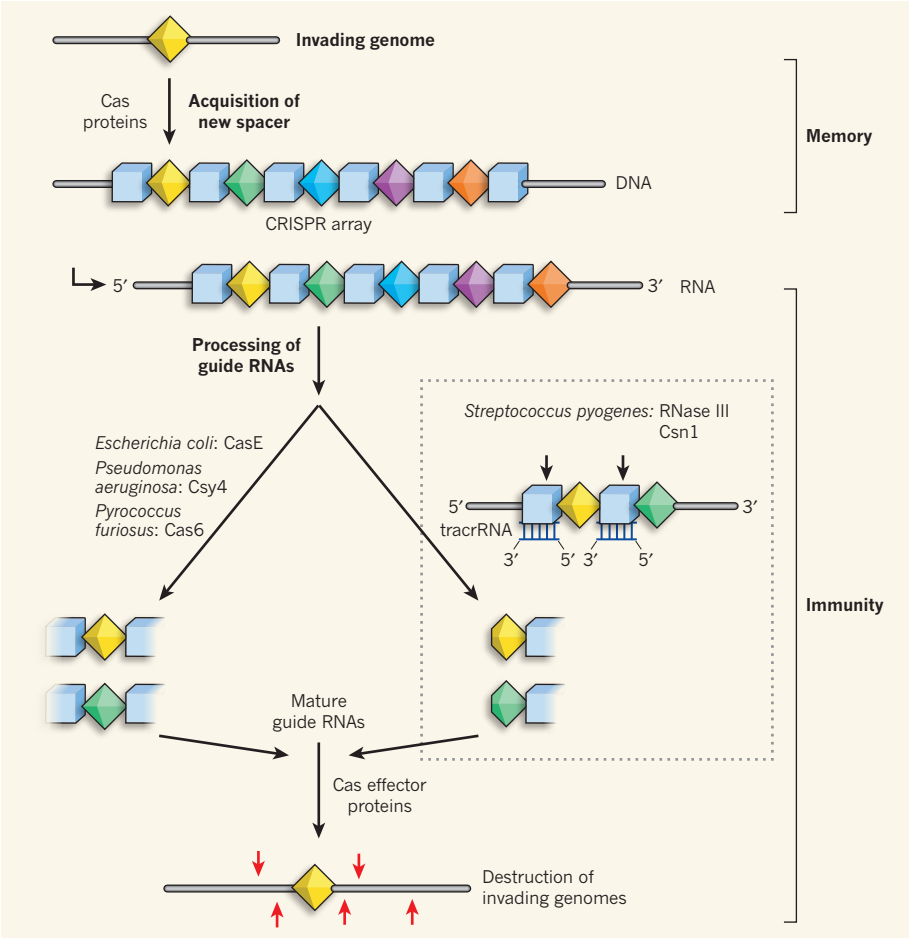

Figure 1 | Flexibility in CRISPR processing systems. In CRISPR arrays of DNA, conserved repeats (squares) alternate with variable spacers (diamonds). During the acquisition/memory step, new spacers are incorporated as a result of invasion of the cell by a phage or plasmid. During the processing steps required to confer immunity, RNA transcripts of the CRISPR array are cleaved to give mature guide RNAs, which then target for destruction invading genomes that match the spacer. The related proteins CasE, Csy4 and Cas6 carry out the initial processing step in many organisms. Deltcheva *et al.*[2] describe a different pathway for guide-RNA maturation that operates in *Streptococcus pyogenes* and certain other bacteria (boxed area). This pathway involves a *trans*-acting RNA (tracrRNA) that hybridizes with the spacers, leading to cleavage by RNase III. Csn1 aids in the process, and an undefined step yields mature guide RNAs.

the authors find that *S. pyogenes* uses the bacterial RNase III to process CRISPR RNA; targeting of cleavage is determined by an antisense RNA, tracrRNA, with complementarity to the CRISPR spacer (boxed area in Fig. 1).

RNase III is a conserved endonuclease that cleaves double-stranded RNA and is involved in the maturation of ribosomal RNA. It is also the bacterial equivalent of the Dicer endonuclease. The new work[2] demonstrates the flexibility in CRISPR systems and, for the first time, links RNase III to the bacterial CRISPR defence system. Finally, the use of a core RNase rather than a Cas-specific protein provides a clear example of crosstalk between the CRISPR system and bacterial metabolism.

The antisense RNA that plays an important part here was identified when Deltcheva and colleagues' deep sequencing of *S. pyogenes* revealed abundant short RNAs all containing a region of complementarity to the CRISPR repeat. These antisense RNAs were encoded close to the CRISPR cluster but on the opposite strand. Deleting the gene for the antisense RNA prevented processing of the CRISPR RNA. *In vitro*, this antisense RNA can anneal to CRISPR RNA, making a double-stranded RNA that is a substrate for RNase III. *In vivo*, cleavage requires RNase III and Csn1, one of the Cas proteins specific to this family of CRISPR clusters; Csn1 may facilitate pairing of tracrRNA and CRISPR RNA, and also a second processing step. Finally, Deltcheva *et al.* find that all of these elements (tracrRNA, Csn1 and RNase III) are necessary for immunity — that is, for *S. pyogenes* to destroy an invading plasmid containing sequences complementary to the CRISPR spacer.

When the family of CRISPRs containing Csn1 was first defined, Haft *et al.*[7] noted that "A characteristic feature of this subtype is a single copy of the repeat (sometimes direct, sometimes inverted), which appears upstream of the first gene in the locus". Now we know that Csn1 is essential, as is the nearby upstream repeat, to this unique pathway for guide RNA maturation. The many bacterial species containing this family of proteins all express tracrRNA[2], and so are highly likely to use this RNA, and RNase III, to mature their CRISPR RNAs.

We do not yet know whether this CRISPR variant is an evolutionary remnant, predating the acquisition of dedicated Cas proteins, or a more recent variation. Certainly the small number of Cas proteins necessary for the *S. pyogenes* system to work, coupled with the enticing similarity of the tracrRNA-dependent processing pathway to the eukaryotic Dicer pathway, favours this as an ancestral minimal system. Curiously, all of the species that harbour the genes in this pathway are associated with vertebrates, either as pathogens or commensals[7]. Perhaps this is a system that has evolved to deal not only with phage invaders, but also with invasion by eukaryotic RNAs (possibly microRNAs).

The recognition of CRISPR was a direct outcome of the sequencing of multiple microbial genomes. Now, deep sequencing[2] of the RNA complement of *S. pyogenes* has demonstrated the existence of a novel pathway for an antisense RNA in CRISPR maturation. We can expect many more roles for regulatory RNAs to emerge as this approach continues to be applied to eukaryotes, bacteria and archaea. ■

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CLARIFICATION

"Reproductive biology: Progesterone's gateway into sperm" by Steve Publicover and Christopher Barratt (*Nature* **471,** 313–314; 2011). The following sentence in this article — "The second, more recent, advance was the development of a method for applying to sperm the technique of whole-cell patch clamping, which records ionic currents across the entire plasma membrane of a cell" — should have been accompanied by a reference to P. V. Lishko, I. L. Botchkina, A. Fedorenko & Y. Kirichok *Cell* **140,** 327–337 (2010).