase protein, MHhaI, by expressing it in two parts. Only the two parts together could form an enzyme that performs the native methylation activity on the sequence, GCGC. They then fused each half-enzyme to a different zinc-finger-type DNA binding domain. Each domain directs the half-enzymes to specific 9-nucleotide DNA sequences in the cell. The study tested whether these sequence-enabled DNA methylases performed as they were designed to. It is impressive that the only sites that were efficiently methylated were those that contained all the correct bits of information: the methylation site and both of the flanking 9-nucleotide sequences. Previous studies from this laboratory and others have shown that the nucleotide specificity of zinc fingers can be engineered, so it is presumed that novel methylases could be targeted to many loci in the genome. Making this clever trick work in a eukaryotic cell will undoubtedly be a next-generation extension of this interesting finding. **Jason G. Underwood, Ph.D.**

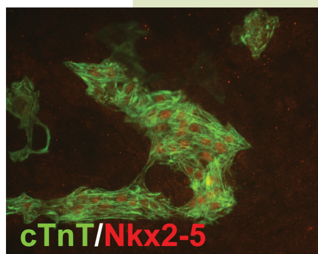
Of Mouse and Man

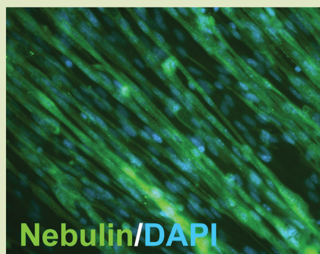
Mouse models of human diseases have been instrumental in helping us understand the causes of and develop treatments for many human conditions. In theory, mouse embryonic stem (ES) cells should be an excellent model for human ES cell research, especially given the ethical debate surrounding the generation of human ES cells. However, distinct differences in the growth conditions necessary and the signaling pathways used by mouse and human ES cells to maintain their pluripotency have brought into question the accuracy of using mouse ES cells as a model for the human system. Now, two reports by Brons *et al*. (*Nature* 2007, *448*, 191–195) and Tesar *et al*. (*Nature* 2007, *448*, 196–199) describe the generation of new pluripotent mouse stem cell lines that exhibit striking similarities to human ES cells.

Unlike mouse ES cells, which are derived from the mouse embryo before implantation into the uterus, the new mouse stem cell lines come from post-implantation tissue called the epiblast. Extensive analysis of these cell lines by both research groups, including investigations into their growth conditions, gene expression analysis, differentiation potential, and epigenetic state, revealed enlightening insight into the regulatory mechanisms governing pluripotent cells.

The first clue that these epiblast-derived stem cells, referred to in both papers as EpiSCs, were different from mouse ES cells arrived when the authors observed that the EpiSCs could not be propagated under the same conditions as mouse ES cells, in which leukemia inhibitory factor and bone morphogenetic protein 4 are key medium supplements. However, EpiSCs grew beautifully in an undifferentiated state under conditions used for human ES cells, in which the proteins activin A and fibroblast growth factor 2 are present. Furthermore, global gene expression analysis *via* DNA microarrays revealed distinct differences in expression patterns between EpiSCs and mouse ES cells. Perhaps not unexpectedly, EpiSCs displayed increased expression of genes associated with the epiblast and decreased expression of genes associated with the inner cell mass, which is the source of mouse ES cells.







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The differentiation potential of EpiSCs cells was assessed in several ways. First, injection of EpiSCs into immunodeficient mice resulted in the formation of teratomas, which are tumors that contain multiple differentiated cells types. In addition,

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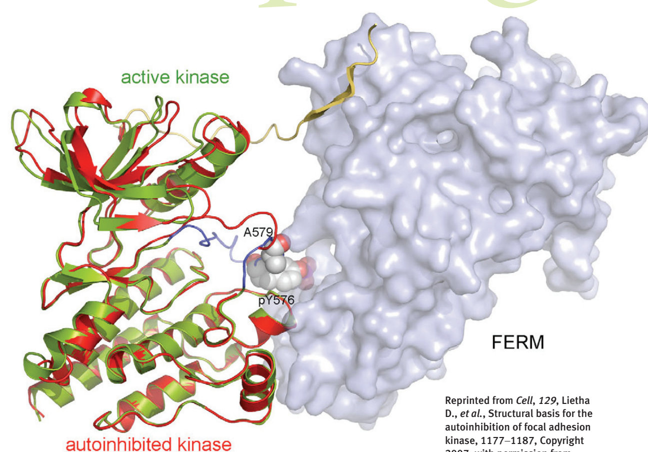
A FERM Grip on FAK Regulation



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Focal adhesions are the points at which a cell attaches to the extracellular matrix through its integrin receptors. On the inside of the cell, these structures are linked to the cytoskeleton and contain an array of structural and signaling proteins that influence many functions. Focal adhesion kinase (FAK), a tyrosine kinase, is a central mediator of these signals, and its activation affects not only adhesion but migration, survival, and cell division as well. Although some elements of FAK activation are known, a detailed model of this process has been lacking. Lietha *et al*. (*Cell* 2007, *129*, 1177–1187) now present a physical basis for its autoinhibition, and subsequent activation, on the basis of crystal structures of both the inhibited and activated states.

After an appropriate stimulus, catalytic activation of FAK occurs *via* a multistep process. First, although it cannot act upon substrates, it phosphorylates itself, providing a binding site for another tyrosine kinase, Src. Src is then able to phosphorylate, and fully activate, FAK. Mechanistically speaking, then, how is the activation of FAK regulated? The crystal structures show that the answer lies with the enzyme's FERM domain, so named because of its presence in four other proteins: band 4.1, ezrin, radixin, and moesin. In the inhibited state, the FERM domain physically blocks the kinase domain, occluding the catalytic cleft and blocking the Src phosphorylation site. The FERM domain also sequesters the FAK autophosphorylation site that is necessary for Src recruitment. The structure of the active form, however, is no longer constrained by the FERM domain. Further, disruption of the FERM/kinase domain interaction in biochemical assays led to increased catalytic activity. Thus, the authors provide an intriguing model in which activation is initiated by physical displacement of the FERM domain, perhaps by activated receptors. This would liberate the kinase and Src recruitment domains, allowing for the full catalytic activation of FAK. **Eric Martens, Ph.D.**

Of Mouse and Man, *continued from page 508*

EpiSCs differentiated into derivatives of all three germ layers when grown as aggregates in solution or as monolayers in which a human ES cell protocol was used. Notably, unlike mouse ES cells, injection of EpiSCs into preimplantation embryos did not support generation of chimeric mice, an indication that despite their developmental potential, EpiSCs are not compatible with the preimplantation environment.

Finally, analysis of the epigenetic state of EpiSCs revealed that certain histone methylation patterns in EpiSCs are more closely matched with human ES cells than with mouse ES cells. These data imply that distinct mechanisms for epigenetic regulation of transcription exist, depending on the temporal origin of the cells.

Notably, the prominent differences between mouse and human ES cells had previously been attributed to species-related distinctions. The efforts of these two research groups provide an exciting revelation that the various regulatory mechanisms governing the developmental potential of pluripotent cells are profoundly affected by their temporal origin. This information will help delineate the many factors contributing to pluripotency, including dissecting species-related *versus* developmental differences in the mouse and human systems. **Eva J. Gordon, Ph.D.**

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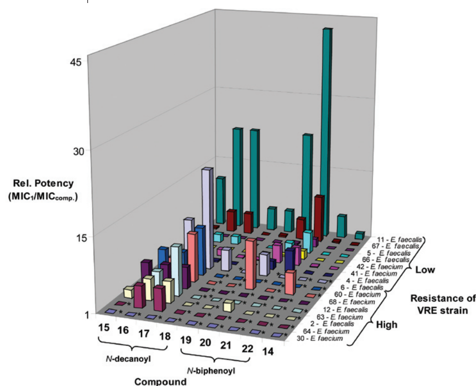
Spotlight

Vanquishing Vancomycin Resistance

The frightening emergence of bacterial strains resistant to certain antibiotics demands the creation of new compounds impervious to these mutants. Vancomycin is a glycopeptide antibiotic to which an alarming number of strains of *Enterococci* and *Staphylococcus* have recently acquired resistance. Efforts to create vancomycin derivatives effective against these strains have revealed that the replacement of the natural disaccharide on vancomycin with a lipid-containing 2′-*N*-acyl glucosyl moiety yields compounds that retain some activity. However, the generation of molecules of this type has been a challenging task for synthetic chemists. Now, Griffith *et al.* (*J. Am. Chem. Soc.* 2007, *129*, 8150–8155) describe a simple, rapid neoglycosylation strategy for creating lipoglycopeptide vancomycin derivatives.



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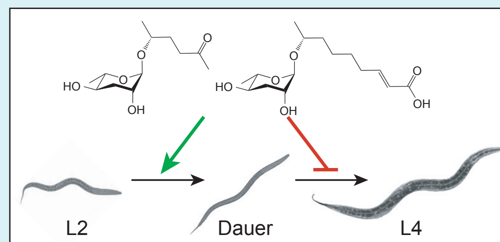
The authors exploit the exquisite stereoselectivity and simplicity of the neoglycosylation reaction between an alkoxyamine and unprotected, unactivated glucose, in which the β-anomer forms exclusively, to access lipoglycopeptide vancomycin variants. The installation of an alkoxyamine to the vancomycin aglycone, followed by the neoglycosylation reaction with lipid-containing glucose derivatives, enabled the generation of eight compounds that varied in the position of either an *N*′-decanoyl or an *N*′-biphenoyl group on the glucose residue. Notably, these compounds were generated on a large scale with limited purification and thus provided distinct advantages over other methods for generating similar molecules. Evaluation of the activity of these compounds against 15 different vancomycin-resistant strains of *Enterococci* uncovered telling details about their structure–activity relationships. For example, compounds containing the *N*′-decanoyl group were more effective overall than those containing the *N*′-biphenoyl group, although both classes of compounds showed a preference for either the 3′ or 4′ position of the sugar. This innovative approach for diversifying a key position of vancomycin provides access to potentially significant new antibiotics not easily accessible through other methods. Eva J. Gordon, Ph.D.

Deciphering Dauers

The nematode *Caenorhabditis elegans* can sense when conditions are not favorable for its own growth and development. The worms produce signaling molecules in the form of the dauer pheromone to monitor population density, food supply, and temperature. If conditions are unfavorable for growth, the worms move into a phase called the dauer stage, instead of progressing through normal development. Despite years of hunting, scientists have had little luck in determining the identities of the components of the dauer pheromone. By searching through crude dauer pheromone, Butcher *et al.* (*Nat. Chem. Biol.* 2007, *3*, 420–422) now identify two small molecules with potent dauer-inducing activity.



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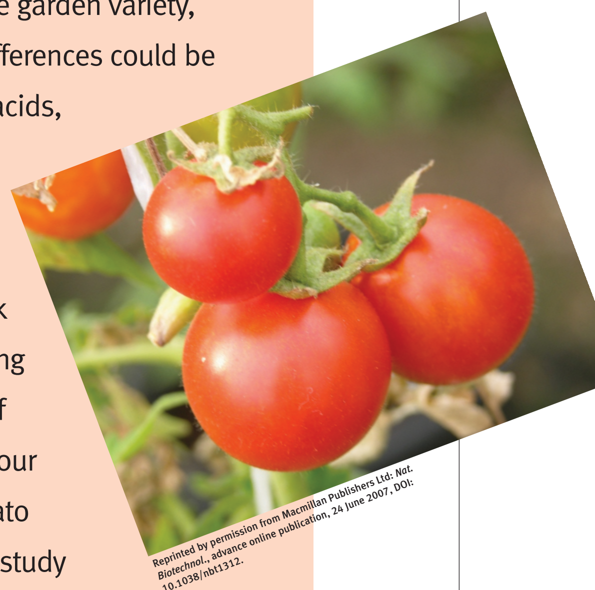
The authors used activity-guided fractionation and detailed NMR analysis to find molecules in the crude dauer pheromone that could cause formation of and inhibit recovery from the dauer stage. Two dideoxysugar members of the ascaroside family were isolated that are related in structure to another ascaroside previously identified as a component of dauer pheromone. To examine the biological activity of these compounds, the authors synthesized and tested the molecules in a dauer formation assay. Notably, the compounds were 2 orders of magnitude more potent than the previously identified ascaroside. Because it seems likely that a concoction of small molecules makes up the dauer signal, the identification of all relevant components will help in the discovery of their molecular target (or targets) and the pathways triggered by their presence. More detailed knowledge of the signaling pathways involved in dauer formation could also contribute to our understanding of related pathways, such as those that control metabolism and aging, in higher organisms. **Eva J. Gordon, Ph.D.**

Transgenic Tomatoes Catch Up

Even as advocates and opponents of genetically modified organisms remain firmly entrenched in warring positions, researchers continue to develop transgenic plants with new properties. Because the tomato is an economically important crop with a short shelf-life, it has always been a prime target for modification. The Flavr Savr transgenic, slow-ripening tomato, for example, was the first genetically engineered food authorized by the U.S. Food and Drug Administration. Now, Davidovich-Rikanati *et al.* (*Nat. Biotechnol.*, published online June 24, 2007; DOI: 10.1038/nbt1312) introduce the lemon basil geraniol synthase gene to create a transgenic tomato with a modified flavor and aroma.

Geraniol synthase catalyzes the conversion of geranyl diphosphate, an intermediate in carotenoid biosynthesis, to geraniol. Geraniol is an acyclic monoterpene alcohol that is a precursor of numerous volatiles with distinctive aromas. In a blind taste test, the majority of participants chose the transgenic tomato over the garden variety, noting that the former had novel aromas not present in the latter. Often, the panelists described the transgenic tomatoes as sweeter than the garden variety, even though no differences could be noted in pH, total acids, soluble solids, or sugar content. However, you might want to think twice before heading out to pluck one off the vine to put in your time-honored tomato sauce. The current study also notes that depletion in geranyl diphosphate levels, which is also a precursor of lycopene, results in the loss of the deep red color associated with the common tomato. This may bode well for the palate, but supermarket consumers accustomed to visually striking, yet comparatively bland, tomatoes (ripened with ethylene) might not be buying into it just yet. **Anirban Mahapatra**



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