CLINICAL IMPLICATIONS OF BASIC RESEARCH

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A CRISPR Path to Cutting-Edge Materials

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Delivery systems that can be triggered to release a drug in situ have advantages over conventional methods of drug delivery and have become more desirable with the development of large-molecule drugs, such as biologics. Biosensing is fundamental to diagnostic testing, which includes laboratory-based medical diagnostics and non–laboratory-based point-of-care testing critical to managing the health of persons living in resource-limited areas and for preventing the spread of infectious disease. English et al.,[1] in a recent report on research from James Collins' group, therefore bring welcome news: they describe a means to molecularly program traditional materials so that they can be used to deliver a drug or can be used as biosensors.

Many materials have been developed and are synthesized for biologic medical applications. An early example is poly(lactic-co-glycolic acid) (PLGA), a biodegradable polymer approved by the Food and Drug Administration and the European Medicines Agency and consisting of repeating units of two monomers (lactic acid and glycolic acid). It is used in vehicles for drug delivery. PLGA is generally biocompatible — not a surprise, given that both monomers are synthesized by cells. Like PLGA, DNA is also a polymer. It is a long molecular chain consisting of four types of monomers (i.e., the nucleotides adenosine, thymine, cytosine, and guanine) and can be used as a delivery vehicle. Indeed, using only DNA as a polymer, scientists have made hydrogels for the delivery of drugs and the production of proteins in a cell-free fashion.[2]

When teamed up with enzymes, DNA has impressive functions. The clustered regularly interspaced short palindromic repeats (CRISPR) system is especially powerful. The most commonly used CRISPR system is called CRISPR-Cas9, in which the Cas9 enzyme (an endonuclease) cleaves double-stranded DNA at a specific site: the location of the cleavage site is determined by the sequence of the guide RNA, so called because it guides the Cas9 endonuclease to the DNA sequence that is complementary to the guide RNA (Fig. 1). Once bound to double-stranded DNA, the enzyme becomes activated and cleaves the DNA. This is the first step in modifying or deleting the gene (i.e., gene editing). The CRISPR system has been augmented with the discovery and incorporation of Cas12a, an endonuclease similar to Cas9. Like activated Cas9, activated Cas12a cuts target double-stranded DNA, but unlike Cas9, Cas12a then becomes activated and unleashes a frenzy of indiscriminate cleavage of single-stranded DNA (at a rate of about 1200 cuts per second), causing collateral damage. It is this CRISPR-Cas12a system that English et al. used to create new materials for drug delivery and biosensing.[1]

They first designed guide RNA to complement "trigger" double-stranded DNA (Fig. 2); cleavage by Cas12a could therefore occur only in the presence of this specific trigger DNA. Then they fabricated hydrogels with the guide RNA–Cas12a complex embedded in a lattice structure made up of single-stranded DNA. When the gel systems were exposed to trigger double-stranded DNA, the Cas12a nuclease became activated. The more trigger double-stranded DNA there was (or the longer the duration of exposure of the gel to the trigger), the greater the number of activated Cas12a molecules, the greater the collateral damage to single-stranded DNA, and the greater the extent of digestion of gel scaffolding and release of encapsulated cargos, such as enzymes, drug-containing nanoparticles, and live cells.

As for biosensing, the authors showed that they could detect fragments of *mecA*, a gene conferring resistance to methicillin in *Staphylococcus*

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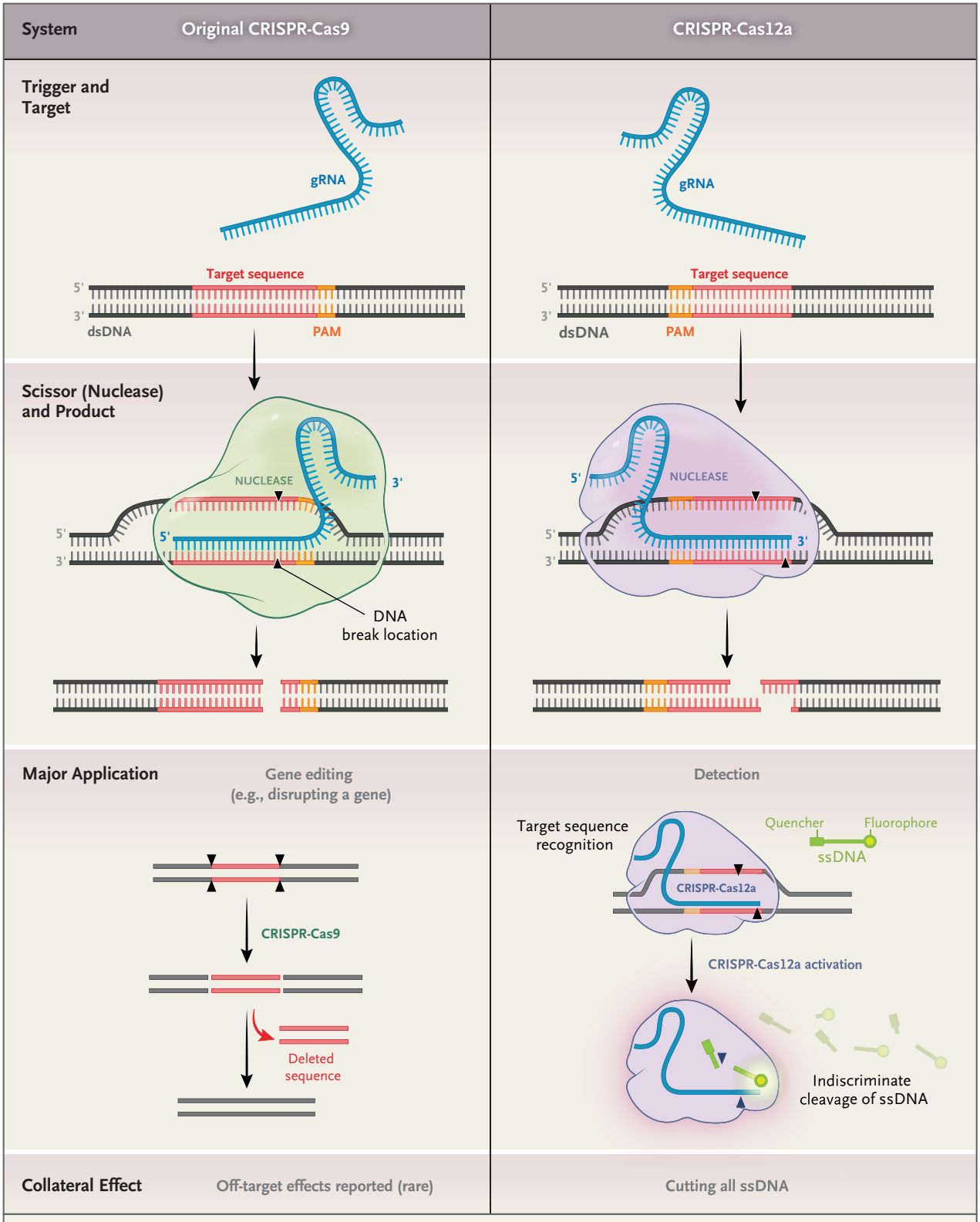
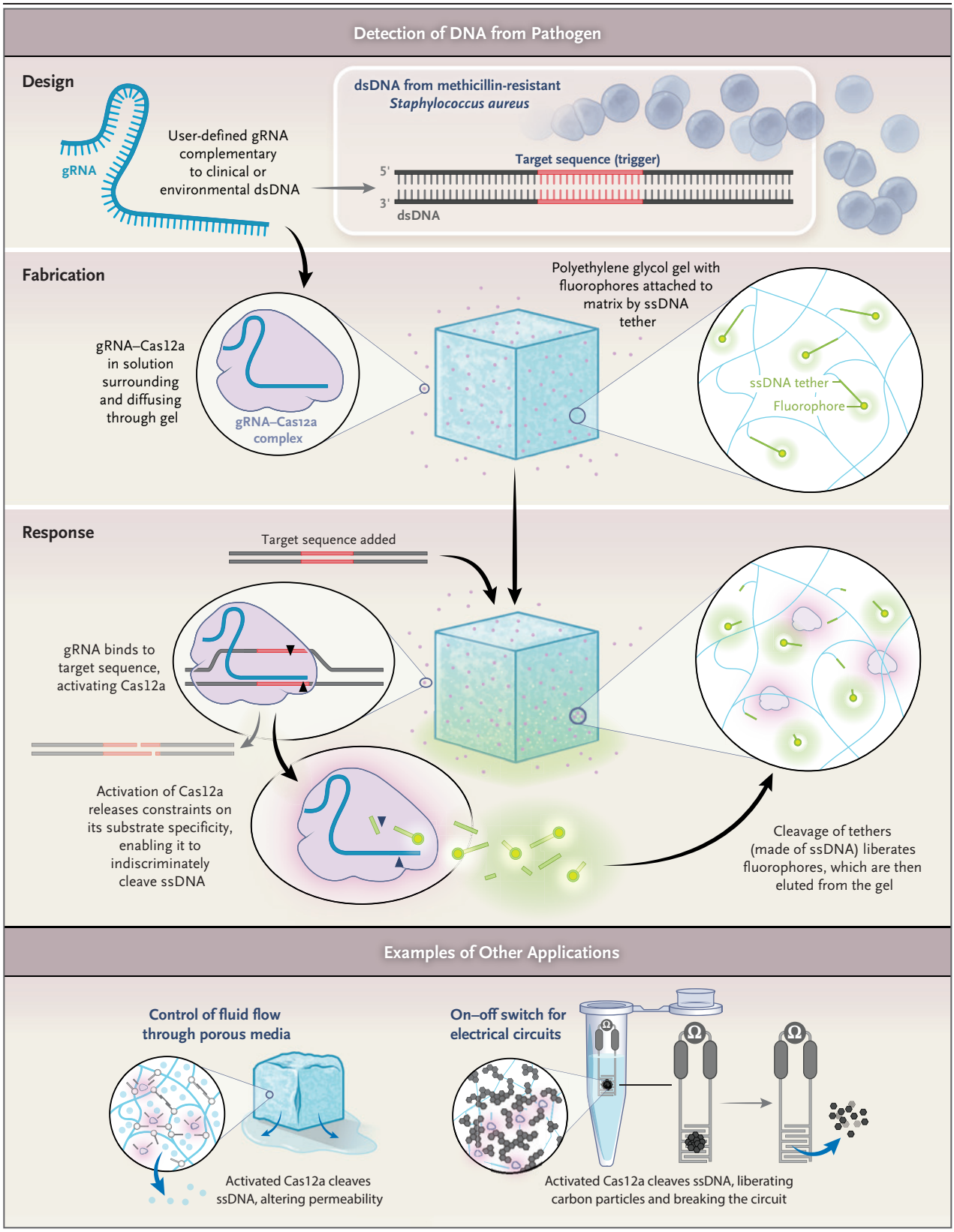


Figure 1. Comparison of Two Cas Endonucleases.

CRISPR denotes clustered regularly interspaced short palindromic repeats, dsDNA double-stranded DNA, gRNA guide RNA, PAM protospacer-adjacent motif, and ssDNA single-stranded DNA.

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Figure 2 (previous page). CRISPR Gel as Biosensor.
English et al.[1] described the fabrication of different types of CRISPR hydrogels that could potentially be used as biosensors (e.g., to detect methicillin-resistant *Staphylococcus aureus* or Ebola virus) or as drug- and cell-delivery systems. In the first example depicted here, activated Cas12a cleaves the ssDNA that is tethering quenched fluorescein to a polyethylene glycol gel, resulting in liberation of the fluorescence. English et al. also showed that the permeability of a polyacrylamide–ssDNA gel increased (thereby increasing the rate at which fluid flows through it) on exposure to activated Cas12a (lower panel, left) and that a fuse made of conductive carbon black nanoparticles cross-linked with ssDNA was tripped on exposure to activated Cas12a (lower panel, right).

aureus, by attaching a quenched fluorescein (the reporter) to one end of a single-stranded DNA (Fig. 2), and the other end of the single-stranded DNA to a polyethylene glycol hydrogel (forming a kind of molecular "pendant" made up of a single-stranded DNA tether with quenched fluorescein at the end). In the presence of double-stranded *mecA* (the trigger) and Cas12a guide RNA (with the sequence of the guide RNA being complementary to part of *mecA*), the fluorescein cargo was released. Collins and colleagues also designed gels such that single-stranded DNA cutting (triggered, for example, by synthetic Ebola virus genomic RNA) resulted in altered permeability or even complete degradation of the gel. Using these property changes, they showed that a CRISPR gel, embedded in a microfluidic paper-based device, could detect synthetic Ebola virus RNA. They tested this method by asking each of three experimenters in different geographic locations to use the device to analyze 12 samples of synthetic Ebola virus RNA (11 attomoles per liter — i.e., 10^{-18} moles per liter) or blanks. All samples were correctly assigned.

The greatest strengths of the system described by English et al. are the ease with which it can be tailored and its capacity to render programmable gels from traditional materials such as polyacrylamide and polyethylene glycol. With dozens of known naturally occurring Cas proteins, facile means of tweaking them through mutagenesis, and many — perhaps thousands — of different DNA-processing enzymes, one can only imagine how many more materials could be designed for clinical settings.

Disclosure forms provided by the authors are available at NEJM.org.

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