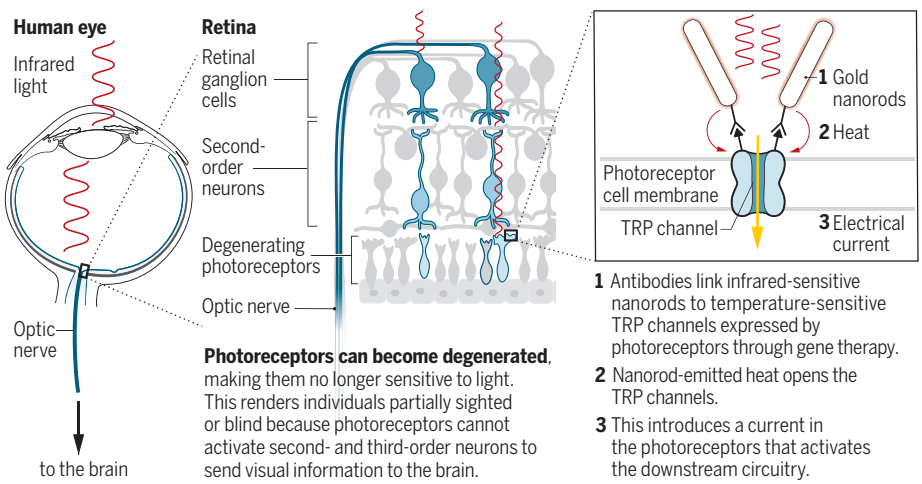
behavior. The authors tested their nanorod–TRP channel approach in cultured, light-insensitive postmortem human retinas, demonstrating that it introduces infrared light sensitivity to this tissue—a critical step in evaluating its relevance for human patients.

A different nanotechnology, called up-conversion nanoparticles, which binds to photoreceptors and "up-converts" infrared into visible light, can make photoreceptors virtually infrared-sensitive (*10*). Although degenerating photoreceptors would likely not be able to use up-conversion nanoparticles, mice treated with this technology used their infrared sensitivity to perform complex visual tasks including shape recognition. Such detailed behavioral evaluation is critical, because it is not clear to what extent the already-developed brain can interpret a new sensory modality to guide behavior—although studies support some plasticity of the adult mammalian brain for integrating new sensory input (*11*, *12*). Both examples demonstrate the strengths of nanotechnology tools over other methods. These tools are more light-sensitive than conventional optogenetics, approaching the sensitivity needed to work under normal daylight levels. Because the nanoparticles harness a different wavelength of light, it might be possible for normal vision and infrared vision to operate in parallel.

The nanorod–TRP channel approach used by Nelidova *et al.* faces further challenges before it can reach the clinic. It is promising that gold nanorods have, so far, appeared to be safe in humans (*13*). Similarly, ocular gene therapies seem to be low-risk and effective (*3*, *4*). However, the main challenge of any ocular gene therapy is to improve the efficiency and completeness of gene introduction (*3*) as well as to provide long-term effectiveness (*14*). More specifically, because nanorods and TRP channels cannot currently be targeted selectively to degenerating photoreceptors, the interaction between induced infrared sensitivity and the intrinsic light sensitivity of healthy photoreceptors requires further investigation. In addition, many objects that humans see are not necessarily infrared-emitting or infrared-reflecting; thus, goggles to convert visible light to infrared light would likely be necessary. Nonetheless, this system has exceptional promise for basic research. Tools that can reintroduce light sensitivity to postmortem human retinas (*15*) offer the potential for studying human retinal function in much greater detail than was previously possible. This basic knowledge is important for any approach to vision restoration, as it would reveal what kinds of functions need to be restored. ■

Nanorods and heat-sensing proteins for infrared detection

Injecting the eye with infrared-sensitive nanorods and genetic constructs to induce the expression of temperature-sensitive transient receptor potential (TRP) channels in photoreceptors may confer infrared vision.



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SYNTHETIC BIOLOGY

Follow the barcoded microbes

Genetically engineered spores give object provenance technology new avenues

By **Jeff Nivala**

Tracking where a physical object originated and where it has been, known as object provenance, is becoming increasingly important with the globalization of supply chains (*1*). Object-labeling technologies that are scalable, robust, and difficult to falsify would support, for example, the mitigation of foodborne illness outbreaks and the manufacture of counterfeit goods. However, there currently exists no single tagging method that satisfies all these requirements. On page 1135 of this issue, Qian *et al.* (*2*) describe a new tagging technique, called the barcoded microbial spores (BMS) system, that uses genetically engineered microbes as molecular tags to address the object provenance problem. The authors demonstrate that BMS can label a range of surfaces and persist for months in real-world conditions. Furthermore, they show how this technology can tag objects that come into only brief contact with BMS-labeled surfaces, suggesting the utility of BMS in forensic surveillance applications (*3*).

Microbes are ubiquitous in our everyday environments, with different geographical locations having distinct microbial populations. Physical objects can even take on the microbial signature of their environments over time (*4*, *5*), which has led to the suggestion that this naturally occurring signature could be used for object provenance (*6*). Although it would be convenient to have environmental microbes acting as automatic label-makers, actually implementing such an approach would pose a number of challenges. For instance, extensive environmental mapping of microbes would have to be done first. This would be expensive and

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time-consuming. Furthermore, environmental microbial abundances can be similar at disparate locales and also change over time, making the use of natural microbe labels a nonstarter for many applications.

Instead, Qian *et al*. take this concept into the realm of synthetic biology by engineering synthetic strains of microbes to function as molecular labels. To do this, they inserted short, specific DNA sequences (barcodes) into the genomes of the bacterium *Bacillus subtilis* and the yeast *Saccharomyces cerevisiae*. They designed and experimentally validated dozens of barcode sequences that could be used in combinations to generate an essentially infinite number of potential tag sets. To tag an object, a set of barcoded strains were mixed together in spore form and sprayed on the surface of an object. A spore is a physically tough, inactive cell state that can persist in harsh environmental conditions without growth (*7*). This resilience allows the barcoded microbial spores to endure in diverse ecosystems without rupturing and exposing their DNA barcodes to the elements, risking tag loss.

To identify the barcodes, the surface of the object is swabbed to collect a sample, which is prepared for input to one of a number of different decoding devices, including a DNA sequencer or a quantitative polymerase chain reaction machine. In addition to these readout options, Qian *et al*. also focused on using a nascent DNA detection technology called SHERLOCK (*8*), which is more amenable to deployment in field settings. This feature is critical to many potential provenance applications. BMS decoding should also be compatible with other field-deployable DNA-sensing technologies, such as commercial nanopore devices (*9*), although the authors did not explore this compatibility.

The release of genetically modified organisms into uncontrolled environments may come with risks. So, Qian *et al*. built clever safeguards into the BMS system to prevent the unintended spread and proliferation of their microbial tags in the environment, beginning with careful selection of the microbial species themselves. Both *B. subtilis* and *S. cerevisiae* are commonly found in the environment and in food samples, and products derived from them have already been granted "generally recognized as safe" (GRAS) status by the U.S. Food and Drug Administration. To prevent their proliferation in native environments, Qian *et al*. used auxotrophic *B. subtilis* and *S. cerevisiae* strains that require supplementation of key amino acids for growth. The authors took additional steps to prevent the microbial spores from returning to an active cellular state by either removing genes essential to this process (for *B. subtilis*) or by boiling the spores to permanently heat-inactivate them (*S. cerevisiae*). These measures were adequate to ensure that the spores did not replicate even in the most favorable laboratory conditions. As there is still the potential for auxotrophs to grow by scavenging for metabolites in the environment, these additional safeguards are prudent. An alternative strategy to biocontainment would be the use of so-called "recoded" cell strains that have been engineered to be dependent on completely synthetic amino acids that are not found in natural ecosystems (*10*, *11*).

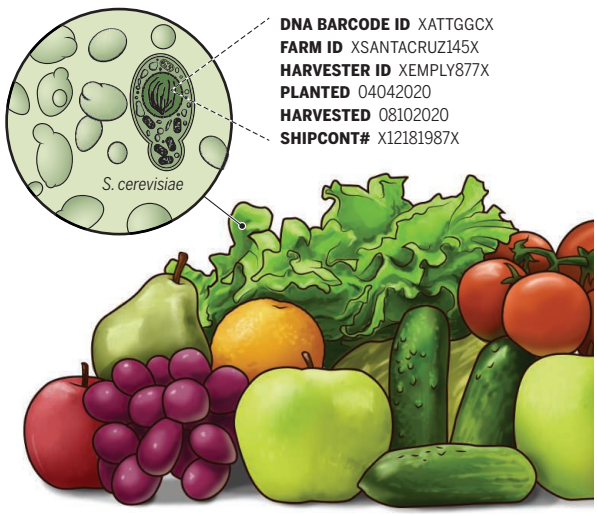
The value of a provenance system depends on its lifetime and applicability to different objects. The BMS system was prototyped on an impressive number of surfaces and simulated environments, including sand, soil, carpet, and wood, in addition to an outdoor grass area. Notably, the barcoded microbes persisted and remained detectable on these surfaces for months, even after real or simulated weather conditions and physical perturbations such as vacuuming and sweeping. Beyond typical object provenance, in which the labels are applied directly to the object being tracked, Qian *et al*. also demonstrated that BMS tags could be transferred to other objects that come in transient contact with a labeled surface. The authors found that they could even trace the path of shoes that had walked on a BMS-tagged floor. The potential use for such a technology by, for example, law enforcement is compelling, although additional work is needed to determine how the BMS system withstands more rigorous real-world stresses, such as the application of cleaning agents to the surface.

Perhaps the most powerful use of microbial tags will come from their application to agricultural and other food supply chains. For example, the tags could safely be sprayed directly on food products, as Qian *et al*. showed with leafy plants (see the figure). *Bacillus thuringiensis*, which is closely related to *B. subtilis*, is already in common use as an insecticide for agricultural products that are commercially available today (*12*). The authors found that *B. thuringiensis* genomic DNA can be detected on store-bought produce even after washing, boiling, frying, and microwaving, thus highlighting the hardiness of DNA-based tags. As would be expected, other microbe and DNA-based provenance systems are also being developed, in both academia (*13*) and industry. We may well be on a path to a brave new world in which food provenance tracking goes not just from the farm to the table, but all the way to the sewer (*14*). ■

"Perhaps the most powerful use of microbial tags will come from their application to agricultural and other food supply chains."

Produce provenance data encoded in the DNA of a synthetic microbe

Genetically barcoded spores could be used for a new generation of object (e.g., food) provenance applications.



GRAPHIC: V. ALTOUNIAN/*SCIENCE*

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Follow the barcoded microbes

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