cell lineages as organoids. Incorporation of additional endocrine lineages, such as glucagon-secreting alpha cells[12], might give rise to more functional islets. Certainly, residual alpha cell function is abnormal in type 1 diabetes, so both cell types may be beneficial for optimal cell therapy. Alpha cells generated *in vitro* should also be useful for pharmaceutical development of new agents to combat hypoglycemia.

Among the challenges common to many stem cell protocols are how to synchronize differentiation across all cells and how to maintain the terminal cell type in culture. Synchronized differentiation is important to allow confident analysis of cells at defined stages of a protocol and mass production. During human development, *NEUROG3* expression in individual cells spans at least a 3-month period. Rezania *et al.*[4] have managed to corral the transient presence of the transcription factor into 7–10 days of stage 5 and early stage 6. This seemingly provided a relatively homogeneous, synchronized culture thereafter, such that by transplanting cells after stage 7, acinar and duct cell lineages had been largely eliminated. Although perhaps less glamorous than developing new differentiation protocols, devising conditions that maintain the terminally differentiated cell type is equally important. This has been challenging for hepatocytes as well as for pancreatic beta cells or islets. Interestingly, whereas ALK5 inhibition was part of the stage 7 differentiation cocktail here, others have recently shown that it prevents loss of key transcription factors, including MAFA, to counter dedifferentiation of human and mouse beta cells[13].

As *Nature Biotechnology* was going to press, a study[14] similar to Rezania *et al.*[4] was published. Pagliuca *et al.*[14] also used thyroid hormone and ALK5 inhibition during the maturation phase, and the resulting insulin-secreting cells showed promising phenotypic characteristics and prevention of hyperglycemia when transplanted into diabetic mice, although MAFA expression was not assessed. How their insulin-secreting cells would respond to an incretin hormone or hypoglycemic challenge, or in a wider range of physiological assessments against fully functional adult human beta cells, is unclear for the moment. Nevertheless, it is very encouraging that the two groups[4,14] worked with different human embryonic stem cell lines using some similar additives, supporting the reproducibility of the general approach across multiple lines.

In summary, although it is imperative not to underestimate the differences that seemingly remain with healthy adult beta cells and the importance of addressing them, this latest work by Rezania *et al.*[4], complemented by the work of Pagliuca *et al.*[14], marks a significant advance in differentiating hPSCs *in vitro* toward a mature beta cell phenotype. There is still much work to do, but the goal of widely available functional human beta cells is certainly closer to realization.

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Antibacterials for any target

Lynn L Silver

RNA-guided nucleases provide a strategy for killing specific bacterial species in complex populations.

The alarming resurgence of antibiotic-resistant bacteria has focused attention on the need for new approaches to antibiotic discovery. Two reports[1,2] in this issue explore the potential of antibacterials fashioned from RNA-guided nucleases (RGNs) and delivered by phagemids, which are engineered plasmids carried in bacteriophage capsids. Because RGNs can be directed to cleave specific genomic sequences simply by providing a complementary 'guide' RNA, in principle they allow selective killing of specific bacterial subpopulations as long as a sufficiently specific target sequence can be identified. As the new studies[1,2] suggest, the approach may help address two central challenges in antibiotic development—the need for agents that are effective against drug-resistant bacteria and for those that have a narrow, rather than a broad, spectrum of activity.

In addition to overuse of antibiotics, another reason antibiotic resistance is rising is that most antibiotics indiscriminately kill a broad spectrum of bacteria well beyond the disease-causing species. By selecting for resistance in many bacterial species, such antibiotics accelerate the exchange of antibiotic-resistance genes (often carried on plasmids). In contrast, narrow-spectrum antibiotics can kill pathogenic bacteria without harming or applying selective pressure to commensal species. In this sense narrow-spectrum antibiotics are desirable, although they cannot be used unless accompanying rapid diagnostics are available, and they would not be appropriate for the treatment of polymicrobial infections.

Discovery and development of narrow-spectrum agents are often challenging, and few such agents are in use, although there are three in the drug-development pipeline (**Table 1**). Targets that are species-specific are generally single enzymes prone to rapid development of drug resistance. In addition, cell impermeability to small-molecule, narrow-spectrum antibiotics is difficult to overcome. RGNs could therefore provide a new path to narrow-spectrum agents, especially if they are extremely safe, in which case they might be used empirically if the specific pathogen is suspected to be present or if cocktails of RGNs could be applied against several species. Methods for delivering RGNs into bacteria, such as phagemids, would obviate the problem of cell impermeability to small molecules.

Both Citorik *et al.*[1] and Bikard *et al.*[2] use the Cas9 nuclease of the bacterial type II clustered, regularly interspaced, short palindromic repeats (CRISPR) system to cleave bacterial genomes or plasmids in a sequence-specific manner. In bacteria, RGNs are part of a rudimentary, adaptive immune system that destroys incoming foreign DNA in the form of bacterial viruses, plasmids or transposons. Since the first report revealing the detailed

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Table 1 Antibacterial candidates with novel mechanisms and/or narrow spectra in the pipeline

Name	Mechanism	Spectrum and/or target organisms	Development phase
Debio 1450/1452	FabI inhibitor	Narrow/Staphylococci	1/2
CG-400549	FabI inhibitor	Narrow/Staphylococci	2
POI7080	LptD inhibitor	Narrow/*Pseudomonas aeruginosa*	2
AZD0914	GyrB/ParE inhibitor	*Neisseria gonorrhoeae* and some Gram-positive bacteria	1
CRS3123	MetRS inhibitor	*C. difficile* and other Gram-positive bacteria	1
Brilacidin	Defensin mimetic	Broad/Gram-positive and Gram-negative bacteria)	2
Lefamulin	Protein synthesis inhibitor	Gram positive	2
GSK-2140944	Topoisomerase inhibitor	Gram positive	2

This table is based on data from ref. 6.

mechanism by which type II CRISPR-Cas9 cleaves DNA and the possibility of targeting this nuclease with a single guide RNA rather than the two RNA strands of the native system[3], RGNs have been exploited for programmable genome editing in organisms from bacteria to higher mammals.

In early 2014, Gomaa *et al.*[4] showed that a designed type I RGN could be delivered by transformation to remove one *Escherichia coli* strain from a mixed population of two strains *in vitro*, opening up the possibility of designing RGNs as bespoke antibacterials. Citorik *et al.*[1] and Bikard *et al.*[2] take this idea further by engineering RGNs to target chromosomal genes of specific pathogens, or antibiotic-resistance plasmids, and delivering them with phagemids or by conjugation with probiotic bacteria that harbor mobilizable plasmids. Both papers show antibacterial activity *in vivo*—increased survival of the moth *Galleria mellonella* infected with enterohemorrhagic *E. coli*[1] and decolonization of *Staphylococcus aureus* in a mouse model of skin infection[2]. Importantly, they also demonstrate effective killing of antibiotic-resistant strains.

In cell culture experiments, both groups demonstrate the selectivity of their approach. Bikard *et al.*[2] find that an RGN targeting the chromosomal *mecA* gene of methicillin-resistant *Staphylococcus aureus* (MRSA) reduces the proportion of MRSA cells in a mixed population of MRSA and methicillin-sensitive *Staphylococcus aureus* from 50% to 0.4%[2] (**Fig. 1a**). Remarkably, Citorik *et al.*[1] show that an RGN targeting a specific fluoroquinolone-resistance mutation, $gyrA_{D87G}$, in *E. coli*, effectively reduces the resistant, but not the isogenic wild-type *E. coli*, by 4 logs[1]. RGNs are also used to remove antibiotic-resistance plasmids from a cultured population of *E. coli* (harboring plasmids carrying the *ndm-1* or *shv-1* β-lactamase genes[1]) and from MRSA strain USA300 (harboring a tetracycline-resistance plasmid, pUSA02)[2] (**Fig. 1b**). Moreover, an RGN targeting plasmid pUSA02 can 'immunize' a naive MRSA strain from subsequent introduction (by transduction) of this plasmid[2].

The recent history of antibiotic drug discovery is notable for a scarcity of new mechanisms of action[5], but there are several candidates with new mechanisms of action in the pipeline, the most promising of which are detailed in **Table 1**. Only two classes in development have a truly narrow spectrum, the FabI inhibitors, which are active against Staphylococci, including MRSA, and POL7080, whose activity is limited to *Pseudomonas aeruginosa*. Do RGN antibacterials have sufficient practical potential to advance as either therapeutics or as tools to combat the spread of antibiotic resistance? The key to practical deployment of this technology, aside from safety, is most likely the efficiency of delivery.

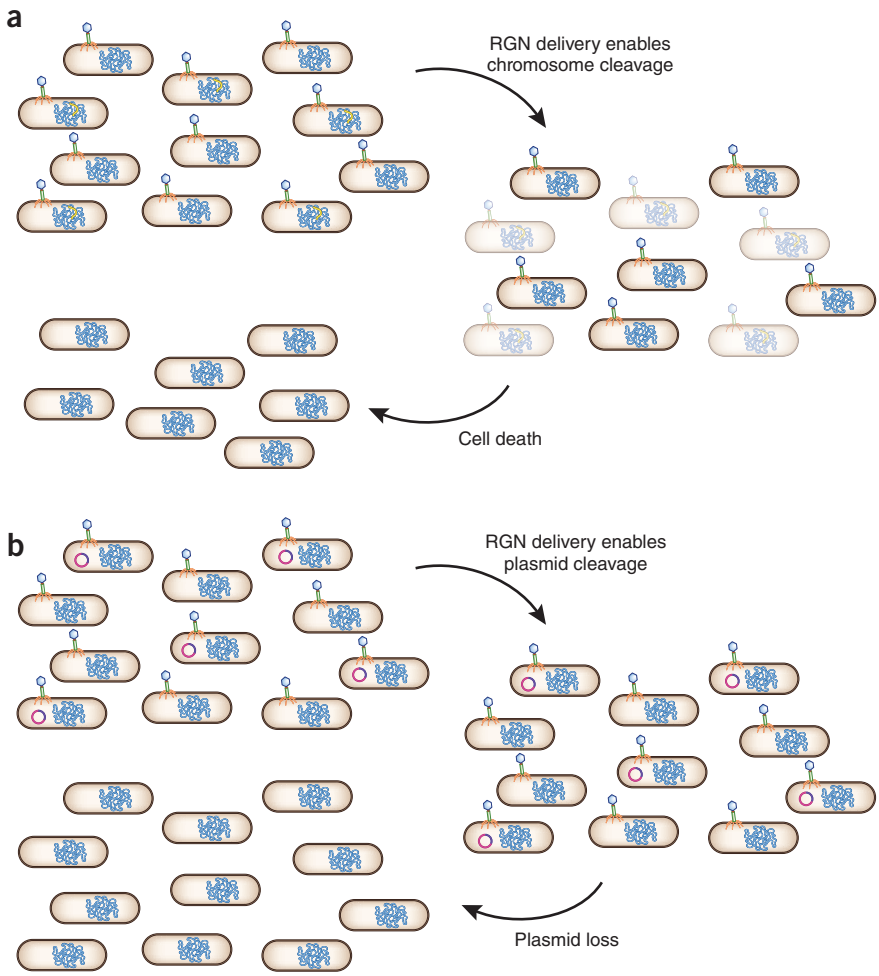

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Figure 1 Directed cleavage and loss of targeted DNA. (**a**) A mixed population of bacteria of a single species, some of which harbor a virulence gene such as *mecA* (yellow line) on the chromosome. Phage carrying RGN phagemids infect the whole population, but the RGN cleaves only the chromosomes of cells carrying the targeted virulence gene. Targeted cells die, leaving a population in which the virulent bacteria have been eliminated. (**b**) A population of bacteria of a single species, some of which harbor a plasmid containing an antibiotic resistance gene (blue), is infected with phage carrying RGN phagemids targeting the antibiotic resistance gene. The plasmid is cleaved and lost, leaving a population that is fully sensitive to the antibiotic.

Although the current data[1,2] are promising, it will be important to optimize delivery further, whether by phagemids, conjugated plasmids or other vehicles such as nanoparticles. One problem that could arise is that if killing is not rapid enough, or does not affect all members of the targeted population, some bacteria may survive the treatment. Among bacteria targeted with ΦNM1 carrying an RGN, ~10^{-4} survived[2]. The survivors had either not received the phagemid, had lost the phagemid or had received a corrupted phagemid. The number of bacteria that acquired resistance to infection by the phage-delivery vehicles owing to loss or mutation of their respective receptors is not evaluated in either paper, but phage resistance can be expected to add to the surviving population—and to be selected for expansion upon retreatment. For RGNs delivered by conjugation, the efficiency of killing is limited by conjugation efficiency, not RGN efficiency[1]. As Citorik *et al.*[1] discuss, next-generation delivery vehicles should also be designed to avoid limitations of host range, phage resistance and immunogenicity.

The increasing urgency of the problem of antibiotic resistance requires creative new approaches to drug discovery. At the same time, recognition of the importance of the gut microbiome in human health has underscored the desirability of narrow-spectrum antibiotics that minimally affect native flora. RGN antibacterials may indeed address these challenges. But their success is not guaranteed and will require further leaps in efficacy and delivery efficiency. They will have to show efficacy equivalent to that of standard-of-care antibiotics, ideally without disturbing the microbiome. This is exciting work—but it is just the beginning.

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Making biology transparent

Burkhard Höckendorf, Luke D Lavis & Philipp J Keller

The molecular and cellular architecture of the organs in a whole mouse is revealed through optical clearing.

In the last several years, scientists have begun to visualize the cellular architecture of entire organs using techniques for making tissues transparent, allowing microscopes to peer into animals at depths of millimeters to centimeters. One of the more promising 'optical clearing' methods is CLARITY, which involves stabilizing specimens with an acrylamide hydrogel and then depleting them of lipids[1]. Lipids and blood scatter and absorb light, obstructing microscopy, but proteins and nucleic acids are intrinsically transparent, so spectacular images can be acquired after lipids and blood have been removed. In a recent extension of CLARITY, Yang *et al.*[2] pumped hydrogel monomers and lipid-clearing agents into animals directly through the vasculature and the cerebrospinal fluid. The advantage of their approach is speed, as large organs and even entire mice can be cleared within a few days.

Observing cells and molecules within the intact architecture of the tissues and organs they form is crucial for our understanding of the structure and function of complex biological systems. However, light absorption, scattering and sample-induced aberrations limit the optical accessibility of biological tissues. Several approaches to make tissues transparent have been developed (**Table 1**). Fixed tissues can be dehydrated and immersed in organic solvents that minimize refractive index mismatches. Such methods initially suffered from tissue-shrinking artifacts and incompatibility with transgenic fluorescent reporters, but these limitations have been largely mitigated[3]. In parallel, aqueous solutions have been developed that compensate for refractive index mismatches and that dissociate collagen fibers of the extracellular matrix while preserving fluorescent markers[4,5].

CLARITY is a relatively new approach that results in extensive optical clearing. However, its reliance on electrophoresis to remove lipids from large tissue samples introduces some complexity to the procedure. Recently developed passive methods for clearing lipids provide a simpler alternative[2,6]. For example, in the passive method of Yang *et al.*[2], an optimized clearing reagent delivered by transcardiac perfusion cleared large samples within a few days. The authors carefully analyzed the degree of acrylamide cross-linking required to balance the large pore sizes desirable for relatively rapid lipid clearing with undesired tissue swelling. For imaging, the resulting hydrogels were immersed in a specific solution that eliminates the mismatch in refractive indices[2,6]. Yang *et al.*[2] demonstrate their method by clearing a variety of mouse organs, including brain, heart, lung, intestine and kidney (**Fig. 1**) as well as the body of a whole mouse.

It is difficult to assess comprehensively how this optimized form of CLARITY compares with other emerging techniques. Generally, clearing methods should be fast, scalable, versatile and easy to use, and they should maintain tissue structure, preserve native fluorescence and generate reproducible results. As competing methods are being developed (**Table 1**), a set of benchmark experiments that quantitatively compare performance in these categories and evaluate the reproducibility of protocols would provide a valuable resource for the community.

Beyond the need to further improve clearing methods, the field faces several additional challenges. For example, spectral overlap of the available fluorophores limits the number of recordable labels in a single imaging pass. One way to circumvent this problem is to perform sequential rounds of staining and label elution, but this introduces additional time-consuming steps[1]. It is also unclear how many staining rounds can be carried out without compromising molecular integrity, antigenicity or gross sample morphology. Overall, there is a need for specifically optimized labels that are spectrally nonoverlapping or selectively activatable, and that are long-lived in the presence of clearing agents.

The samples produced by optical tissue clearing are often very large in size and very rich in information content. Clearing procedures open the door to imaging with maximum contrast and resolution, but an imaging modality is needed that delivers such performance at high data-acquisition speeds.

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