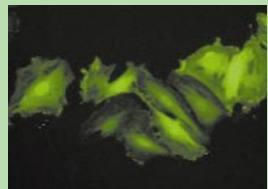


## IN BRIEF

## THIS MONTH IN NATURE BIOTECHNOLOGY



Gene therapy may prove useful for progressive re-obstruction of coronary angioplasties,

a widespread condition that has proved difficult to treat pharmacologically. On page 1181, Levy and colleagues address the problem of how to achieve localized and sustained delivery of therapeutic DNA to sites of angioplasty. They developed a polymer impregnated with the plasmid DNA that, when coated onto a stent (a metallic tube that expands to open blocked coronary arteries) releases DNA without causing inflammation or interfering with stent mechanics. When implanted into pig coronary arteries, a stent coated with the polymer releasing the GFP gene transfected ~1% of arterial cells, as shown by immunofluorescence. Once it's clear which genes will have the best therapeutic effect, such sustained-release localized delivery methods can improve treatment for a variety of vascular injuries. *ND*

## An edible hepatitis vaccine

Vaccines against infectious disease can only be truly effective if they overcome obstacles of costs of production and storage that limit their use in parts of the world where they are truly needed. Now on page 1167, Richter et al. report progress in developing an edible hepatitis B vaccine in potatoes, taking a step toward overcoming these hurdles. Using a tuber-specific promoter to drive transcription of hepatitis B surface antigen (HBsAg), they produced potato plants that express HBsAg in their tubers. Mice fed the tubers developed a humoral antibody response, a surprising result given that HBsAg is a non-enteric pathogen. Because a limitation in edible vaccines is attaining high enough dosages, they focused on increasing the amount of antigen expressed in tubers, using alternative polyadenylation signals to enhance translation, and signal peptides to enhance endoplasmic reticulum retention (see also p. 1141). *JJ*

*This Month in Nature Biotechnology* written by Natalie DeWitt, Judy Jamison, Meeghan Sinclair and Andrew Marshall.

## Wavelength shifters

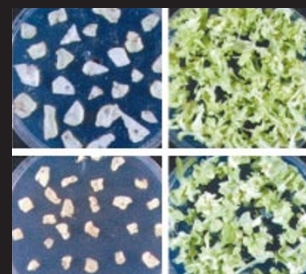
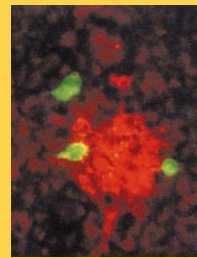
On page 1191, Tyagi et al. describe "wavelength-shifting molecular beacons," a technology that expands the number of molecular beacons that can be used to detect different complementary nucleic acid targets simultaneously. Molecular beacons are hairpin-shaped nucleic acids with a fluorophore and a nonfluorescent quencher attached to either end. When the hairpin is closed, fluorescence is quenched, but upon annealing to a complementary sequence the molecule becomes linear and fluorescence is restored. The researchers set out to develop a series of fluorophores that could be stimulated by the same monochromatic light, but could be distinguished from one another by the different wavelengths of light they emit. In addition to the quencher and emitter fluorophores, the modified molecular beacons contained an additional "harvester" fluorophore that absorbs monochromatic light, and when the beacon is bound to a target, transfers energy to the emitter fluorophore. This shifts the emission spectrum of the emitter by fluorescence energy transfer so that each fluoresces in its own characteristic color. *ND*



Damage by bacteria and fungi causes serious losses every year in both cultivated and stored in crops. Plant

genetic engineering could offer a helpful alternative to the antimicrobials and pesticides, but until now the spectrum of microbial protection achieved has been fairly narrow. In this issue, Osusky et al. describe their engineering of broad-spectrum antimicrobial resistance into two potato cultivars. They added a hexapeptide to the N terminus of the cationic peptide chimera CEMA, which contains parts of two different insect antimicrobial peptides. Molecular modeling predicted that the new peptide (MsrA1) would maintain a positively charged N terminus, essential for antifungal activity, and an overall amphipathic  $\alpha$ -helical character. They cloned the *msrA1* gene into potato cultivars Russet Burbank and Desiree, and stable MsrA1 expression brought about strong resistance to several common phytopathogens with no effect on morphology or yield. (page 1162) *JJ*

On page 1177, Lorenzen et al. report that a DNA vaccine encoding a recombinant antibody protected fish against viral pathogens, a step in developing safer and more efficacious animal vaccines. They prepared single-chain antibody (ScAb) gene constructs coding for neutralizing antibodies to the fish viral hemorrhagic septicemia virus (VHSV), and inserted them into a eukaryotic expression plasmid. They showed that rainbow trout fingerlings injected intramuscularly with the recombinant plasmid were protected against VHSV challenge. If immunoprophylaxis by DNA vaccines delivering antibody genes can be extended beyond aquaculture to mammals, it may provide a valuable tool in situations where conventional vaccination is ineffective or impractical (see also p. 1145). *JJ*



In transformation of crop plants, the transfer of antibiotic marker genes or vector sequences to the environment or gut microbes is increasingly viewed as posing an unacceptable risk. Inserting foreign genes into plastid DNA is one way of minimizing transgene escape; however, conventional methods still leave behind marker and vector sequences. In this issue, Day and Iamtham describe a procedure that uses homologous recombination to generate transplastomic tobacco plants free of these controversial sequences. In their plastid expression cassettes, reporter (*uidA*) and herbicide resistance (*bar*) coding regions flank the antibiotic resistance marker (*aadA*), and include direct repeats that mediate the antibiotic marker gene's excision through homologous recombination and plastid segregation. The first-generation plants were free of *aadA*, and subsequent crosses produced plants free of both *aadA* and *bar* genes (see p. 1172). *JJ*