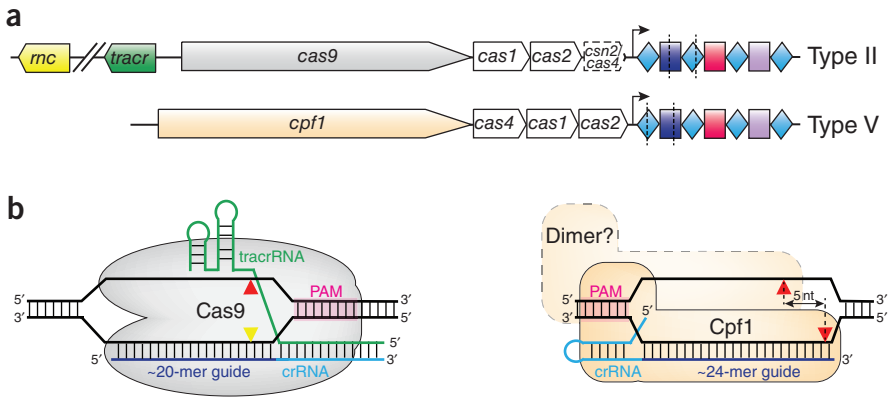

Figure 1 Cas9, Cpf1 and their respective interference mechanisms. (**a**) Architecture of type II and type V CRISPR-*cas* loci. Diamonds, CRISPR repeats; rectangles, CRISPR spacers; chevrons, genes. In type II systems, the tracrRNA (green) is an essential cofactor, and most also employ RNase III (encoded by the *rnc* gene; yellow) for crRNA processing. Genes shown in white are involved in the acquisition of new spacers but are dispensable for the interference function of existing spacers. The arrows denote the direction of pre-crRNA transcription, and the dashed lines indicate the boundaries of the mature crRNA from the first transcribed spacer. (**b**) DNA target recognition and cleavage by Cas9, Cpf1 and their respective RNA partners. In type II systems, Cas9 forms an RNA-protein complex with the crRNA (blue) and tracrRNA (green), where the repeat region (light blue) of the crRNA is 3′ to the spacer (guide; dark blue) sequence. In type V systems, Cpf1 forms a complex with its crRNA, which has the guide following the repeat region of the crRNA. Both nucleases identify their target sites on the basis of complementarity of the guide sequence (which is assessed through heteroduplex formation) as well as the presence of a neighboring PAM (magenta). However, the PAM is found on opposite sides of the heteroduplex in these systems. Cas9 cleaves the DNA strand complementary to the guide using its HNH domain (yellow arrowhead) and the noncomplementary strand using its RuvC domain (red arrowhead). Cpf1 appears to contain only a single nuclease domain (RuvC) and may cleave both strands through the participation of the RuvC domain of another monomer via a dimeric complex.

cells. They generated lesions with efficiency similar to *Streptococcus pyogenes* Cas9, suggesting that these Cpf1 orthologs could be repurposed as genome-engineering tools.

The study by Zetsche and colleagues[1] highlights the diversity within class II systems, where independent nuclease solutions (Cas9 and Cpf1) have evolved as components of adaptive defense systems. Other divergent class II crRNA-effector systems are beginning to be characterized (such as C2c1; ref. 7), and these promise to increase the number of Cas9 'classmates'. The absence of strong conservation among the crRNA-effector proteins suggests that further variations in interference strategy will continue to emerge. Given that bacteria can harbor multiple, distinct class II systems with different interference properties, combinations of these systems may achieve synergistic effects by targeting different vulnerabilities within the bacteriophage lifecycle.

Beyond the implications for bacterial immunology, the Cpf1 system also has attributes that should make it a useful addition to the genome-engineering toolbox. Cpf1 uses a compact A/T-rich PAM with specificities different from those of known type II systems (nearly all of which include one or more G residues), providing access to distinct target sites. It uses a single, small crRNA, which could simplify guide production and delivery, in particular when using synthetic modified RNAs to increase stability[8]. As noted by Zetsche *et al.*[1], the production of DSBs with 5′ overhangs could be leveraged for the directional insertion of DNA via the nonhomologous end joining DSB repair pathway[9]. This could be valuable for genome editing in cell types in which homologous recombination has proven challenging (such as post-mitotic cells). The accuracy of target-site cleavage by Cpf1 orthologs, in comparison to characterized Cas9 orthologs, will be an important factor in their adoption as genome-editing tools.

The study by Zetsche and colleagues, like any good scientific exploration, also raises several intriguing questions. How are type V pre-crRNAs processed? If a Cpf1 dimer is responsible for DNA cleavage, is the presumptive second subunit also loaded with a crRNA? What is the allocation of responsibilities for each monomer, such that target recognition is not hindered by cross-talk between distinct crRNAs loaded into each monomer? How does the Cpf1 RuvC domain efficiently cleave different substrates (the crRNA-paired and unpaired DNA strands)? How does Cpf1 define the position of the break formation on each strand given the large distance of the DSB from the PAM element, which provides a reference point for Cas9? Whatever the answers to these questions, it is likely that Cpf1 is but the first of many upcoming additions to the RNA-guided DNA endonuclease toolbox that is transforming biomedical science.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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