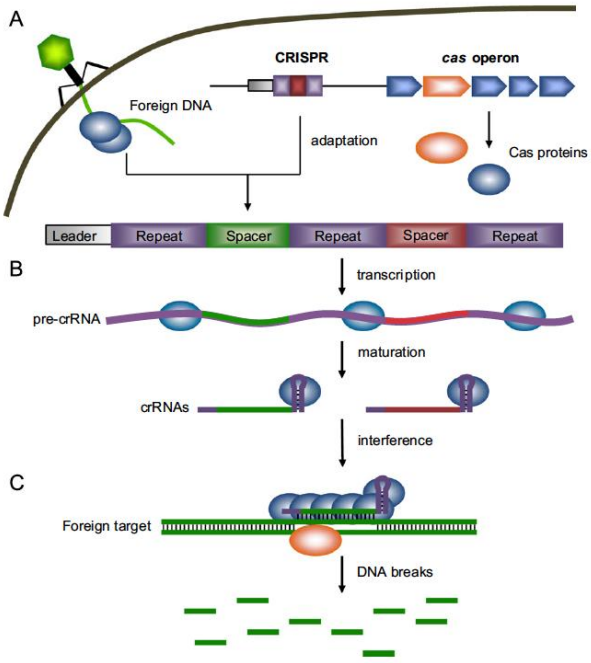
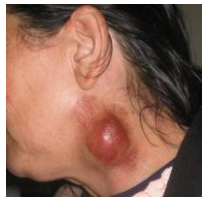
A CRISPR/Cas system mediates bacterial innate immune evasion and virulence

Sampson et al., Nature 479, page 254-257, 2013

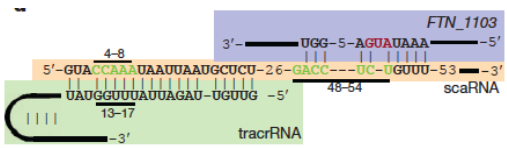


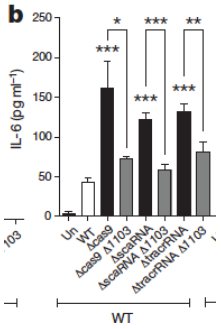
The CRISPR/Cas (clustered regularly interspaced short palindromic repeats/CRISPR-associated) system uses the genetic information of invading DNA, integrated in the genome, to fight against re-infection by the same DNA. The system was first proposed as adaptive immune system in 2006 (Makarova et al., 2006) and shown to protect against reinfection in 2007 (Barrangou et al., 2007). Foreign DNA is cleaved by Cas proteins and integrated as short spacers into a region containing remnant information of other abortive infections separated by repeat regions (adaptation stage). The repeat regions are transcribed and Cas proteins process this pre-crRNA (expression stage), which will guide endonucleases to cleave the infectious DNA (interference stage). Depending on the processing of the pre-crRNA and the cleavage mechanism of target DNA, three types of CRISPR systems can be defined. This system of defense can therefore be considered as an adaptive immune system against infectious agents in bacteria. Although the "classical" target of the CRISPR interference is foreign DNA, targeted degradation of RNA has also been reported (Hale et al., 2012). (Figure : Fineran & Charpentier, 2012).

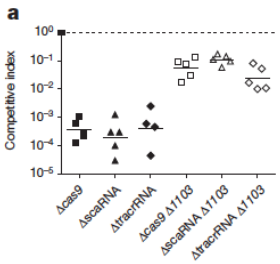
Francisella novicida is a intracellular pathogen closely related to the highly infective *Francisella tularensis* (and sometimes considered as a subspecies of *F.tularensis*). Tularemia was discovered in 1911 in California in the county Tular and the human disease first described in 1914 by E. Francis. Tularemia is a zoonotic disease (also called rabbit fever, see figure), acquired by the manipulation of infected rabbit meat or through scratches by infected cats or dogs, but can also transmitted by ticks or other biting insects, and acquired through aerosols. The symptoms can range, depending on the infection site, from ulceroglandular papuls (figure), pneumonia, oropharyngeal or gastrointestinal infection, to typhoidal fever. *Francisella* are Gram-negative, non-motile, obligate aerobe, bacteria that cause bacteremia associated with fever (Images: www.rayur.com, www.ncwildlife.org).

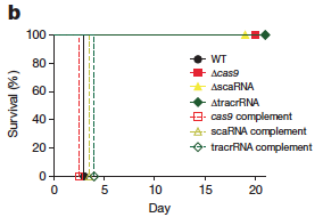


The success of infection by *Francisella* is in part due to its ability to dampen the innate immune response, as for example by reducing the TLR2 activation by lipoproteins. The authors reported previously that a particular gene of *F. novicida* is involved in the reduction of lipoprotein synthesis and by bioinformatic analysis this gene was found to be similar to known *cas9* genes and moreover is present in a locus with several *cas* genes. Deletion of the *cas9* homologue, but not other *cas* genes or the crRNA locus, resulted in increased expression of the lipoprotein gene *FTN_1103*. Whereas the deletion of the crRNA locus did not affect the expression, deletion of tracrRNA, involved in crRNA maturation, or scaRNA (small CRISPR associated RNA), did increase the expression of the lipoprotein. Analysis of *FTN-1103* mRNA turnover showed that the deletion of *cas9* increases the stability of the FTN_1103 mRNA and that an arginine rich motif (ARM) in Cas9, but intriguingly not the previously defined nuclease motifs, were necessary for this activity. Immunoprecipitation of Cas9 enriched the tracrRNA, the scaRNA and the *FTN_1103* mRNA, but not in the ARM mutant. Bioinformatic analysis and mutational analysis with single and complementary substitutions, indicate an imperfect alignment of the tracrRNA with the scaRNA, and the scaRNA with the target RNA (right figure). Consistent with a *cas*-dependent dampening of the TLR2 response, the tracrRNA and scaRNA as well as cas9 were induced 1h after infection, whereas the expression of the lipoprotein diminished. The analysis of TLR2 mediated IL-6 secretion in membrane fractions or macrophage infection showed a TLR2 dependent increase in the *cas9* mutant but could abrogated in a *cas9 FTN_1103* double mutant (left figure). In accordance, the *cas9* and scaRNA and tracrRNA mutants were highly attenuated in a mice competition assay (Figure a, below) and mice survived upon infection (Figure b, below). Interestingly, the infection by the mutant strains rendered the mice immune to subsequent challenge by the wild type strain. Finally, the authors report preliminary data on the reduction of virulence (adhesion, invasion, and replication) of *Neisseria meningitidis* of a *cas9* mutant strain.









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