traveled. Eliav *et al.* suggest a feed-forward model whereby the multiscale fields in CA1 result from convergence of inputs from multiple CA3 place cells with different spatial scales onto each CA1 place cell. Predictions of this model that still need to be tested are that CA3 neurons should not show multiple fields in large environments and that either grid cells should not show multiple fields or grid cell inputs do not contribute to the firing of CA1 place fields in large environments.

A second question is whether there is a continuum of multiscale coding across environments of all sizes or whether (as suggested by Eliav *et al.*) multiscale coding occurs only in sufficiently large environments. And if the latter, what behavioral, perceptual, and neural mechanisms trigger the transition from small-scale to large-scale encoding of space?

The study of Eliav *et al.* provides a marker for the need to examine spatial coding in ethologically relevant environments. The multiscale place cell coding mechanism that they demonstrate may allow both fine-scale spatial localization and localization on a more extended scale, which would be required for navigating accurately between very distant locations hundreds of meters or kilometers apart. It will be interesting to see whether similar multiscale spatial representations occur in humans or nonhuman primates navigating (virtual or real) large, open spaces and whether multiscale coding by individual neurons occurs in other, nonspatial domains, such as the coding of time (*11*). ■

"...the multiscale coding mechanism seen in the bats is a particularly efficient mechanism for coding large environments."

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MEDICINE

CRISPR diagnostics

New CRISPR enzyme activities add to the nucleic acid detection arsenal

By **Omar O. Abudayyeh** and **Jonathan S. Gootenberg**

Although clinical diagnostics take many forms, nucleic acid–based testing has become the gold standard for sensitive detection of many diseases, including pathogenic infections. Quantitative polymerase chain reaction (qPCR) has been widely adopted for its ability to detect only a few DNA or RNA molecules that can unambiguously specify a particular disease. However, the complexity of this technique restricts application to laboratory settings. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has underscored the need for the development and deployment of nucleic acid tests that are economical, easily scaled, and capable of being run in low-resource settings, without sacrifices in speed, sensitivity or specificity. CRISPR-based diagnostic (CRISPR-dx) tools offer a solution, and multiple CRISPR-dx products for detection of the SARS-CoV-2 RNA genome have been authorized by the US Food and Drug Administration (FDA). On page 941 of this issue, Jiao *et al.* (*1*) describe a new CRISPR-based tool to distinguish several SARS-CoV-2 variants in a single reaction.

There are multiple types of CRISPR systems comprising basic components of a single protein or protein complex, which cuts a specific DNA or RNA target programmed by a complementary guide sequence in a CRISPR-associated RNA (crRNA). The type V and VI systems and the CRISPR-associated endonucleases Cas12 (*2*, *3*) and Cas13 (*4*, *5*) bind and cut DNA or RNA, respectively. Furthermore, upon recognizing a target DNA or RNA sequence, Cas12 and Cas13 proteins exhibit "collateral activity" whereby any DNA or RNA, respectively, in the sample is cleaved regardless of its nucleic acid sequence (*4*, *6*). Thus, reporter DNAs or RNAs, which allow for visual or fluorescent detection upon cleavage, can be added to a sample to infer the presence or absence of specific DNA or RNA species (*4*–*8*).

Initial versions of CRISPR-dx utilizing Cas13 alone were sensitive to the low picomolar range, corresponding to a limit of detection of millions of molecules in a microliter sample. To improve sensitivity, preamplification methods, such as recombinase polymerase amplification (RPA), PCR, loop-mediated isothermal amplification (LAMP), or nucleic acid sequence–based amplification (NASBA), can be used with Cas12 or Cas13 to enable a limit of detection down to a single molecule (*8*). This preamplification approach, applicable to both Cas12 and Cas13 (*6*, *7*), enabled a suite of detection methods and multiplexing up to four orthogonal targets (*7*). Additional developments expanded CRISPR-dx readouts beyond fluorescence, including lateral flow (*7*), colorimetric (*9*), and electronic or material responsive readouts (*10*), allowing for instrument-free approaches. In addition, post–collateral-cleavage amplification methods, such as the use of the CRISPR-associated enzyme Csm6, have been combined with Cas13 to further increase the speed of CRISPR-dx tests (*7*). As an alternative to collateral-cleavage–based detection, type III CRISPR systems, which involve large multiprotein complexes capable of targeting both DNA and RNA, have been used for SARS-CoV-2 detection through production of colorimetric or fluorometric readouts (*11*).

FDA-authorized CRISPR-dx tests are currently only for use in centralized labs, because the most common CRISPR detection protocols require fluid handling steps and two different incubations, precluding their immediate use at the point of care. Single-step formulations have been developed to overcome this limitation, and these "one-pot" versions of CRISPR-dx are simple to run, operate at a single temperature, and run without complex equipment, producing either fluorescence or lateral flow readouts. The programmability of CRISPR makes new diagnostic tests easier to develop, and within months of the release of the SARS-CoV-2 genome, many COVID-19–specific CRISPR tests were reported and distributed around the world.

The broader capability for Cas enzyme–enhanced nucleic acid binding or cleavage has led to several other detection modalities. Cas9-based methods for cleaving nucleic acids in solution for diagnostic purposes have been combined with other detection platforms, such as destruction of undesired amplicons for preparation of next-generation sequencing libraries (*12*), or selective removal of alleles for nucleotide-specific detection

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(*13*). Alternatively, the programmable cleavage event from the Cas nuclease can be used to initiate an amplification reaction (*14*). Cas9-based DNA targeting has also been used for nucleotide detection in combination with solid-state electronics, promising an amplification-free platform for detection. In this platform, called CRISPR-Chip, the Cas9 protein binds nucleotide targets of interest (often in the context of the native genome) to graphene transistors, where the presence of these targets alters either current or voltage (*15*). By utilizing additional Cas9 orthologs and specific guide designs, CRISPR-Chip approaches have been tuned for single–base-pair sensitivity (*15*). Because they are integrated with electronic readers, CRISPR-Chip platforms may allow facile point-of-care detection with handheld devices.

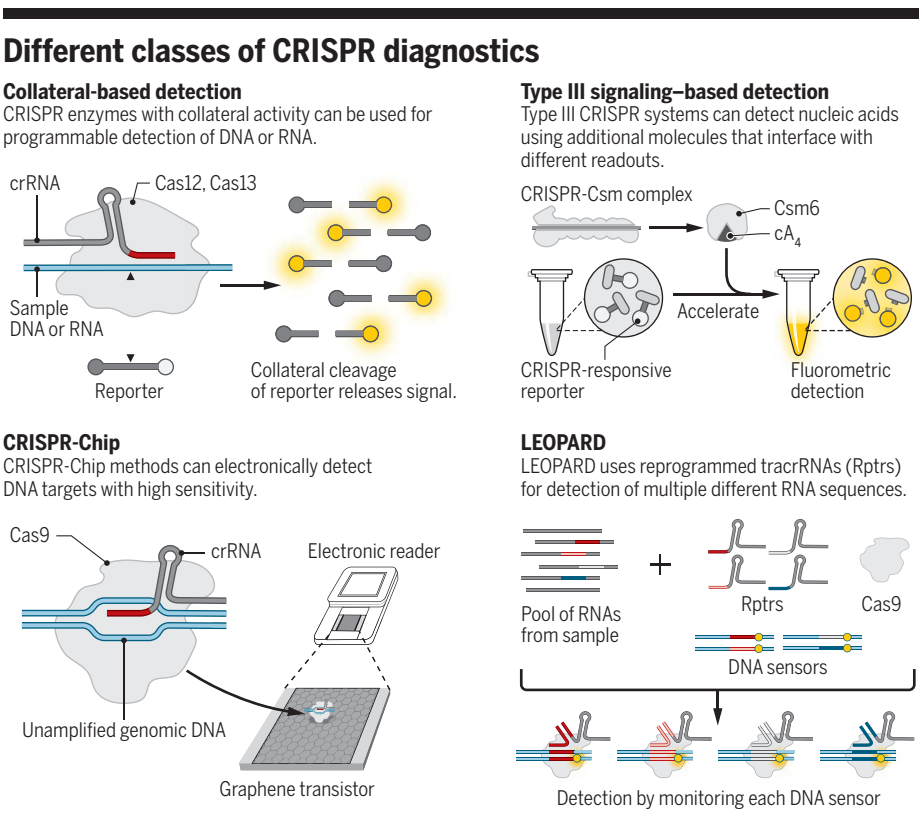
Jiao *et al.* use a distinct characteristic of type II CRISPR systems, which involve Cas9, to develop a new type of noncollateral based CRISPR detection. Unlike Cas12s and Cas13, Cas9-crRNA complex formation requires an additional RNA known as the trans-activating CRISPR RNA (tracrRNA). By sequencing RNAs bound to Cas9 from *Campylobacter jejuni* in its natural host, the authors identified unexpected crRNAs, called noncanonical crRNA (ncrRNA), that corresponded to endogenous transcripts. Upon investigation of this surprising observation, it became clear that the tracrRNA was capable of hybridizing to semi-complementary sequences from a variety of RNA sources, leading to biogenesis of ncrRNAs of various sizes. Recognizing that they could program tracrRNAs to target a transcript of interest, the authors generated a reprogrammed tracrRNA (Rptr) that could bind and cleave a desired transcript, converting a piece of that transcript into a functional guide RNA. By then creating fluorescent DNA sensors that would be cleaved by the Rptr and ncrRNAs, the sensing of RNA by Cas9 could be linked to a detectable readout. This platform, called LEOPARD (leveraging engineered tracrRNAs and on-target DNAs for parallel RNA detection), can be combined with gel-based readouts and enables multiplexed detection of several different sequences in a single reaction (see the figure).

Jiao *et al.* also combined LEOPARD with PCR in a multistep workflow to detect SARS-CoV-2 genomes from patients with COVID-19. Although more work is needed to integrate this Cas9-based detection modality into a single step with RPA or LAMP to create a portable and sensitive isothermal test, an advantage of this approach is the higher-order multiplexing that can be achieved, allowing multiple pathogens, diseases, or variants to be detected simultaneously. More work is also needed to combine this technology with extraction-free methods for better ease of use; alternative readouts to gel-based readouts, such as lateral flow and colorimetric readouts, would be beneficial for point-of-care detection.

In just 5 years, the CRISPR-dx field has rapidly expanded, growing from a set of peculiar molecular biology discoveries to multiple FDA-authorized COVID-19 tests and spanning four of the six major subtypes of CRISPR systems. Despite the tremendous promise of CRISPR-dx, substantial challenges remain to adapting these technologies for point-of-care and at-home settings. Simplification of the chemistries to operate as a single reaction in a matter of minutes would be revolutionary, especially if the reaction could be run at room temperature without any complex or expensive equipment. These improvements to CRISPR-dx assays can be achieved by identification or engineering of additional Cas enzymes with lower-temperature requirements, higher sensitivity, or faster kinetics, enabling rapid and simple amplification-free detection with single-molecule sensitivity.

Often overlooked is the necessity for a sample DNA or RNA preparation step that is simple enough to be added directly to the CRISPR reaction to maintain a simple workflow for point-of-care testing. In addition, higher-order multiplexing developments would allow for expansive testing menus and approach the possibility of testing for all known diseases. As these advancements are realized, innovative uses of CRISPR-dx will continue in areas such as surveillance, integration with biomaterials, and environmental monitoring. In future years, CRISPR-dx assays may become universal in the clinic and at home, reshaping how diseases are diagnosed. ■

Different classes of CRISPR diagnostics



cA_4, cyclic tetra-adenylate; Cas, CRISPR-associated endonuclease; crRNA, CRISPR-associated RNA; LEOPARD, leveraging engineered tracrRNAs and on-target DNAs for parallel RNA detection; tracrRNA, trans-activating crRNA.

GRAPHIC: ERIN DANIEL

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CRISPR diagnostics

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