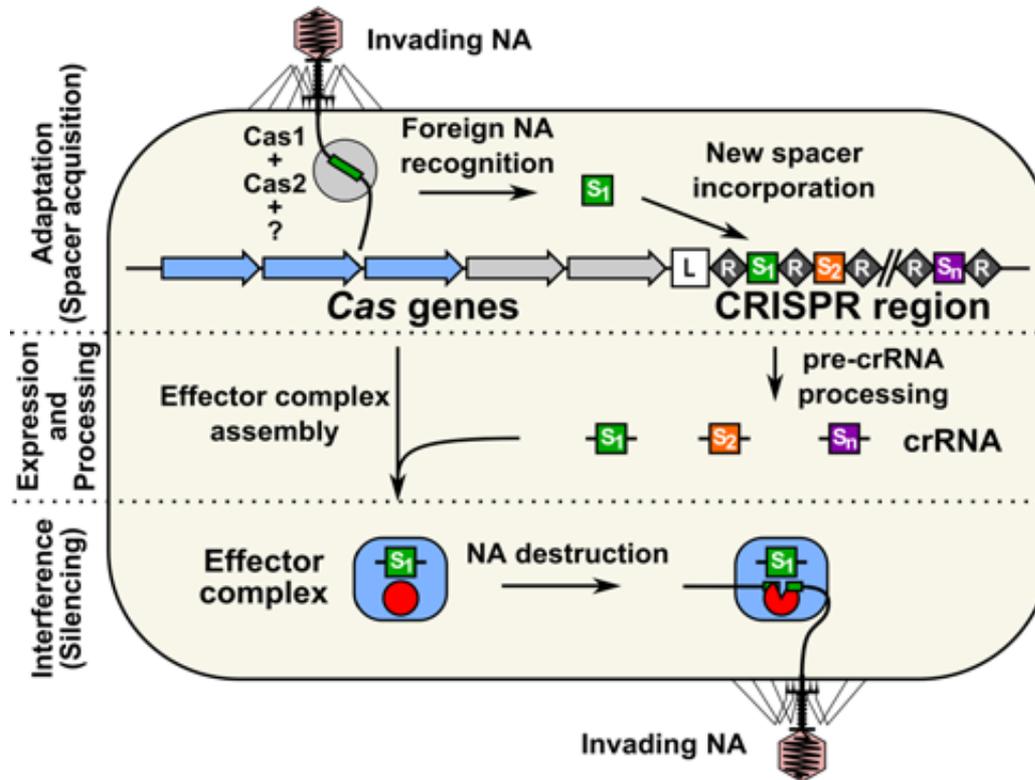


The CRISPR/Cas system is a bacterial immune mechanism vs “viruses”



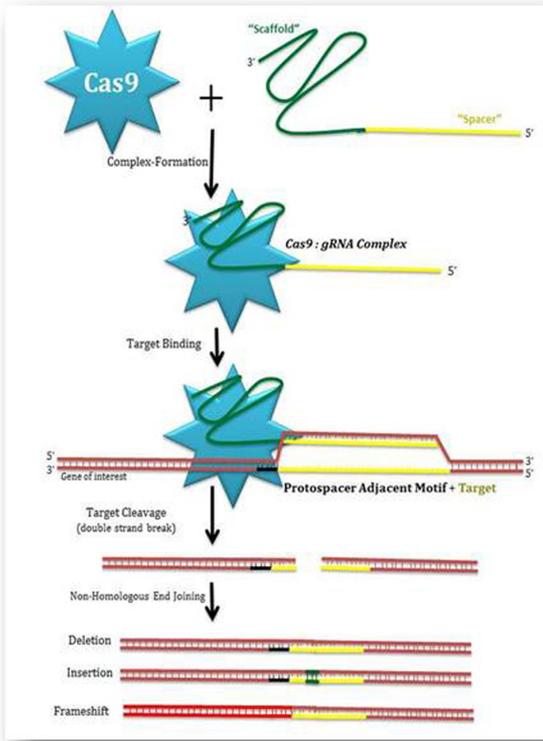
CRISPR = Clustered Regularly Interspaced Short Palindromic Repeats

CAS = CRISPR associated genes (nucleases and DNA binding proteins)

These regions of the bacterial genome are the “memory” of an immune system that recognizes and destroys invading DNA from a bacteriophage/virus. The “spacers” are pieces of phage DNA that direct a nuclease (CAS protein) to invading DNA sequences.

CRISPR-Cas9 can cause deletions/insertions to knock out a gene (A)
 OR
 can introduce a new sequence to replace or mutate a gene (B)

A



B

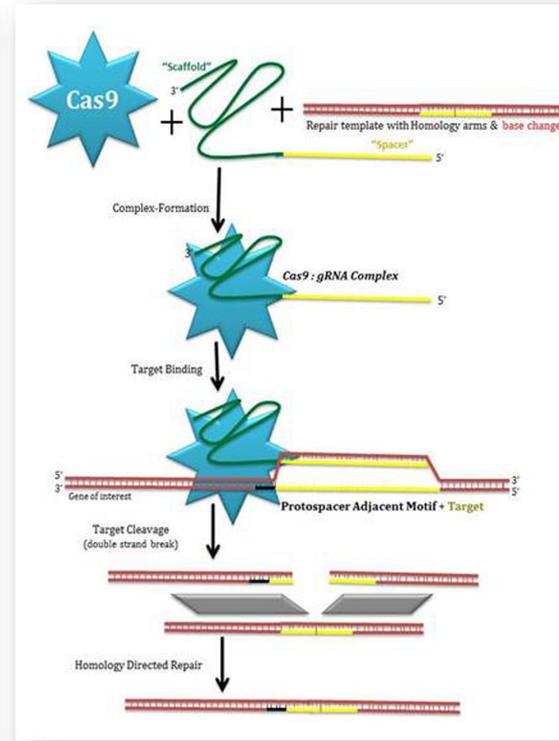
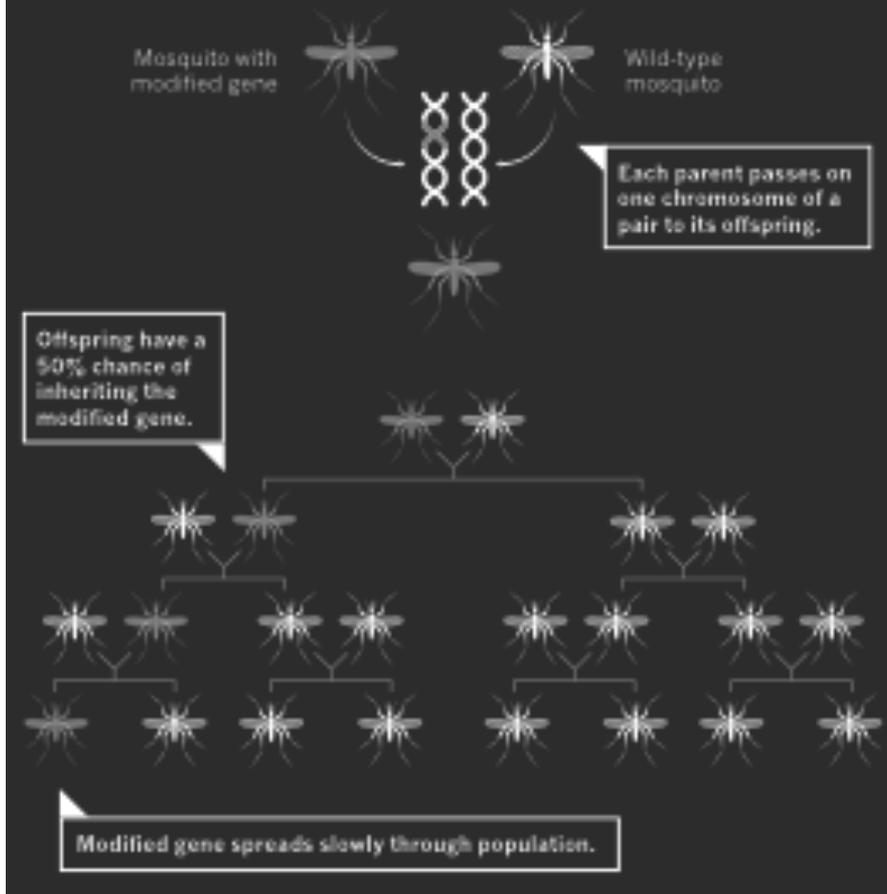


FIGURE 2 | How CRISPR-Cas9 perform genome editing. Cas9 induce double stranded breaks (DSBs) at particular site. The resulting DSB is then repaired by one of these two general repair pathways, e.g., by Non-homologous end joining (NHEJ) or by Homology directed repair (HDR). **(A)** The NHEJ repair pathway frequently results in small nucleotide insertions or deletions (InDels) at the DSB site. This may result in gene knock out or gene insertion. **(B)** HDR can be used to generate precise nucleotide modifications (also called gene “edits”) ranging from a single nucleotide change to large insertions.

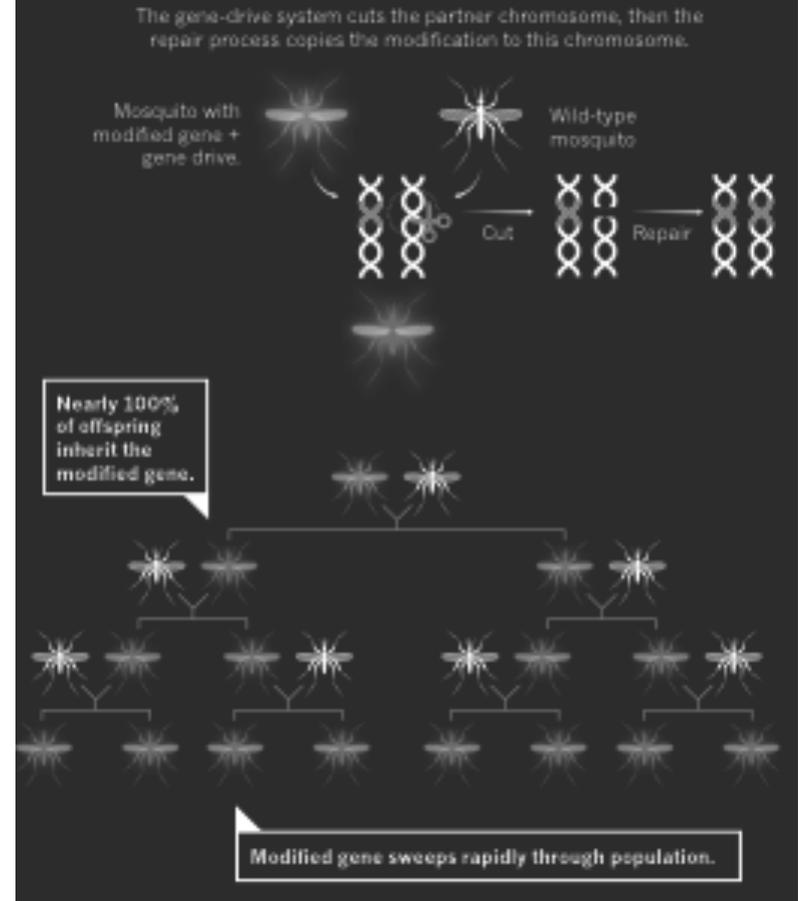
GENE DRIVE

CRISPR gene editing can be used to propagate a genetic modification rapidly through generations. It might be used to eradicate a population of disease-carrying mosquitoes.

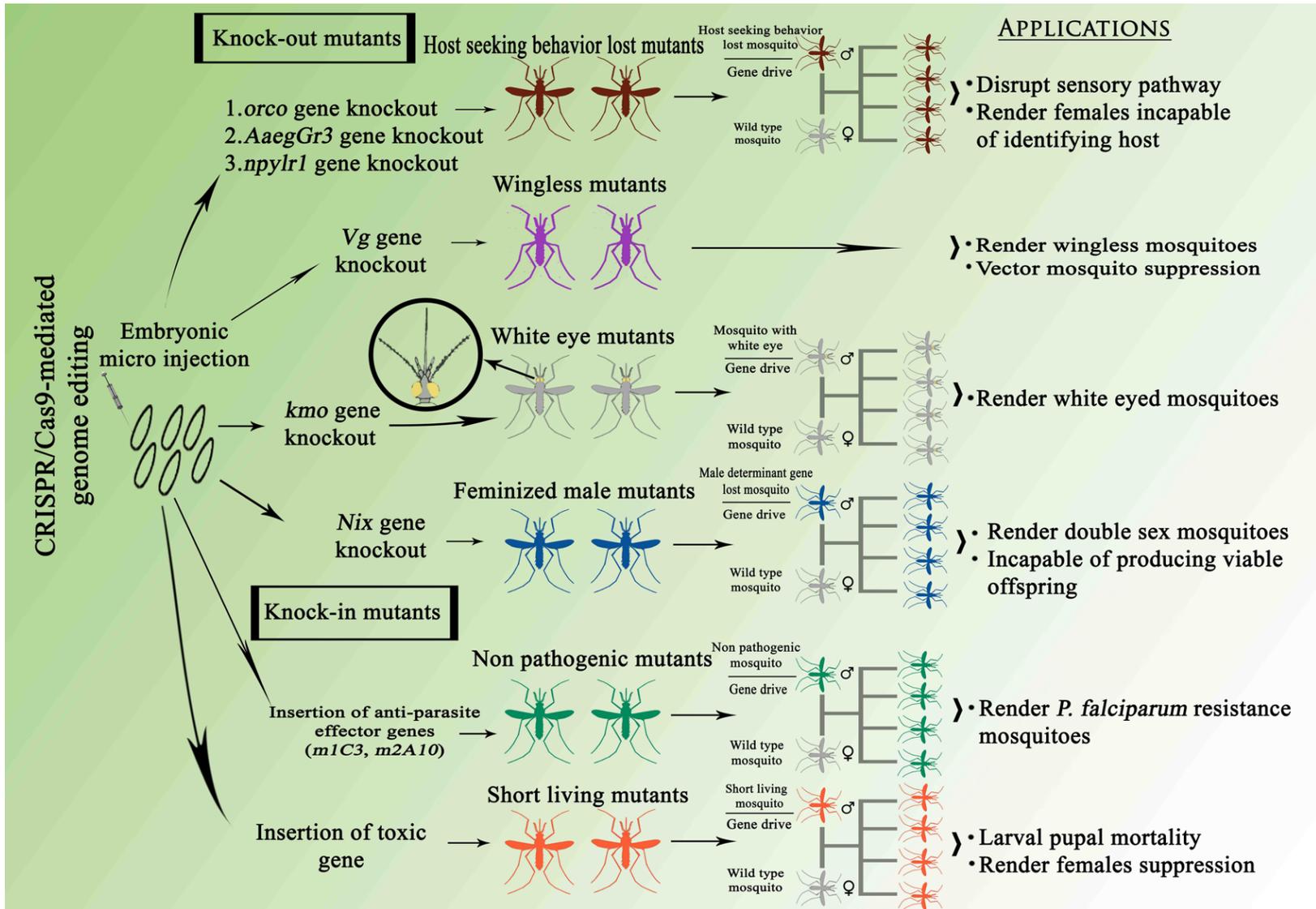
STANDARD INHERITANCE



GENE-DRIVE INHERITANCE



Using CRISPRcas9/Gene Drive to wipe out malaria

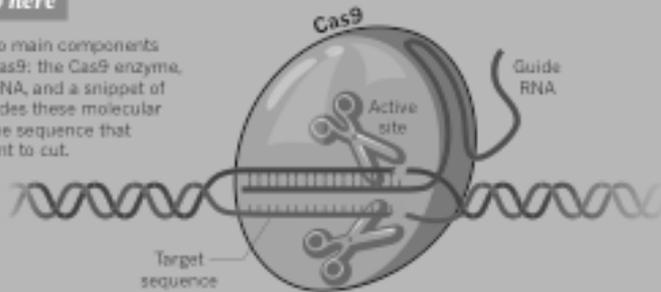


New modifications of CRISPRcas9 broaden its uses in genetic regulation

By modifying the molecular machinery that powers CRISPR-Cas9 gene editing, scientists can probe the functions of genes and gene regulators with unprecedented specificity.

Snip snip here

There are two main components of CRISPR-Cas9: the Cas9 enzyme, which cuts DNA, and a snippet of RNA that guides these molecular scissors to the sequence that scientists want to cut.



Broken scissors

The Cas9 enzyme can be broken so that it no longer cuts DNA. But with the right guide RNA, it can still attach to specific parts of the genome.

CRISPR inhibition

A broken, or 'dead', Cas9 enzyme will block the binding of other proteins, such as RNA polymerase, needed to express a gene.



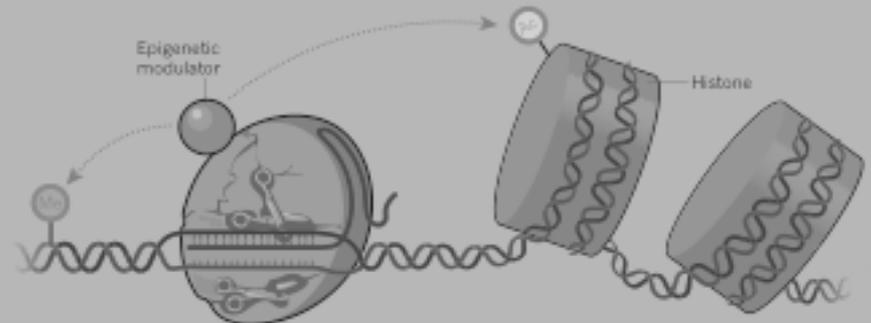
CRISPR activation

An activating protein can be attached to a dead Cas9 protein to stimulate expression of a specific gene.



CRISPR epigenetics

A broken Cas9 enzyme can be coupled to epigenetic modifiers, such as those that add methyl groups (Me) to DNA or acetyl groups (Ac) to histone proteins. This will allow researchers to study how precisely placed modifications affect gene expression and DNA dynamics.



Inducible CRISPR

Cas9 — either dead or alive — can be coupled to switches so that it can be controlled by certain chemicals or, as shown below, by light.

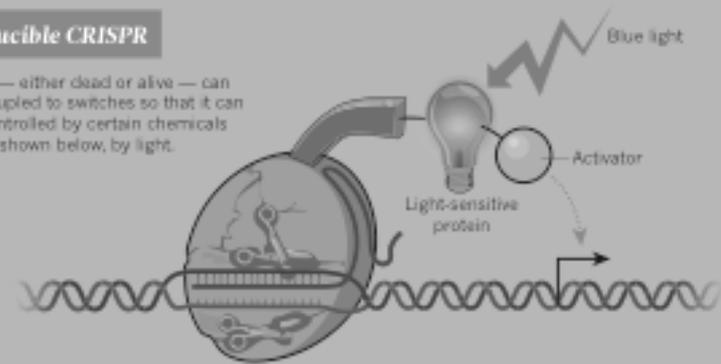
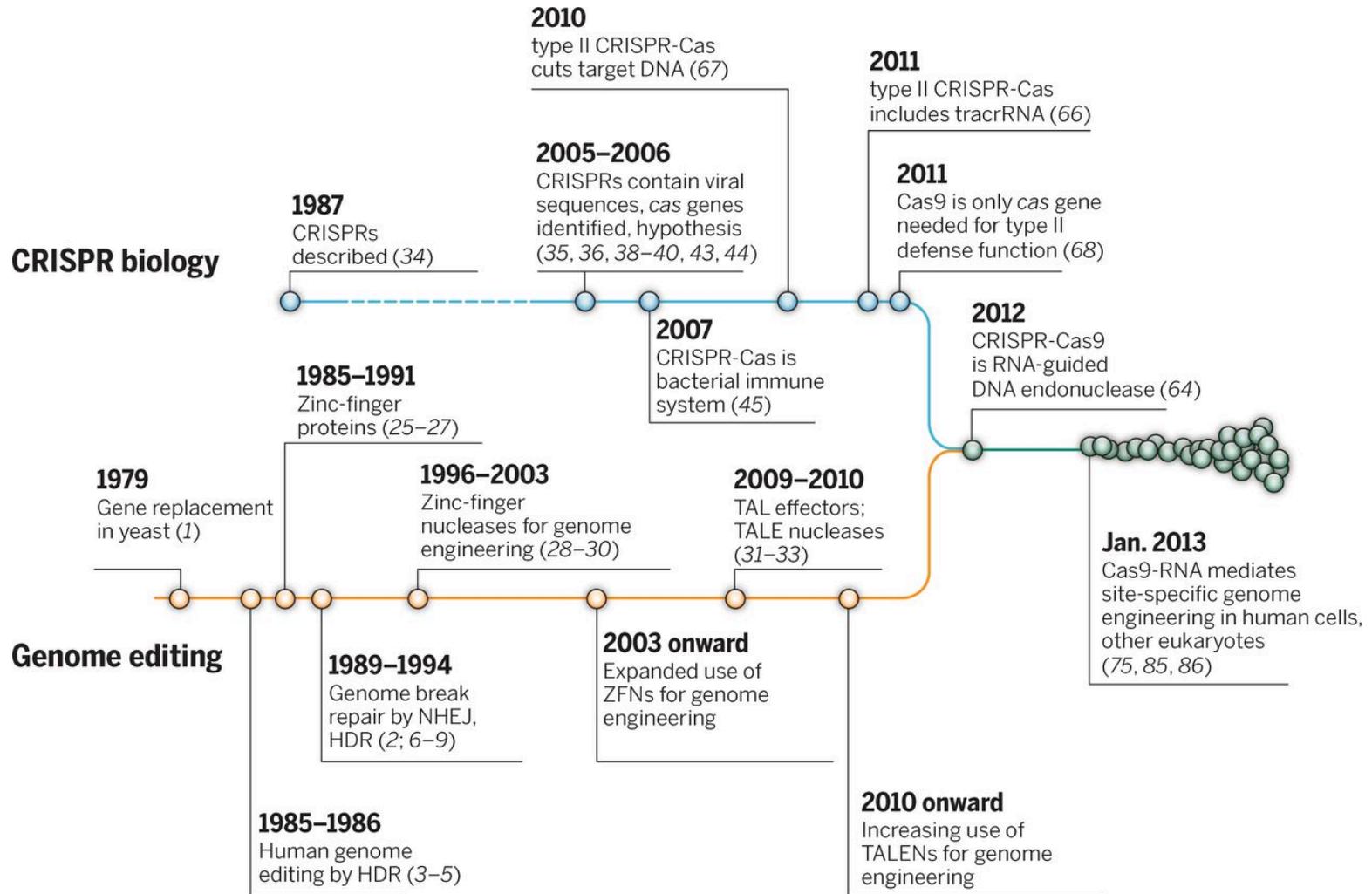


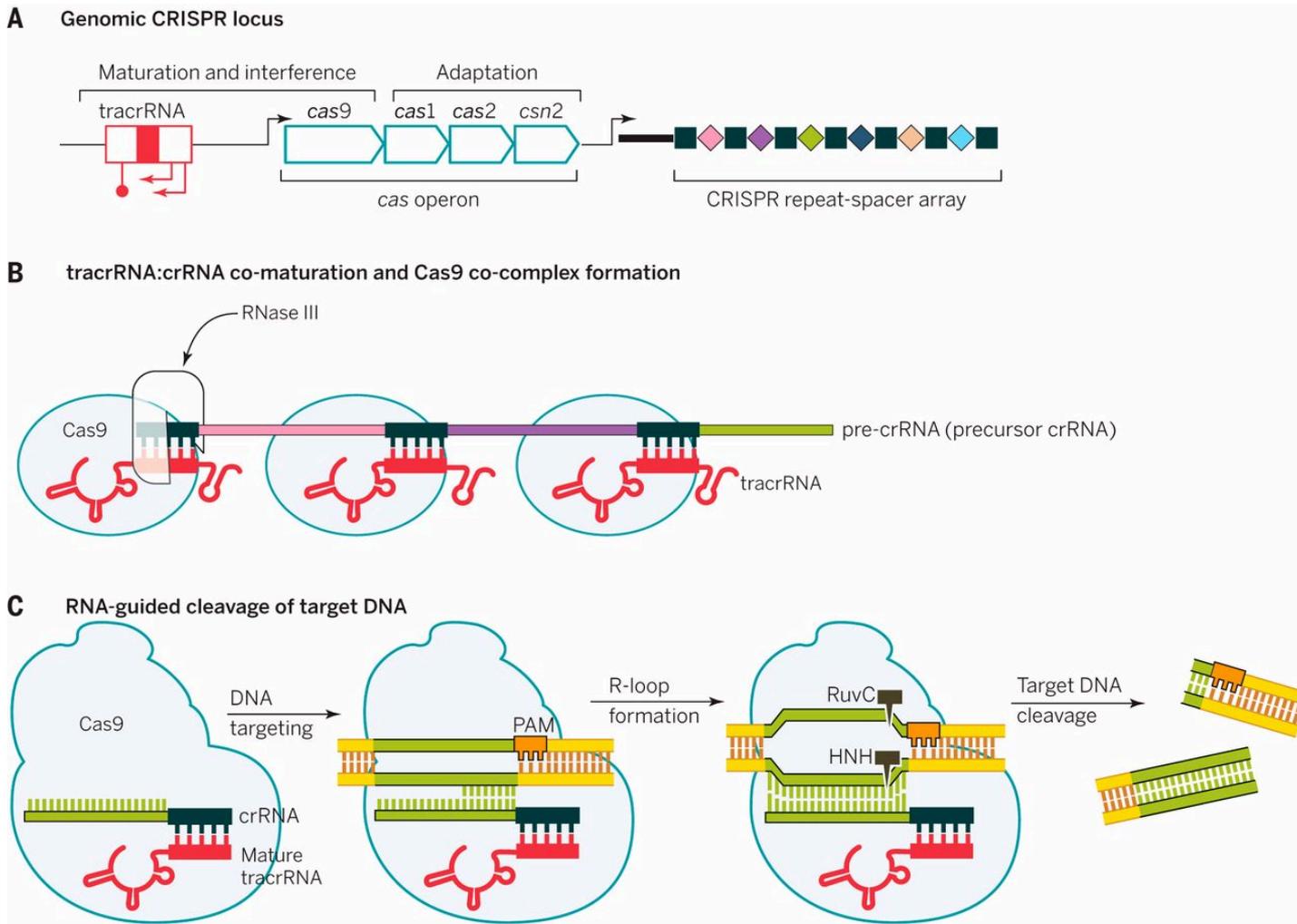
Fig. 1 Timeline of CRISPR-Cas and genome engineering research fields. Key developments in both fields are shown.



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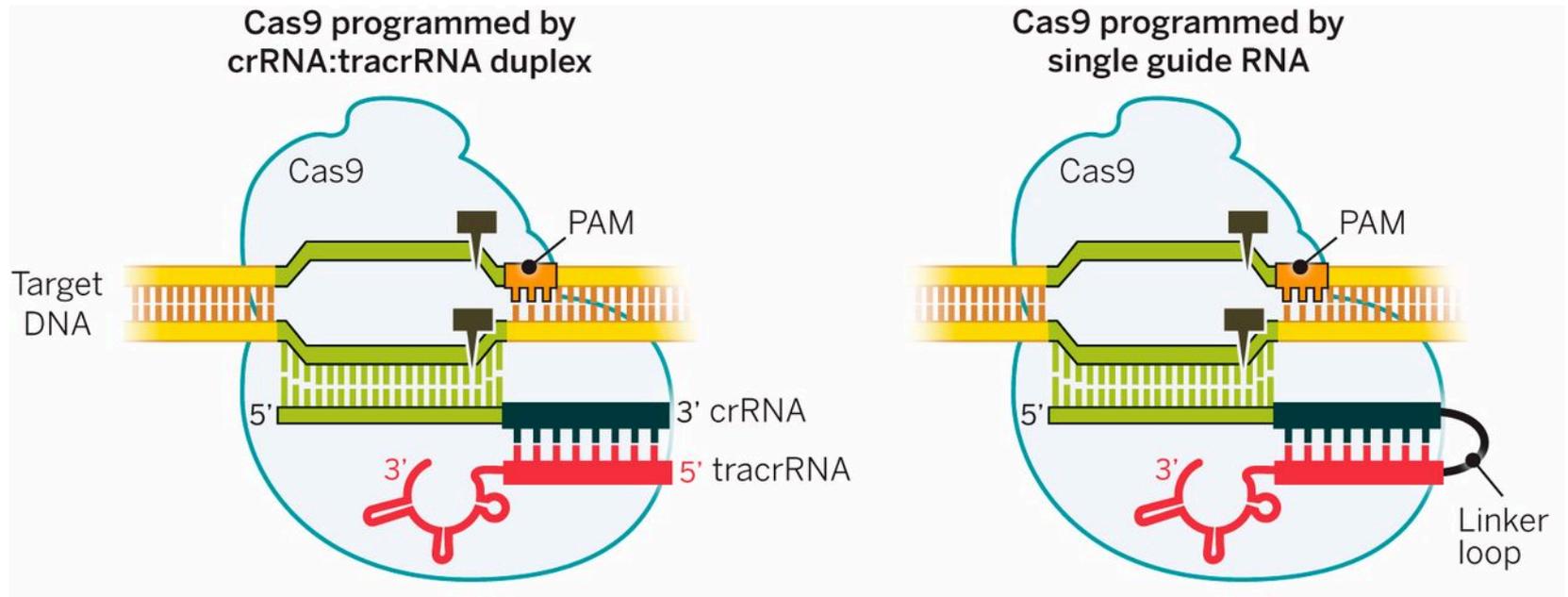
Fig. 2 Biology of the type II-A CRISPR-Cas system. The type II-A system from *S. pyogenes* is shown as an example.



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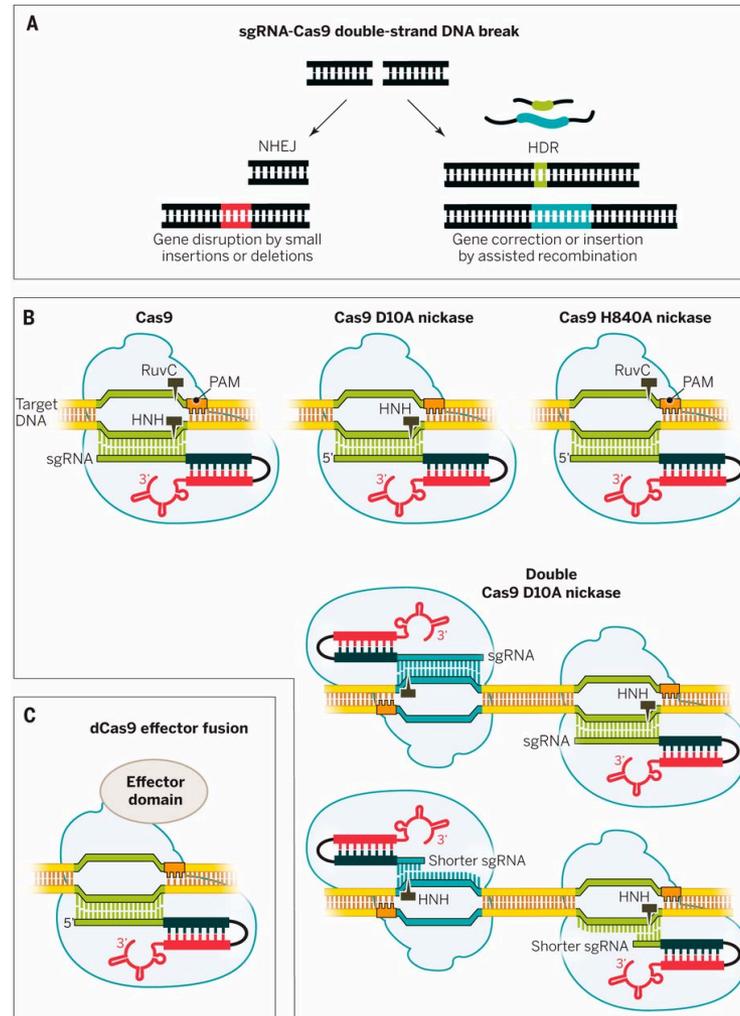
Fig. 3 Evolution and structure of Cas9. The structure of *S. pyogenes* Cas9 in the unliganded and RNA-DNA-bound forms [from (77, 81)].



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Fig. 4 CRISPR-Cas9 as a genome engineering tool.(A) Different strategies for introducing blunt double-stranded DNA breaks into genomic loci, which become substrates for endogenous cellular DNA repair machinery that catalyze nonhomologous end joining (NHEJ) or homology-directed repair (HDR).



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