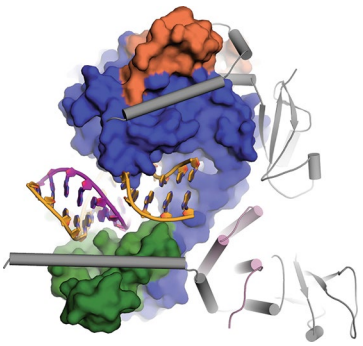
CORONAVIRUS

Groovy RNA polymerase

Science **368**, 779–782 (2020)
Cell https://doi.org/10.1016/j.cell.2020.05.034 (2020)



Credit: Zihe Rao

The coronavirus SARS-CoV-2 is responsible for the disease COVID-19, which has become a global pandemic. The SARS-CoV-2 structural protein Spike and the non-structural protein RNA-dependent RNA polymerase (nsp12) are potential drug targets given their importance in host-cell recognition and viral replication, respectively. To further understand the mechanism of viral polymerase activity, Gao et al. solved the cryo-EM structure of SARS-CoV-2 nsp12 with cofactors nsp7 and nsp8, components of the polymerase processivity clamp. Although the overall architecture is similar to that of the closely related SARS-CoV-1 homolog, the authors noted differences in the nucleotidyltransferase (NiRAN) domain and the existence of an N-terminal β-hairpin that stabilizes the overall structure by insertion into the groove clamped by the NiRAN domain and the palm subdomain of the polymerase domain. Catalytic complex structures from Wang et al. showed structural rearrangements required to accommodate the RNA, and a pre-translocated complex with the nucleotide analog remdesivir clarified the transition of the polymerase primase complex to its catalytic complex. These findings can potentially enable development of new antiviral drugs against a promising target. *MB*

https://doi.org/10.1038/s41589-020-0582-1

SYNTHETIC BIOLOGY

Passing the acid test

ACS Synth. Biol. https://doi.org/10.1021/acssynbio.0c00089 (2020)

The microbial production of certain compounds benefits from the use of specialized conditions, such as temperature and pH extremes, to which commonly used organisms such as *Escherichia coli* are not naturally resistant. To make *E. coli* acid tolerant, de Siqueira et al. drew on metagenome sequences to construct new synthetic 'acid resistance clusters' consisting of three genes encoding extremophile homologs of the DNA-binding protein HU, an RNA-binding protein, and the protease ClpP. Using a combinatorial approach, the authors generated a panel of synthetic operons using these genes with varied ribosome-binding sequences (RBSs) and screened for their ability to confer acid resistance in *E. coli*. While each gene alone improved cellular viability at pH 1.9, the three together exhibited a synergistic advantage and enhanced *E. coli* survival by 100-fold relative to the empty vector. Although future work is needed to construct improved strains with increasingly robust acid tolerance, this engineering effort demonstrates the potential of using synthetic operons to confer the benefit of multiple resistance genes on an industrially useful microbe. *CD*

https://doi.org/10.1038/s41589-020-0580-3

GENOME ENGINEERING

Better editors

Nat. Methods **17**, 600–604 (2020)

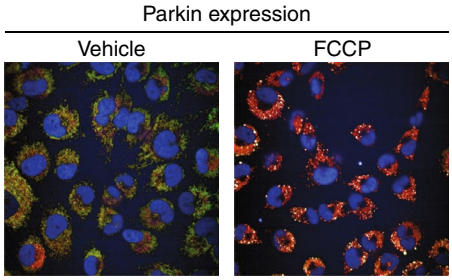
Base editors that direct deaminases to specific genome sites via CRISPR-system enzymes have gained recent attention due to precision base editing without induction of DNA double-strand breaks. However, potential off-target effects on both DNA and RNA may complicate biotechnological and therapeutic applications. To obtain cytosine base editors (CBEs) with increased specificity, Zuo et al. constructed variants of rAPOBEC1, a widely used cytidine deaminase component in CBE, within regions related to DNA and RNA editing activity. Four variants with single or double mutations near putative DNA-binding sites and/or hydrophobic sites required for RNA binding exhibited decreased DNA and RNA off-target effects in embryos and HEK293T cells, yet maintained on-target editing activity. Interestingly, the on-target activity of the variants could be further enhanced by addition of an N-terminal nuclear localization signal and codon optimization. The resulting optimized variant YE1-BE3-FNLS had on-target editing activity comparable to that of the current best base editors, with a nearly basal level of off-target edits. This study demonstrates the use of rational design to improve the performance of base editors for further applications. *YS*

https://doi.org/10.1038/s41589-020-0583-0

IMAGING

Taking out the trash

Cell **181**, 1176–1187 (2020)



Credit: Koji Nagasawa and Yoshiyuki Tsujihata

Mitophagy uses lysosomal degradation to remove defective mitochondria. Although probes to detect and measure mitophagy such as mt-mKeima have been developed, they can only image live specimens due to their reliance on a pH difference inside and outside the lysosome. To develop an improved mitophagy probe, Katayama et al. screened a library of fluorescent proteins and identified a cyan-emitting protein called TOLLES that is resistant to lysosomal environments. They used TOLLES as a FRET donor and a YFP variant (YPet) as a FRET acceptor to develop a ratiometric autophagy indicator called SRAI. Starvation of cells to induce autophagy resulted in movement of SRAI to the lysosome, where YPet is degraded, producing a high TOLLES/YPet ratio that was also retained in fixed samples. A mitochondrially targeted variant, mito-SRAI, could detect mitophagy induced by the uncoupler FCCP and Parkin expression and enabled the identification of a compound that enhances mitophagy on damaged mitochondria while sparing intact mitochondria. Overall, the development of mito-SRAI as a reliable readout of mitophagy offers the potential for further understanding of how defects in mitophagy can result in diseases. *GM*

https://doi.org/10.1038/s41589-020-0581-2

Mirella Bucci, Caitlin Deane, Grant Miura and Yiyun Song