

Mooove over mastitis!

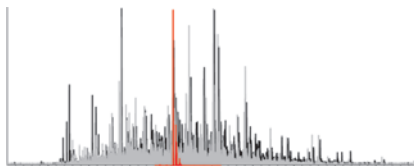
Mastitis has proven to be one of the most economically taxing diseases for the dairy industry, with billions of dollars spent annually in the US alone. Wall and colleagues take a genetic engineering approach to construct transgenic



cows whose mammary glands show enhanced mastitis resistance because they express an antibacterial enzyme, lysostaphin, which is secreted into their milk. Lysostaphin attacks the cell wall of *Staphylococcus aureus*, one of the toughest mastitis-causing bacterial strains to control and one that is responsible for up to 30% of clinical mastitis cases. Transgenic cows exposed to *S. aureus* were resistant to intramammary infection. These results suggest the potential for genetic engineering as a tool to control endemic mastitis in cattle. [Articles, p. 445; News and Views, p. 430] NC

Fluorous proteomics

Functional proteomic analysis requires methodologies that break down proteome complexity into specific fractions. Although



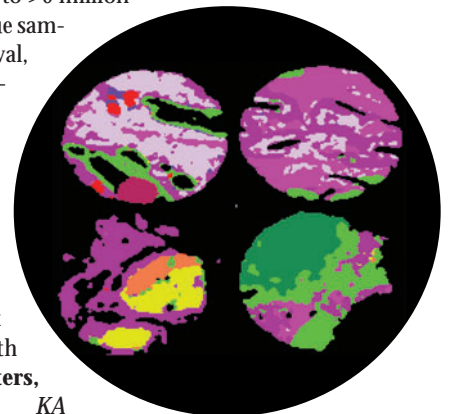
conventional methods, such as biotin-streptavidin, exist, they are often limited by their high cost, inefficient recovery of labeled substrates and their tendency to obscure tandem mass spectrometry data. Peters and colleagues have developed a method that overcomes these limitations and can be tailored to enrich for various classes of peptides with different side-chain functionalities or specific post-translational modifications. Their method is based on tagging peptides, from trypsin-digested protein extracts or whole cell lysates, with highly fluorinated functional groups. These fluorine-containing tags then selectively bind to a fluorine-based resin, and peptides with particular side-chain functionalities can be eluted from the column. Tandem mass spectrometry data are then generated that are not skewed by the presence of the fluorous tag. [Letters, p. 463] NC

In This Issue written by Kathy Aschheim, Nadia Cervoni, Laura DeFrancesco, Michael Francisco and Gaspar Taronger-Oldenburg.

Histopathology fast-forward

Histopathology is carried out today much as it has been for over a century. Trained observers evaluate stained tissue samples one by one under a microscope—an inherently slow and subjective process. Levin and colleagues are seeking to complement traditional histopathology with an automated, high-throughput method based on Fourier transform infrared (FTIR) analysis of microarrayed tissue. FTIR spectroscopy identifies specific chemical bonds, such as amide bonds or phosphodiester bonds, and thus reflects the sample's intrinsic chemical composition rather than added stains or labels. Using prostate tissue, the authors first worked out spectral signatures that are characteristic of specific cell types, such as normal epithelium, fibrous stroma, mixed stroma, muscle, nerve, lymphocyte and blood. The classification scheme, which provides statistical information on accuracy and error, was then applied to >9 million

spectra from >870 tissue samples, including archival, formalin-fixed and paraffin-embedded tissue. The approach should allow fast and reliable analysis of biopsies, as the authors show by distinguishing patient-matched benign and malignant prostate samples with 100% accuracy. [Letters, p. 469] KA



Protein production cleans up its act

As anyone who has produced recombinant proteins in *Escherichia coli* knows, purifying the protein of interest from the other contents of the cells can be a tedious process. Westerlund-Wikström and colleagues have designed a system that secretes recombinant protein into the medium to simplify purification. The *E. coli* flagellar type III secretion apparatus directs the synthesis of flagella by transporting proteins from the cytoplasm to the outside of the cell, bypassing the periplasm. FliC is the major structural flagellar protein. FliD caps the flagellum at its distal end and is essential for flagellar assembly: bacterial strains lacking FliD do not make flagella and secrete FliC monomers into the medium. The new system uses an *E. coli* strain lacking *fliD* and *fliC* in combination with two untranslated *fliC* sequences fused to the recombinant gene to assure secretion. Tests on several proteins show that it achieves protein concentrations in the medium of 1–15 mg/l, well above the ~100 µg/l seen with an existing secretion approach based on α -hemolysin. [Letters, p. 475] KA

siRNA immunity

Research into the therapeutic application of short interfering RNAs (siRNA) has been gathering momentum as of late, but several questions pertaining to their potential toxicity and off-target effects due to stimulation of the immune system remain unanswered. Judge *et al.* report on the potent, sequence-dependent immunostimulatory effect of synthetic siRNAs in mice (*in vivo*) and in human blood (*in vitro*), and most importantly, identify a series of sequence motifs that contribute directly to this effect. These results provide the authors with a means to identify effective siRNAs with minimal immunostimulatory activity, an approach that should be of relevance to other efforts to develop siRNAs for *in vivo* therapeutic interventions. [Letters, p. 457] GTO

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Transgenic mating foils insect pest

In an effort to curb populations of the Mediterranean fruit fly (medfly), one of the world's most important agricultural pest insects, Alpey and colleagues use genetic engineering to complement current sterile insect techniques (SIT). The authors produce male medflies that mate with wild females to produce eggs that are incapable of developing into adult flies. Their system takes advantage of a protein, the tetracycline-repressible transactivator (tTA), which interacts with the antibiotic tetracycline. In the presence of tetracycline, tTA is expressed at low levels and is innocuous to medflies—when tetracycline is withdrawn, however, tTA is expressed to such high levels that it causes severe growth impairment or death in these insects. Male medflies engineered with the tTA construct can be released as part of established SIT programs to control medfly infestations by mating with the natural population of medfly females. [Articles, p. 453; News and Views, p. 432] GTO



A more golden rice

The development five years ago of Golden Rice, transgenic rice containing elevated amounts of β -carotene (provitamin A), promised a means to combat vitamin A deficiency in vast areas of the world. Although certainly a valuable first step, it was soon recognized, however, that the levels of β -carotene would have to be substantially increased to allow this goal to be realized. Drake and colleagues have now developed 'Golden Rice 2,' a strain that accumulates twentyfold more β -carotene than the original Golden Rice. The authors achieved this through methodical screening for alternative versions of phytoene synthase, the first gene of the β -carotene pathway engineered into Golden Rice. The updated version of Golden Rice serves as an excellent starting point for breeding new varieties of rice rich in provitamin A. [Articles, p. 482; News & Views, p. 429] GTO



Rethinking transgenic plant regulations

The regulatory concept of using product rather than process to assess new transgenic plant varieties has been around for nearly 20 years. Yet regulatory agencies around the world still require extensive additional reporting for each new transgenic plant, even when similar varieties have been produced, and without consideration of phenotype and the real risk it poses to the environment or human health. Such policies raise the cost to get new varieties through the regulatory process and in so doing hinder the development and adoption of small market or specialty crops. To counter the tendency for regulations to burgeon, Bradford *et al.* propose a way to rationalize the regulation of transgenic crops by stratifying plants into risk categories based on the knowledge of the traits involved, both from conventional breeding and from the two decades of work with transgenic plants. [Perspective, p. 439] LD

Patent roundup

- Written by two economists, *Innovation and Its Discontents* contends that the US patent system is broken and has reached a point of diminishing returns. [Book Review, p. 421] MF
- Pioneered by industry, material transfer agreements are now in widespread use in academia as well. Rodriguez discusses their characteristics and different uses. [Patent Article, p. 489] MF
- Recently published patent applications in RNA interference. [New Patents, p. 492] MF

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- *In vivo* imaging of Alzheimer-associated plaques
- Gene knockdown by large circular antisense
- Efficient *in vivo* expression of therapeutic monoclonal antibodies
- Quantitative tissue proteomics
- Protein microarrays for characterizing enzyme inhibitors