Modified CRISPR/Cas9 gene editing system used to learn more about the evolution of giant viruses

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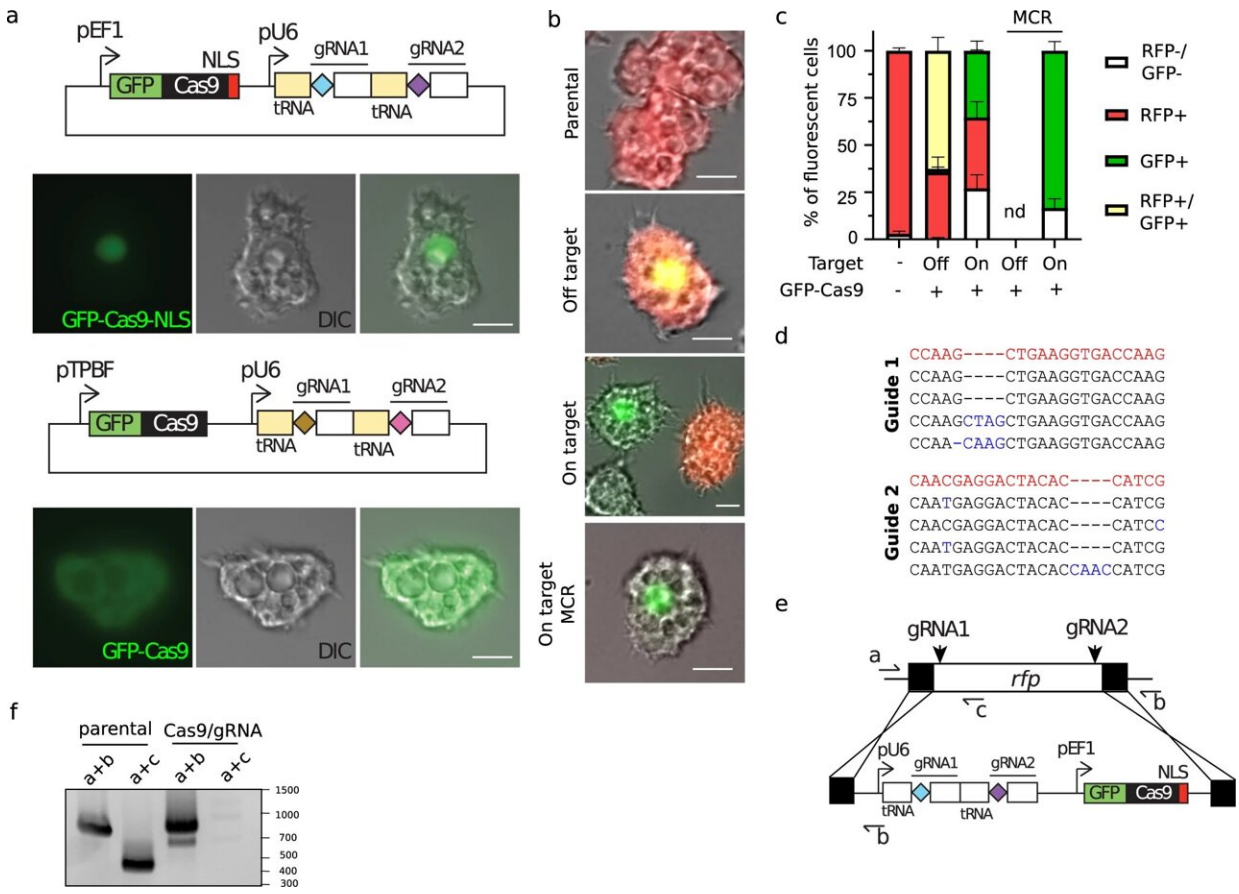

CRISPR/Cas9 allows manipulation of A. castellanii. **a** Constructs used to constitutively express Cas9 in *A. castellanii*. The sequence of the gRNA is depicted with diamonds followed by the Cas9-binding scaffold and an *A. castellanii* tRNA (yellow rectangles). The localization of the GFP-Cas9 fusion for the different constructs is shown by fluorescence. Micrographs are

representative of 3 independent experiments. Scale bar: 10μm. **b** Representative micrographs showing amoebas expressing mRFP (vector Vc241) and GFP-Cas9 after selection for 2–3 weeks with the appropriate drug(s) (refer to materials and methods). mRFP (product of the targeted gene) and GFP (Indicating Cas9 expression) fluorescence are shown. MCR: mutagenic chain reaction. Scale bar: 10μm. **c** The quantification of the micrograph shown in (**b**). The mean ± SD of at least 200 amoebas (3 independent experiments ($n = 3$)) is shown. Amoebas were classified either as non-fluorescent, mRFP+, GFP+ or GFP+mRFP+. MCR: mutagenic chain reaction. Guides targeting pandoravirus rpb1 were used as off-target gRNAs. Nd: not detected. **d** Representative sequencing results of targeted guide sequences on *rfp* upon transfection with on-target gRNAs. PCR were performed on the target sequences as shown in Fig. S1a, b, cloned into a TA cloning vector and single clones were sent for sequencing. The wild type sequence and mutations generated are shown in red and blue respectively. Ten individual clones were amplified and sequenced. **e** Schematic representation of the rfp locus, guide targeting location, homology arms for recombination and primer annealing sites for a disruption using a "mutagenic chain reaction" strategy (see Fig. S1e). **f** Gene disruption of *rfp* by the "mutagenic chain reaction" observed at the population level after 2–3 weeks post-transfection. Expected PCR size: a + b: 837 bp (unmodified locus), a + b 890 bp (recombinant locus), a + c: 500 bp (unmodified locus). Note that the primer b anneals both in the wild type and recombinant locus resulting in PCR products with slight differences in size. Credit: *Nature Communications* (2023). DOI: 10.1038/s41467-023-36145-4

A team of virologists at Aix–Marseille University, has found evidence that suggests the giant virus Pandoravirus neocaledonia evolved from smaller and simpler viruses. In their study, published in the journal *Nature Communications*, the group used a modified version of the CRISPR/Cas9 gene editing system to learn more about the evolutionary history of the giant virus, and perhaps others like it.

Prior research has shown that most viruses are much smaller than

bacteria. But a few are so large that biologists refer to them as giant viruses. Somewhat perplexed by their existence, evolutionary biologists have long debated how such strange viruses might have come to exist.

Currently, there are two main theories: The first is that they evolved as a mix of several smaller viruses. The second is that they devolved from larger, more sophisticated organisms. In this new effort, the team in France took a new approach to solving the mystery—using CRISPR/Cas9 to identify what are known as essential genes in the viruses' genome (those that are needed to reproduce).

Prior research has shown that a good way to discover essential genes is to remove genes one at a time until the organism is no longer able to reproduce. That is where CRISPR/Cas9 came in—it allows for knocking out whatever genes are desired. But Pandoravirus neocaledonia presented a problem—it has 25 copies of each of its chromosomes, and CRISPR/Cas9 is only able to knock out one gene at a time. To overcome this problem, the team modified the gene editing system to generate a chain reaction—whenever a gene was cut, another cut would be instigated along the chain until all of the copies were removed.

After running their chain-reaction gene editing on Pandoravirus neocaledonia until the virus was no longer able to reproduce, they found that the genes needed for reproduction were located on just one end of the genome, somewhat apart from other, less essential genes—evidence, the team suggests, that the virus evolved from several smaller viruses. They suggest that it is likely that other giant viruses evolved in similar ways.

More information: Hugo Bisio et al, Evolution of giant pandoravirus revealed by CRISPR/Cas9, *Nature Communications* (2023). DOI: 10.1038/s41467-023-36145-4

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