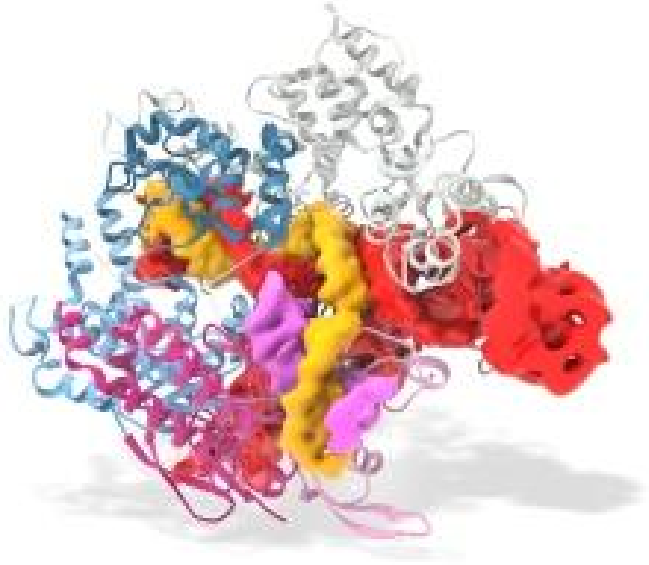
UBBMP Bioquímica y Biología Molecular de Plantas

The Red Queen takes to dance CRISPRs (Clustered Regularly Interspaced Short Palindromic Repeats)

(Repeticiones palindrómicas pequeñas interespaciadas agrupadas regularmente)



Víctor M. Loyola-Vargas

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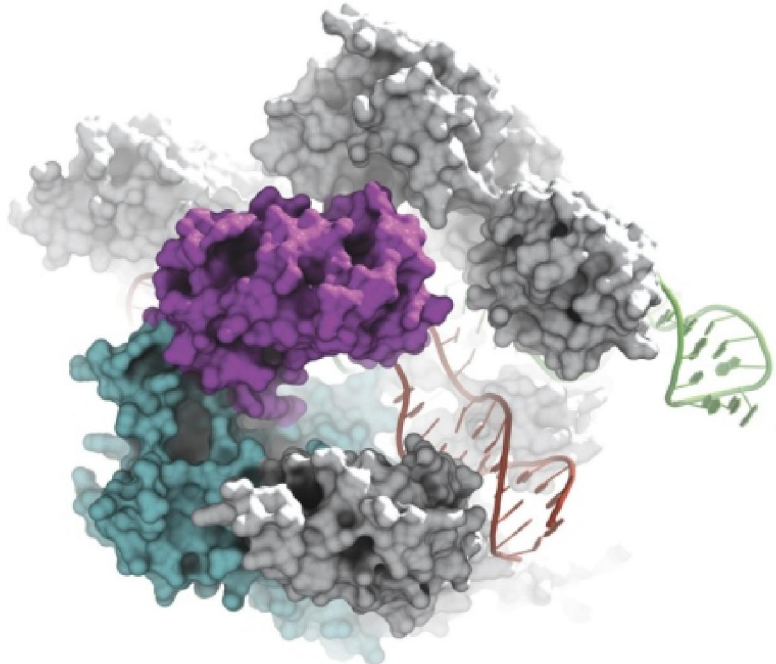
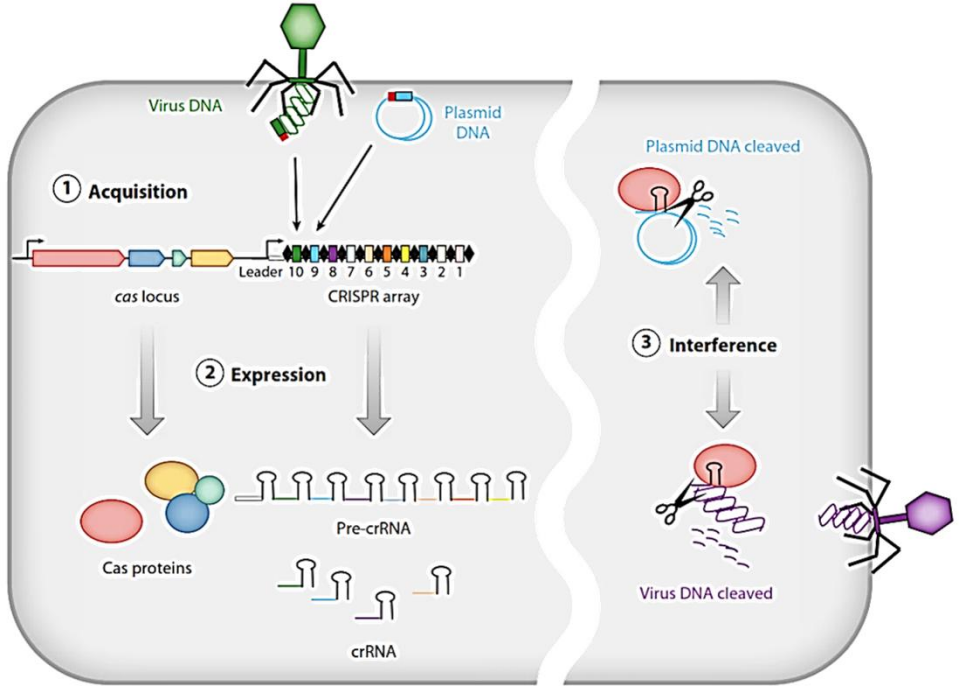


Outline

- Introduction.
- History.
- The mechanism.
- CRISPR and genome modifications.
- Applications in animals.
- Applications in plants.

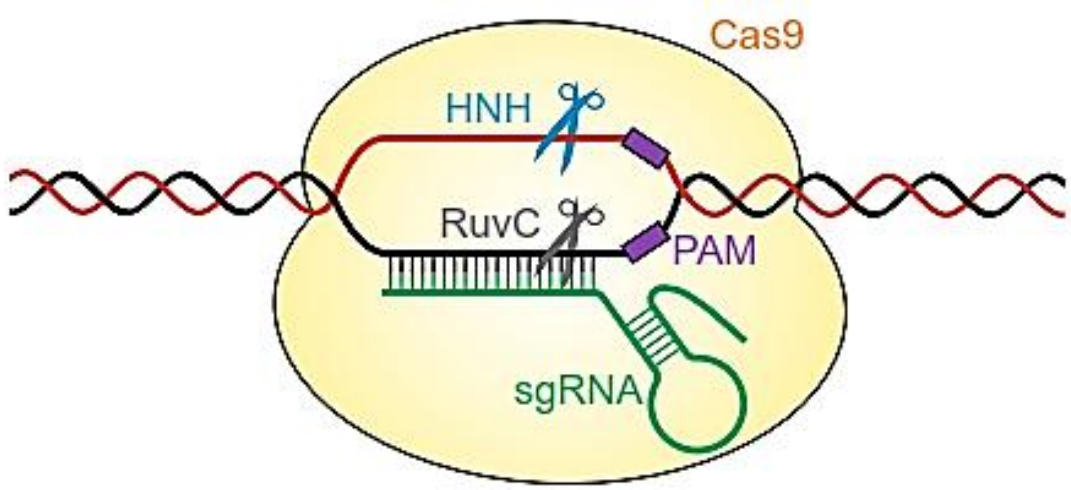


Introduction

The arms race between host and their perpetually evolving predators has fueled the evolution of a defense arsenal.

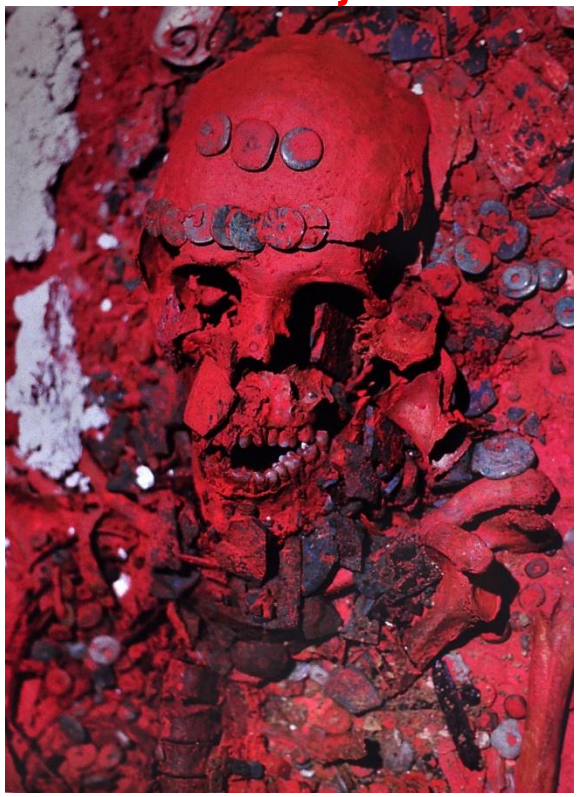
CRISPR: are adaptive immune defense systems found in bacteria and archaea.

The study of natural DNA repair pathways in bacteria and yeast, as well as the mechanisms of DNA recombination, revealed that cells have endogenous machinery to repair double-strand DNA breaks (DSBs) that would otherwise be lethal.



The Red Queen

Tz'akbu Ajaw



Palenque, 672 a.C.; Temple XIII. Wife of Pakal II.

Fanny López Jiménez (1994).
Arnoldo González Cruz

Supercomputer and Head of the Umbrella Corporation. Resident evil.

Helena Bonham Carter

http://www.dailymail.co.uk/tvshowbiz/article-3542632/Pink-releases-Just-Like-Fire-upcoming-Alice-Looking-Glass.html

The Reed Queen

Through the Looking-Glass, and What Alice Found There. Chapter IX.
Charles Lutwidge Dodgson (Lewis Carroll, 1832-1898).

A NEW EVOLUTIONARY LAW

Evol. Theory 1: 1-30, (1973).

Leigh Van Valen
Department of Biology
The University of Chicago
Chicago, Illinois 60637

lini-review J. evol. Biol. 4: 1–7 (1991)

'esting the Red Queen Hypothesis

ntoni Hoffman

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In 1973 Leigh Van Valen observed that the vast majority of approximately 50 organic groups he analyzed – protists, plants, and animals, both extinct and extant – displayed loglinear taxonomic survivorship curves. This result implied, for each organic group, constancy in the probability of extinction of its constituent taxa, regardless of their previous duration. To explain this unexpected phenomenon, termed the Law of Constant Extinction, Van Valen (1973) proposed the Red Queen Hypothesis: "... the effective environment of any homogeneous group of organisms deteriorates at a stochastically constant rate", where the 'effective environment' comprises primarily the biota; and consequently, "The Red Queen does not need changes in the physical environment, although she can accommodate them.

The Reed Queen dynamics

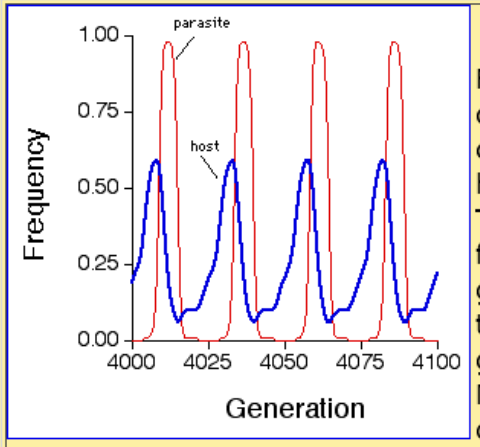


Figure 1. Red Queen dynamics: results from a computer simulation for host-parasite coevolution. The blue line gives the frequency of one host genotype; the red line gives the frequency of the parasite genotype that can infect it. Note that both genotypes oscillate over time, as if they were "running" in circles. The model assumes that hosts have self-nonself recognition systems, which can detect foreign organisms. The model also assumes that hosts and parasites both reproduce sexually.

Through the Looking Glass (chapter 2; Carroll 1872).

Alice decides that it would be easier to see the garden if she first climbs the hill, to which there appears to be a very straight path. However, as she follows the path, she finds that it leads her back to the house. When she tries to speed up, she not only returns to the house, she crashes into it. Hence, forward movement takes Alice back to her starting point (Red Queen dynamics), and rapid movement causes abrupt stops (extinction).

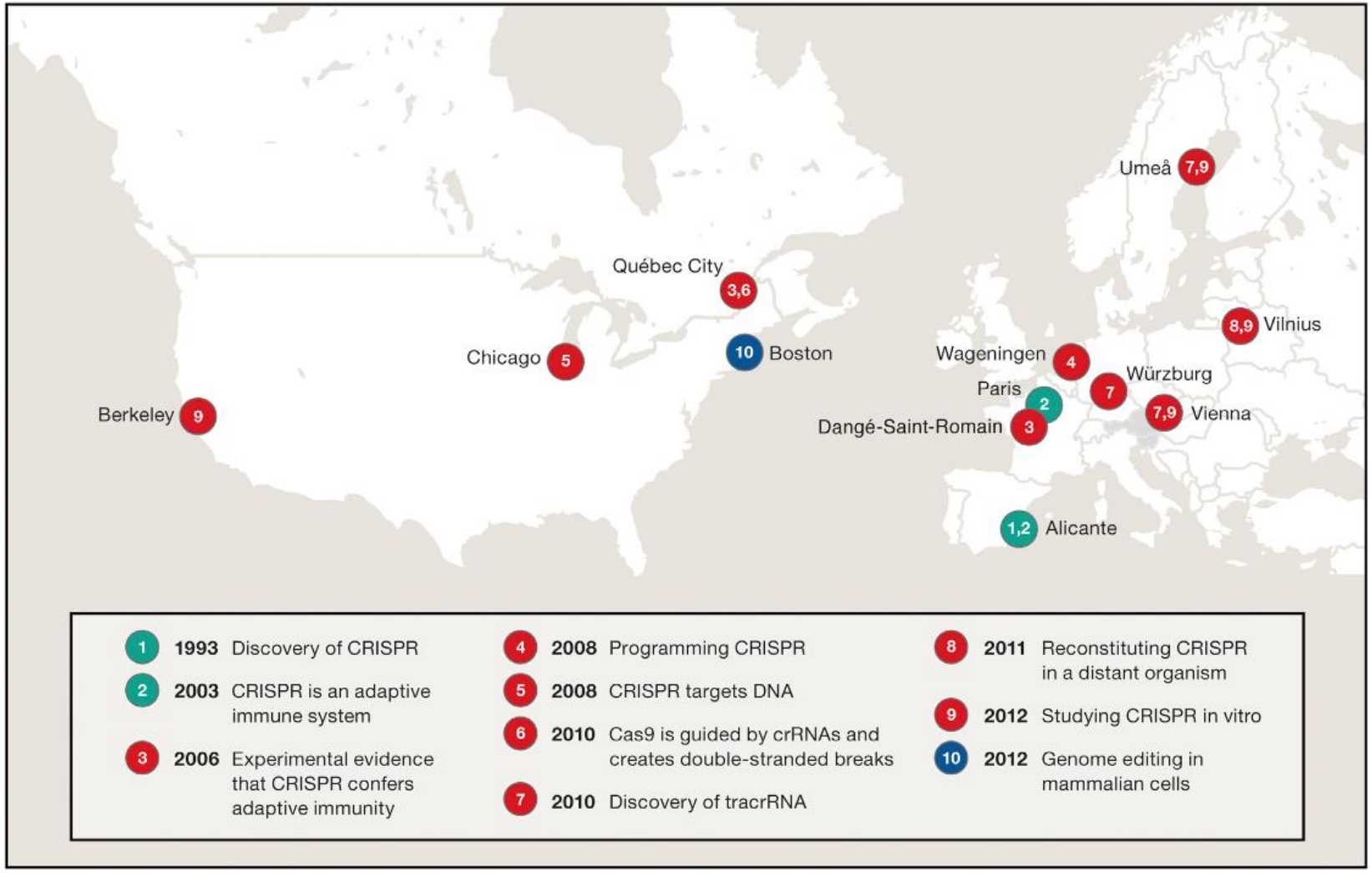
….When Alice spots the Red Queen, she begins moving toward her. But, the Red Queen quickly disappears from sight. Alice decides to follow the advice of the rose, and go the other way ("I should advise you to walk the other way"). Immediately she comes face-to-face with the Red Queen.

The Red Queen then leads Alice directly to the top of the hill. Along the way, the Red Queen explains that hills can become valleys, which confuses Alice. Already, in this world, straight can become curvy, and progress can be made only by going the opposite direction; now, according to the Red Queen, hills can become valleys and valleys can become hills.

At the top of the hill, the Red Queen begins to run, faster and faster. Alice runs after the Red Queen, but is further perplexed to find that neither one seems to be moving. When they stop running, they are in exactly the same place. Alice remarks on this, to which the Red Queen responds: "Now, here, you see, it takes all the running you can do to keep in the same place".

And so it may be with coevolution. Evolutionary change may be required to stay in the same place. Cessation of change may result in extinction.

The twenty-year story of CRISPR unfolded across twelve cities in nine countries



Erik S. Lander, The Heroes of CRISPR, Cell, 164(1-2): 18-28, (2016).

Broad Institute, MIT and Harvard

Table 1.1 Chronology of seminal developments in the CRISPR field

Years	Contribution	Reference
1987	Discovery of the *iap*-associated repeats of *E. coli*	Ishino et al.
1991	Discovery of DRs in mycobacteria	Hermans et al.
1993	Discovery of TREPs in haloarchaea	Mojica et al.
1993	Evidence for TREPs transcription	Mojica et al.
1993	Development of the first typing method based on the repeats	Groenen et al.
1995	Evidence of TREPs activity	Mojica et al.
1998	Repeat array found in an archaeal conjugative plasmid	She et al.
2000	Recognition and description of the SRSR family of repeats	Mojica et al.
2002	Renaming of repeats as CRISPR	Jansen et al.
2002	Identification of core *cas* genes	Jansen et al.
2002	Characterization of CRISPR transcripts and of regular processing within repeats	Tang et al.
2002	Identification of RAMP proteins carrying RNA recognition motifs	Makarova et al.
2003	First experimental identification of a protein interacting with CRISPR DNA repeats	Peng et al.

Mojica F. J. M. y R. A. Garrett, Discovery and seminal developments in the CRISPR field, en: CRISPR-Cas Systems: RNA-mediated Adaptive Immunity in Bacteria and Archaea, (Barrangou R. y J. van der Oost, eds.), Springer, Berlin, Heidelberg, 1-31, (2013).

2005	Unveiling the origin of spacers and a proposal of a universal defence function for CRISPR	Mojica et al. Pourcel et al.
2005	Identification of a conserved PAM motif associated with protospacers	Bolotin et al.
2005	Classification of 45 Cas protein families	Haft et al.
2006	Identification of small spacer-containing crRNAs	Lillestøl et al.
2006	Demonstration that putative protospacers can be located within genes, intergenically, and on either DNA strand	Lillestøl et al.
2006	Characterisation of antisense crRNAs	Lillestøl et al.
2006	Evidence for horizontal transfer of CRISPR systems	Godde et al.
2007	Repeats-based classification of CRISPR-Cas systems	Kunin et al.
2007	First experimental demonstration of CRISPR interference	Barrangou et al.
2007	First experimental demonstration of acquisition of new spacers, leading to CRISPR adaptation	Barrangou et al.
2008	Demonstration of the rapidly changing spacer contents of CRISPR arrays in environmental biofilms	Tyson et al. Andersson et al.
2008	Direct experimental evidence for DNA targeting by a type III-A system	Marraffini et al.
2008	Demonstration of CRISPR interference against plasmids	Marraffini et al.
2008	Identification of a ribonucleoprotein complex (Cascade) responsible for processing of pre-crRNA to crRNA	Brouns et al.
2008	Characterisation of the mature crRNAs of a type I system and proof of their role to guide Cascade to the target sequence, after which Cas3 is recruited to the trigger interference	Brouns et al.

Mojica F. J. M. y R. A. Garrett, Discovery and seminal developments in the CRISPR field, en: CRISPR-Cas Systems: RNA-mediated Adaptive Immunity in Bacteria and Archaea, (Barrangou R. y J. van der Oost, eds.), Springer, Berlin, Heidelberg, 1-31, (2013).

Table 1.1 (continued)

Years	Contribution	Reference
2008	Experimental evidence in support of DNA targeting by a type I system	Brouns et al.
2009	In vitro targeting of RNA by a type III-B system	Hale et al.
2010	In vivo DNA targeting demonstrated for a type II system	Garneau et al.
2010	Unveiling the mechanism of self versus non-self discrimination during interference by a type III-A system	Marraffini et al.
2010	Development of the auto-immune CRISPR concept	Stern et al.
2010	Crystal structure of a complex of Cas6 with a hairpin structured pre-crRNA	Haurwitz et al.
2011	Architecture of type I targeting complexes	Jore et al. Wiedenheft et al.
2011	CryoEM structure determination of type I targeting complex (Cascade)	Jore et al. Wiedenheft et al.
2011	Characterisation of the type II tracrRNA-based processing mechanism	Delcheva et al.
2011	Defining limits of type I DNA targeting specificity	Gudbergsdottir et al. Semenova et al.
2011	Crystal structure of a complex of Cas6 and single stranded pre-crRNA	Wang et al.
2011	Reclassification of Cas proteins and CRISPR systems	Makarova et al.

Mojica F. J. M. y R. A. Garrett, Discovery and seminal developments in the CRISPR field, en: CRISPR-Cas Systems: RNA-mediated Adaptive Immunity in Bacteria and Archaea, (Barrangou R. y J. van der Oost, eds.), Springer, Berlin, Heidelberg, 1-31, (2013).

2012	In vivo cleavage of antisense crRNAs by a type III-B system	Hale et al.
2012	Demonstration of an alternative type III-B interference mechanism	Zhang et al.
2012	EM structural determination of a type III-B interference complex	Zhang et al.
2012	CasA (Cse1) of type I-E system interacts with PAM and is required for target recognition and binding	Sashital et al.
2012	Concerted action of a type I-E interference complex and Cas3	Westra et al.
2012	Induced acquisition in type I-E system by overexpressing Cas1 and Cas2	Yosef et al.
2012	Evidence for a positive feedback between active CRISPR spacers and new spacer uptake in a type I-E system	Swarts et al.
2012	Evidence that prior recognition of protospacers by specific crRNAs stimulates acquisition in a type I-E system	Datsenko et al.
2012	Evidence for a ruler mechanism operating during protospacer excision in a type I-A system	Erdmann and Garrett
2012	Evidence for spacer acquisition throughout a CRISPR array by an alternative mechanism in type I-A system	Erdmann and Garrett

Listed are some of the seminal developments in the CRISPR field. It is not complete and many developments, including, for example, the characterisation of individual Cas proteins are not included for space reasons.


History

The initial three works

JOURNAL OF BACTERIOLOGY, Dec. 1987, p. 5429–5433
0021-9193/87/125429-05$02.00/0

Vol. 169, No. 12

Nucleotide Sequence of the *iap* Gene, Responsible for Alkaline Phosphatase Isozyme Conversion in *Escherichia coli*, and Identification of the Gene Product

YOSHIZUMI ISHINO, HIDEO SHINAGAWA, KOZO MAKINO, MITSUKO AMEMURA, AND ATSUO NAKATA*

Department of Experimental Chemotherapy, The Research Institute for Microbial Diseases, Osaka University, 3-1 Yamadaoka, Suita, Osaka 565, Japan

Received 1 May 1987/Accepted 22 August 1987

VIROLOGY 157, 156–166 (1987)

Two DNA Antirestriction Systems of Bacteriophage P1, *darA*, and *darB*: Characterization of *darA*⁻ Phages

SHIGERU IIDA,[1] MARKUS B. STREIFF,[2] THOMAS A. BICKLE, AND WERNER ARBER

Microbiology Department, Biozentrum, University of Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland

Received September 9, 1986; accepted October 27, 1986

Bacteriophage P1 is only weakly restricted when it infects cells carrying type I restriction and modification systems even though DNA purified from P1 phage particles is a good substrate for type I restriction enzymes *in vitro*. Here we show that this protection against restriction is due to the products of two phage genes which we call *darA* and *darB* (*dar* for *d*efense *a*gainst *r*estriction). Each of the *dar* gene products provides protection against a different subset of type I restriction systems. The *darA* and *darB* gene products are found in the phage head and protect any DNA packaged into a phage head, including transduced chromosomal markers, from restriction. The proteins must, therefore, be injected into recipient cells along with the DNA. The proteins act strictly in *cis*. For example, upon double infection of restricting cells with *dar*⁺ and *dar*⁻ P1 phages, the *dar*⁺ genomes are protected from restriction while the *dar*⁻ genomes are efficiently restricted.

Molecular Microbiology (1995) 17(1), 85–93

Long stretches of short tandem repeats are present in the largest replicons of the Archaea *Haloferax mediterranei* and *Haloferax volcanii* and could be involved in replicon partitioning

F. J. M. Mojica, C. Ferrer, G. Juez and F. Rodríguez-Valera*

Departamento de Genética y Microbiología, Universidad de Alicante, Campus de San Juan, Apartado 374, 03080 Alicante, Spain.

Dr. Francisco J. Martínez Mojica

http://web.ua.es/es/actualidad-universitaria/2016/enero16/7-17/cientificos-internacionales-avalan-al-investigador-de-la-universidad-de-alicante-francisco-mojica-como-descubridor-del-sistema-crispr.html

Proposal of the CRISPR acronym

Asunto: **Re: Acronym**
Fecha: Wed, 21 Nov 2001 16:39:06 +0100
De: "Ruud Jansen" <R.Jansen@vet.uu.nl>
Empresa: Diergeneeskunde
A: "Francisco J. Martínez Mojica" <fmojica@ua.es>

Dear Francis

What a great acronym is CRISPR.
I feel that every letter that was removed in the alternatives made it less crispy so I prefer the snappy CRISPR over SRSR and SPIDR.
Also not unimportant is the fact that in MedLine CRISPR is a unique entry, which is not true for some of the other shorter acronyms.

Mojica F. J. M. y R. A. Garrett, Discovery and seminal developments in the CRISPR field, en: CRISPR-Cas Systems: RNA-mediated Adaptive Immunity in Bacteria and Archaea, (Barrangou R. y J. van der Oost, eds.), Springer, Berlin, Heidelberg, 1-31, (2013).

JOURNAL OF MOLECULAR EVOLUTION

J Mol Evol (2005) 60:174–182
DOI: 10.1007/s00239-004-0046-3

Received: 6 February 2004 / Accepted: 1 October 2004

Nature
PNAS
Microbiology
Nucleic Acid Research

Intervening Sequences of Regularly Spaced Prokaryotic Repeats Derive from Foreign Genetic Elements

Francisco J.M. Mojica, César Díez-Villaseñor, Jesús García-Martínez, Elena Soria

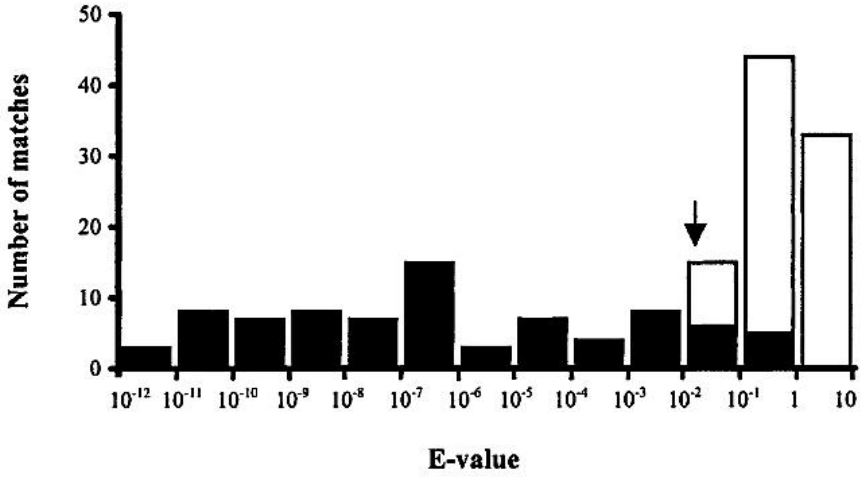
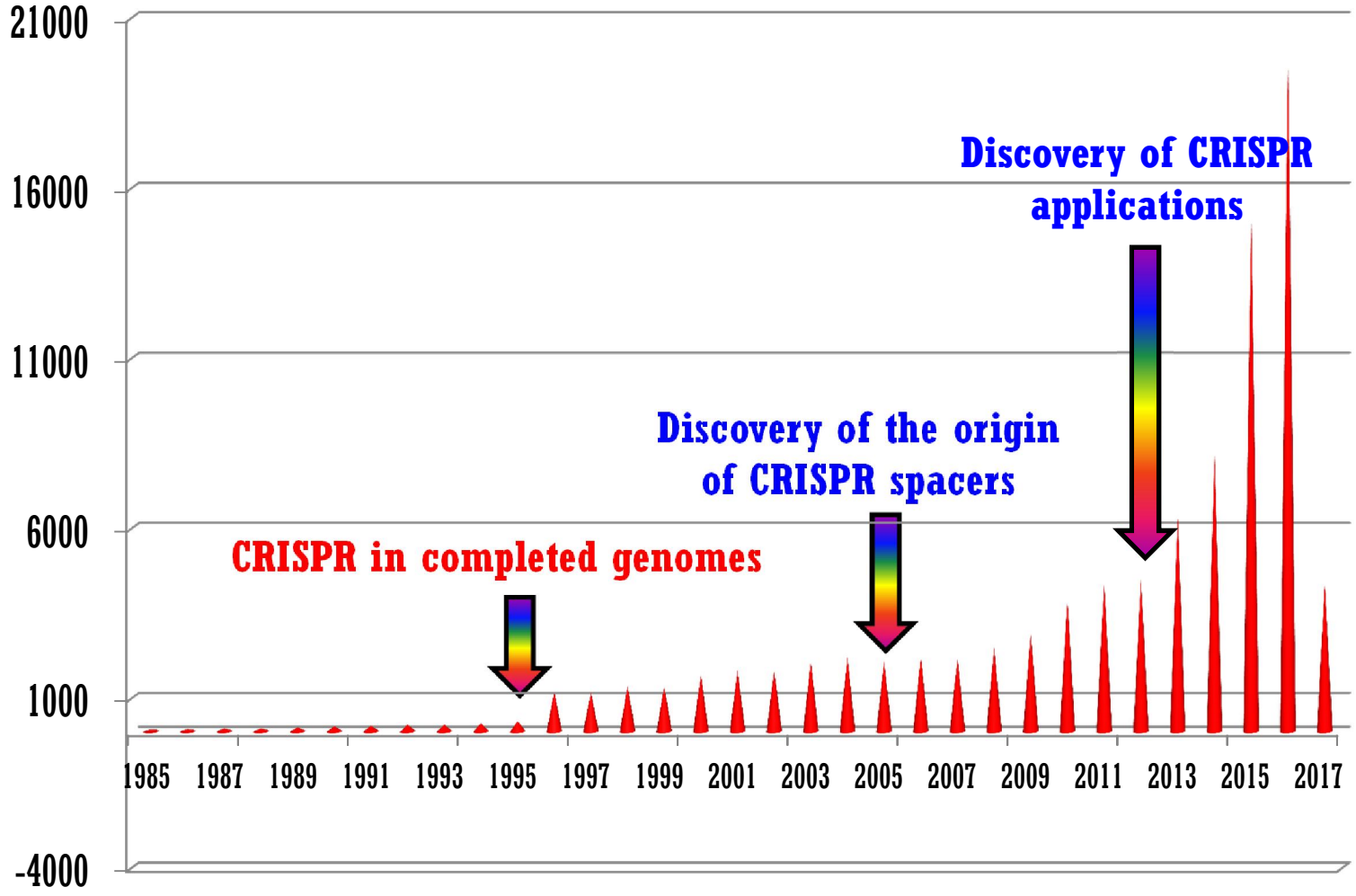


Fig. 1. Distribution of E-values corresponding to validated similarities (solid bars) and best-score discarded alignments for the positive searches (open bars). Cutoff significance value is indicated by an arrow. See Materials and Methods for details.

Abstract. Prokaryotes contain short DNA repeats known as CRISPR, recognizable by the regular spacing existing between the recurring units. They represent the most widely distributed family of repeats among prokaryotic genomes, suggesting a biological function. The origin of the intervening sequences, at present unknown, could provide clues about their biological activities. Here we show that CRISPR spacers derive from preexisting sequences, either chromosomal or within transmissible genetic elements such as bacteriophages and conjugative plasmids. Remarkably, these extrachromosomal elements fail to infect the specific spacer-carrier strain, implying a relationship between CRISPR and immunity against targeted DNA. Bacteriophages and conjugative plasmids are involved in prokaryotic population control, evolution, and pathogenicity. All these biological traits could be influenced by the presence of specific spacers. CRISPR loci can be visualized as mosaics of a repeated unit, separated by sequences at some time present elsewhere in the cell.

Papers published relating to CRISPR (GS)



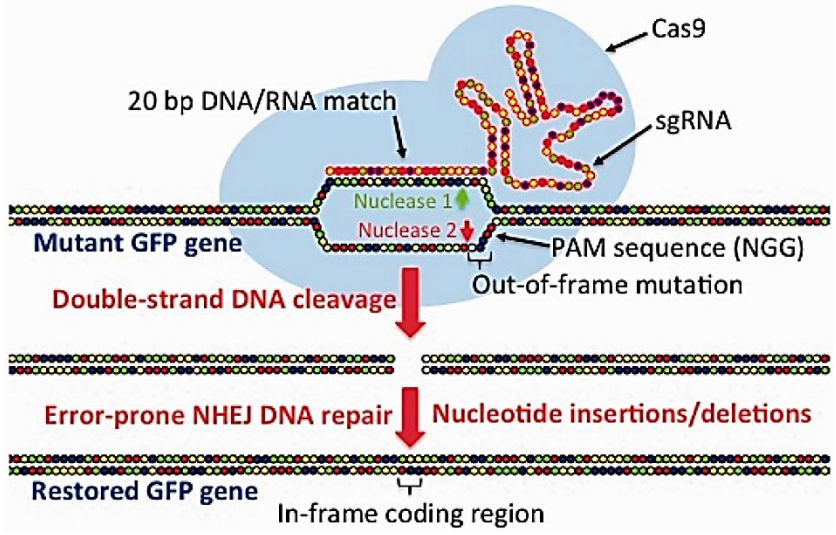
Background

- CRISPR-Cas (clustered regularly interspaced short palindromic repeats-CRISPR associated) adaptive immune systems are found in roughly 50% of bacteria and 90% of archaea (Makarova et al., 2015).[1]
- CRISPR immunity functions analogously to vertebrate adaptive immunity by generating records of previous infections to elicit a rapid and robust response upon reinfection.

[1] Nat. Rev. Microbiol., 13(11): 722-736, (2015).

Wright A. V., J. K. Nuñez y J. A. Doudna, Biology and applications of CRISPR systems: Harnessing nature's toolbox for genome engineering, Cell, 164(1-2): 29-44, (2016).

The mechanism



Jiang W. et al., Nucleic Acids Res., 41(20): e188, (2013).

Emmanuelle Charpentier

One day in March 2011, Emmanuelle Charpentier[48], a geneticist who was studying flesh-eating bacteria, approached Jennifer Doudna[53], an award-winning scientist, at a microbiology conference in Puerto Rico.

Charpentier, a more junior researcher, hoped to persuade Doudna, the head of a formidably large lab at the University of California, Berkeley, to collaborate. While walking the cobblestone streets of Old San Juan, the two women fell to talking.

Charpentier had recently grown interested in a particular gene, known as *crispr*, that seemed to help flesh-eating bacteria fight off invasive viruses. By understanding that gene, as well as the protein that enabled it, called Cas9, Charpentier hoped to find a way to cure patients infected with the bacteria by stripping it of its protective immune system.

https://www.nytimes.com/2015/11/15/magazine/the-crispr-quandary.html

CRISPR-Cas

- These systems are divided into two major classes, six types and 19 subtypes. Each system consists of two components: a locus for memory storage (the CRISPR array) and *cas* genes that encode the machinery driving immunity.
- CRISPR adaptation provides heritable benefits, an attribute that is unparalleled in eukaryotic immune systems.
- The "polarized" addition of spacers into CRISPR loci produces a chronological account of the encounters between phages and bacteria that can provide insights into phage-host co-occurrences, evolution, and ecology.

Jackson S. A. et al., CRISPR-Cas: Adapting to change. Science 356, eaal5056, (2017).

Background

- CRISPR-Cas systems are generally defined by a genomic locus called the CRISPR array, a series of 20–50 base-pair (bp) direct repeats separated by unique "spacers" of similar length and preceded by an AT-rich "leader" sequence (Jansen et al., 2002; Kunin et al., 2007).

Jansen R. et al., Mol. Microbiol., 43(6): 1565-1575, (2002).
Kunin V. et al., Genome Biol, 8(4): R61, (2007).

- Nearly two decades after CRISPR loci were first identified in *Escherichia coli*, spacers were found to derive from viral genomes and conjugative plasmids, serving as records of previous infection (Bolotin et al., 2005; Ishino et al., 1987; Mojica et al., 2005; Pourcel et al., 2005).

Bolotin A. et al., Microbiology, 151(8): 2551-2561, (2005). Ishino Y. et al., J. Bacteriol., 169(12): 5429-5433, (1987).
Mojica F. J. M. et al., J. Mol. Evol., 60(2): 174-182, (2005). Pourcel C. et al., Microbiology, 151(3): 653-663, (2005

- Sequences in foreign DNA matching spacers are referred to as "protospacers."

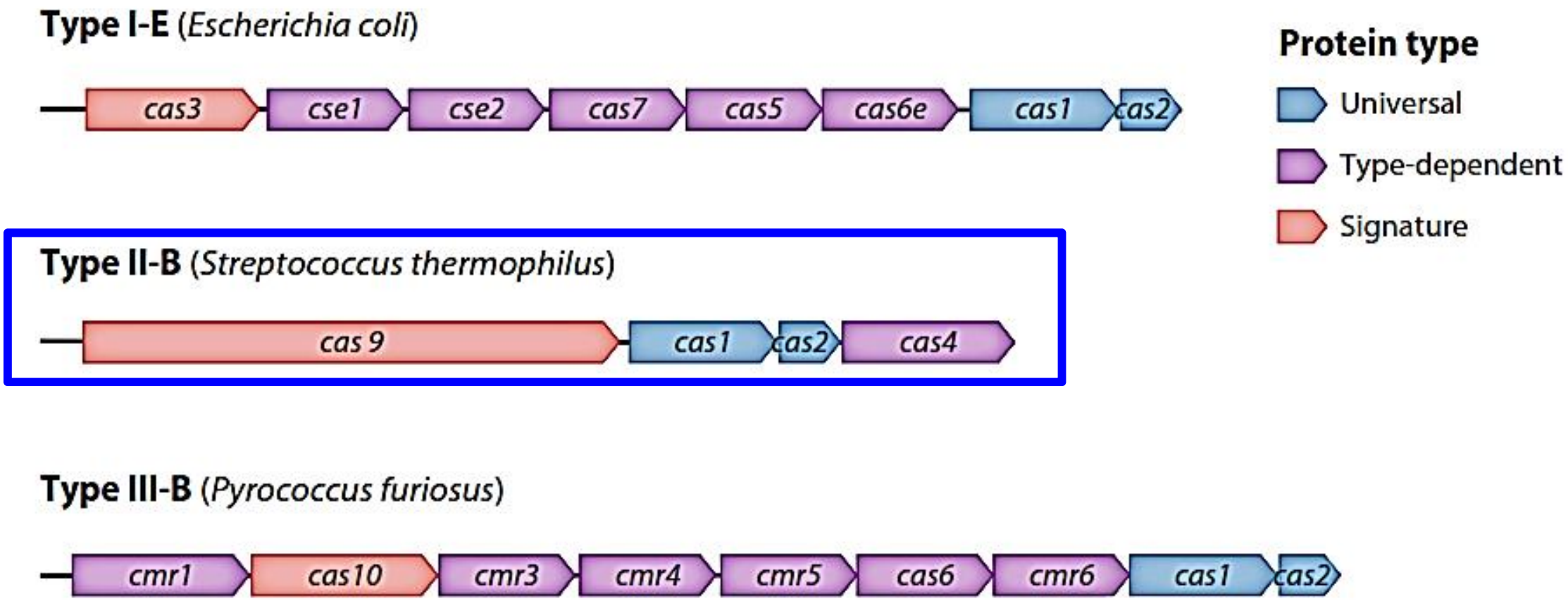
Wright A. V., J. K. Nuñez y J. A. Doudna, Biology and applications of CRISPR systems: Harnessing nature's toolbox for genome engineering, Cell, 164(1-2): 29-44, (2016).

Background

- CRISPR immunity is divided into three stages: spacer acquisition, CRISPR RNA (crRNA) biogenesis, and interference.
- In the interference stage, an effector complex uses the crRNA to identify and destroy any phage or plasmid bearing sequence complementarity to the spacer sequence of the crRNA.
- These steps are carried out primarily by Cas proteins, which are encoded by *cas* genes flanking the CRISPR arrays.

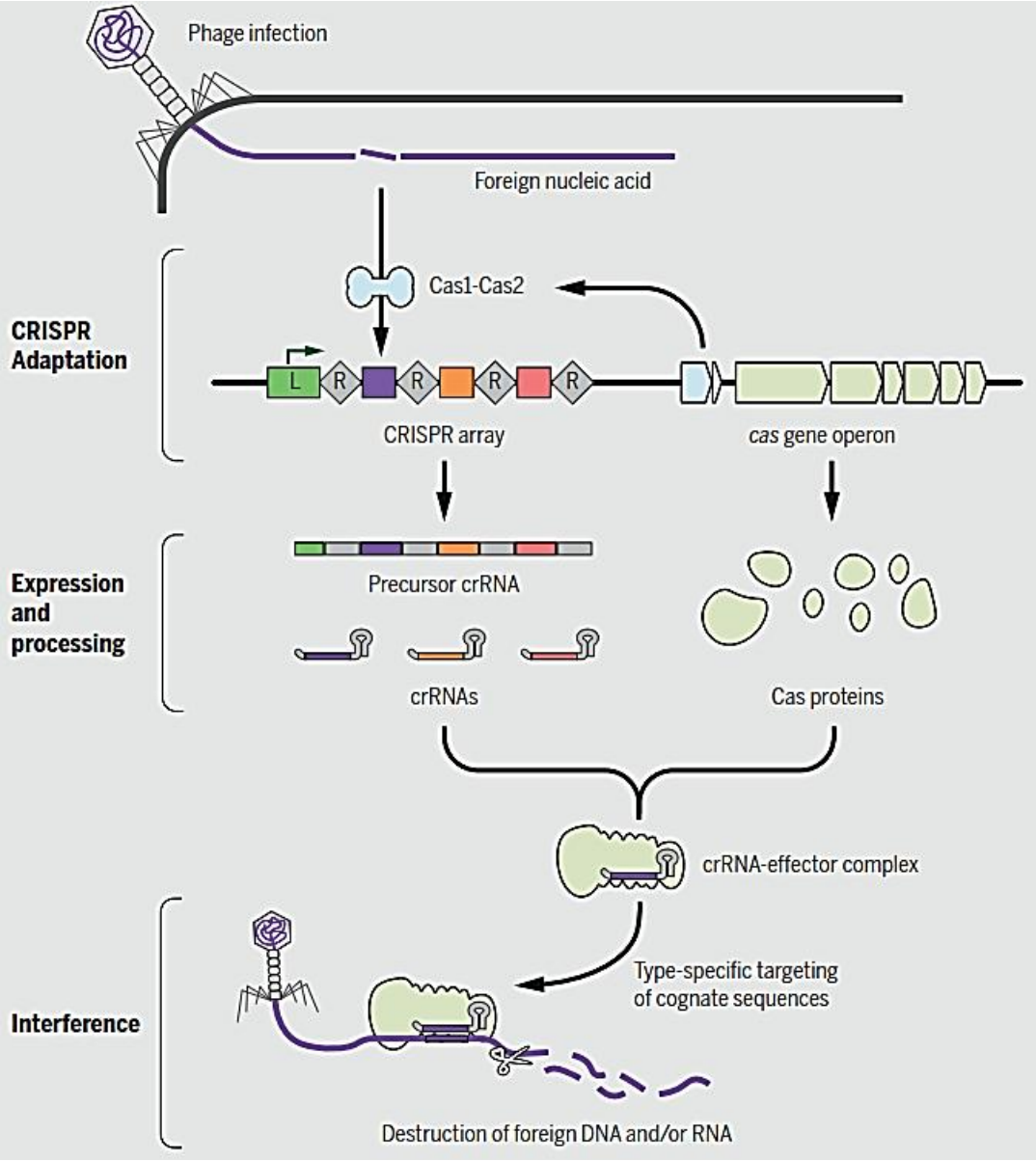
Wright A. V., J. K. Nuñez y J. A. Doudna, Biology and applications of CRISPR systems: Harnessing nature's toolbox for genome engineering, Cell, 164(1-2): 29-44, (2016).

Cas proteins in Type I, II, and III CRISPR-Cas systems

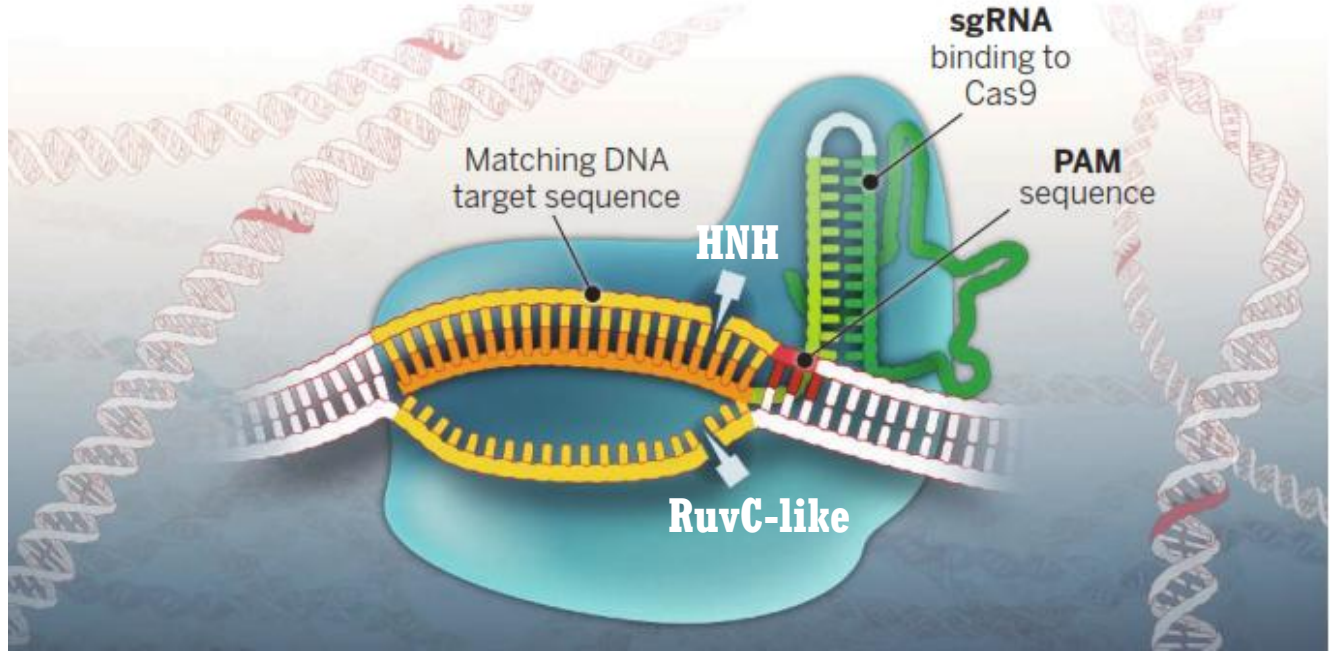


Bhaya D., M. Davison y R. Barrangou, CRISPR-Cas systems in bacteria and archaea: Versatile small RNAs for adaptive defense and regulation, Annu. Rev. Genet., 45(1): 273-297, (2011).

A roadmap of CRISPR-Cas adaptation and defense



Jackson S. A. et al., CRISPR-Cas: Adapting to change, Science, 356(6333): eaal5056, (2017).



Doudna J. A. y E. Charpentier, The new frontier of genome engineering with CRISPR-Cas9, Science, 346(6213): 1258096-1, (2014).

Function and organization of CRISPR systems

- The specific complement of *cas* genes varies widely. CRISPR-Cas systems can be classified based on the presence of "signature genes" into six types, which are additionally grouped into two classes (Makarova et al., 2011; 2015; Shmakov et al., 2015).

Makarova K. S. et al., Evolution and classification of the CRISPR-Cas systems, Nat. Rev. Microbiol., 9(6): 467-477, (2011).
Makarova K. S. et al., An updated evolutionary classification of CRISPR-Cas systems, Nat. Rev. Microbiol., 13(11): 722-736, (2015).
Shmakov S. et al., Discovery and functional characterization of diverse class 2 CRISPR-Cas systems, Mol. Cell, 60(3): 385-397, (2015).

Wright A. V., J. K. Nuñez y J. A. Doudna, Biology and applications of CRISPR systems: Harnessing nature's toolbox for genome engineering, Cell, 164(1-2): 29-44, (2016).

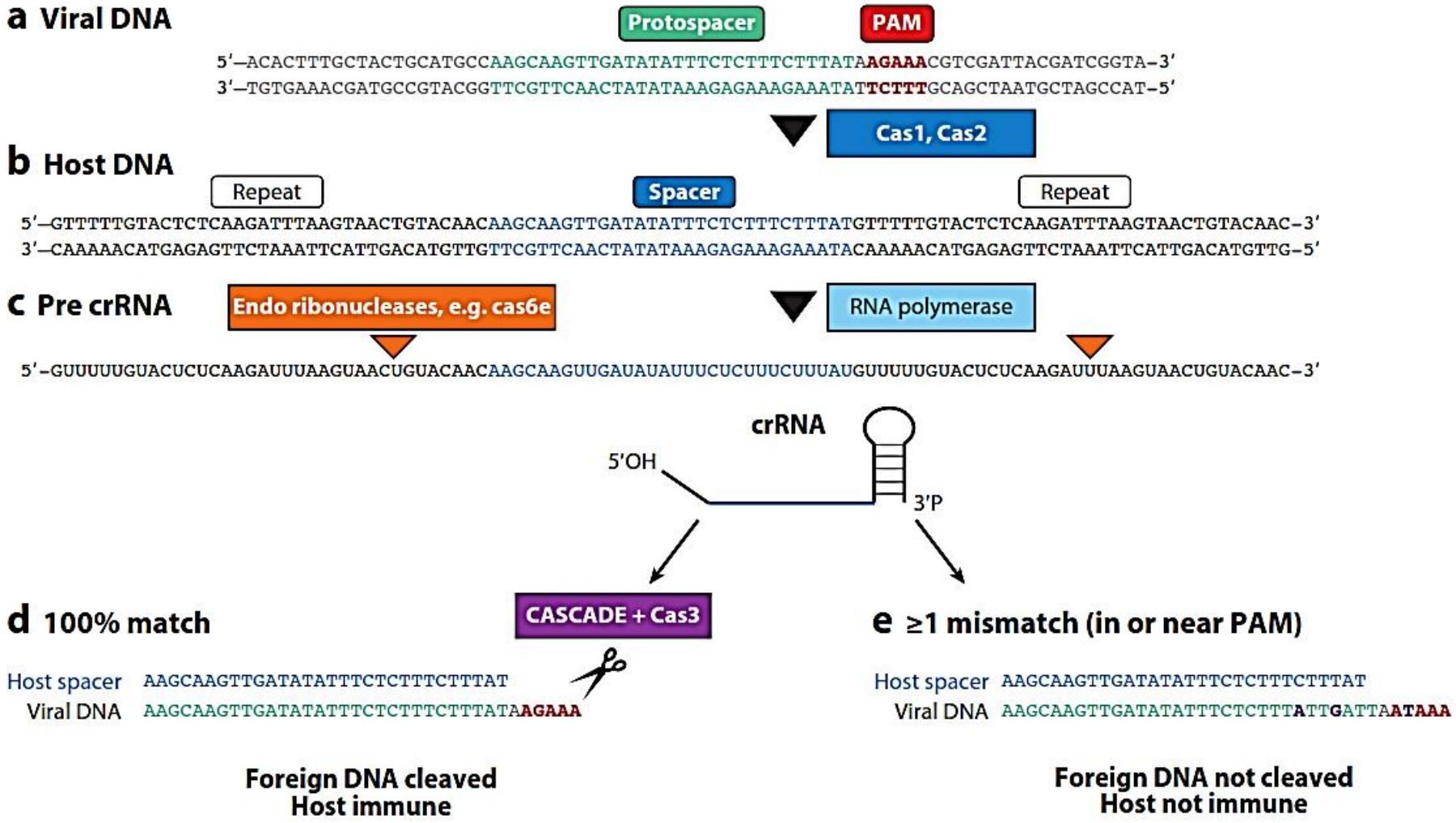
Table 1. Classification and Examples of CRISPR Systems

Class	Type	Subtype	Hallmarks	Example effector	Example organism	Studies Cited
Class 1	Type I		multisubunit effector complex; Cas3	Cascade	*E. coli*	Brouns et al., 2008
	Type III	III-A	multisubunit effector complex; Csm effector module; DNA targeting	Cas10-Csm	*S. epidermidis*	Marraffini and Sontheimer, 2008
		III-B	multisubunit effector complex; Cmr effector module; RNA targeting	Cmr	*P. furiosus*	Hale et al., 2009
Class 2	Type II		single protein effector; tracrRNA	Cas9	*S. thermophilus*	Bolotin et al., 2005; Barrangou et al., 2007; Sapranauskas et al., 2011; Gasiunas et al., 2012
					S. pyogenes	Deltcheva et al., 2011; Jinek et al., 2012; Cong et al., 2013; Mali et al., 2013
	Type V		single protein effector; single-RNA guided	Cpf1	*F. novicida*	Zetsche et al., 2015

CRISPR systems are currently organized into two overarching classes: Class 1, which contain multi-subunit effectors, and Class 2, which contain single protein effectors. These classes are subdivided into five types (Makarova et al., 2015), with type IV remaining a putative type within Class 1. Although only Class 2 systems have been adapted for genome engineering, the results described in this review emerged from studying a diversity of CRISPR-Cas systems. (Type III-B systems are not discussed but represent an unusual system that targets RNA rather than DNA [Hale et al., 2009].)

Lander E. S., The Heroes of CRISPR, Cell, 164(1-2): 18-28, (2016).

A close-up of the CRISPR-Cas system



Bhaya D. et al., CRISPR-Cas systems in bacteria and archaea: Versatile small RNAs for adaptive defense and regulation, Annu. Rev. Genet., 45(1): 273-297, (2011).

Published online 3 August 2011

Nucleic Acids Research, 2011, Vol. 39, No. 21 ***9275–9282***
doi:10.1093/nar/gkr606

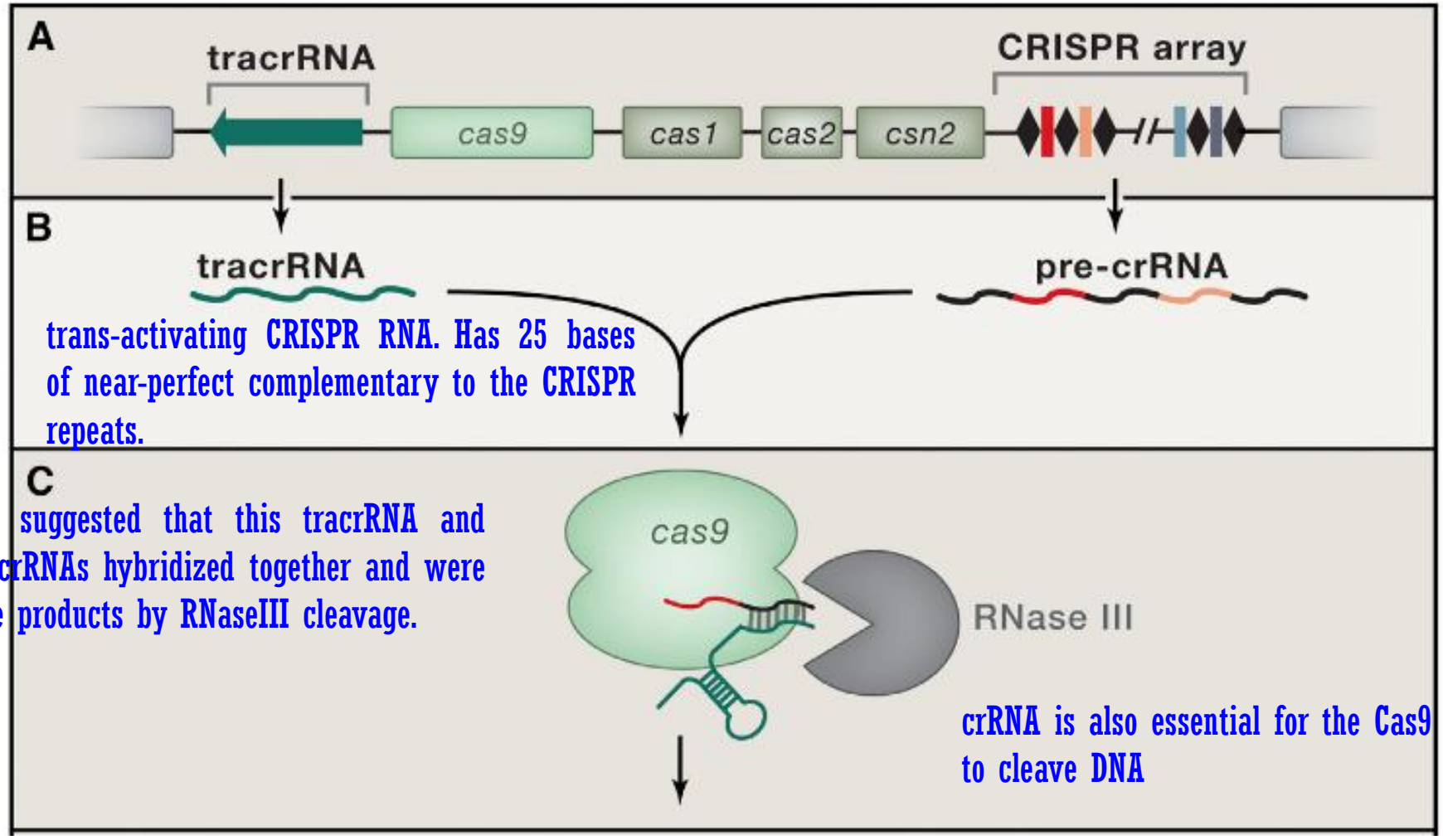
The *Streptococcus thermophilus* CRISPR/Cas system provides immunity in *Escherichia coli*

Rimantas Sapranauskas[1], Giedrius Gasiunas[1], Christophe Fremaux[2], Rodolphe Barrangou[3], Philippe Horvath[2] and Virginijus Siksnys[1,*]

[1]Institute of Biotechnology, Vilnius University, Graiciuno 8, LT–02241, Vilnius, Lithuania, [2]Danisco France SAS, BP 10, F-86220 Dangé-Saint-Romain, France and [3]Danisco USA Inc., Madison, WI 53716, USA

Sapranauskas R. et al., The *Streptococcus thermophilus* CRISPR/Cas system provides immunity in *Escherichia coli*, Nucleic Acids Res., 39(21): 9275-9282, (2011).

Class 2, Type II CRISPR-Cas9 System from *S. thermophilus*

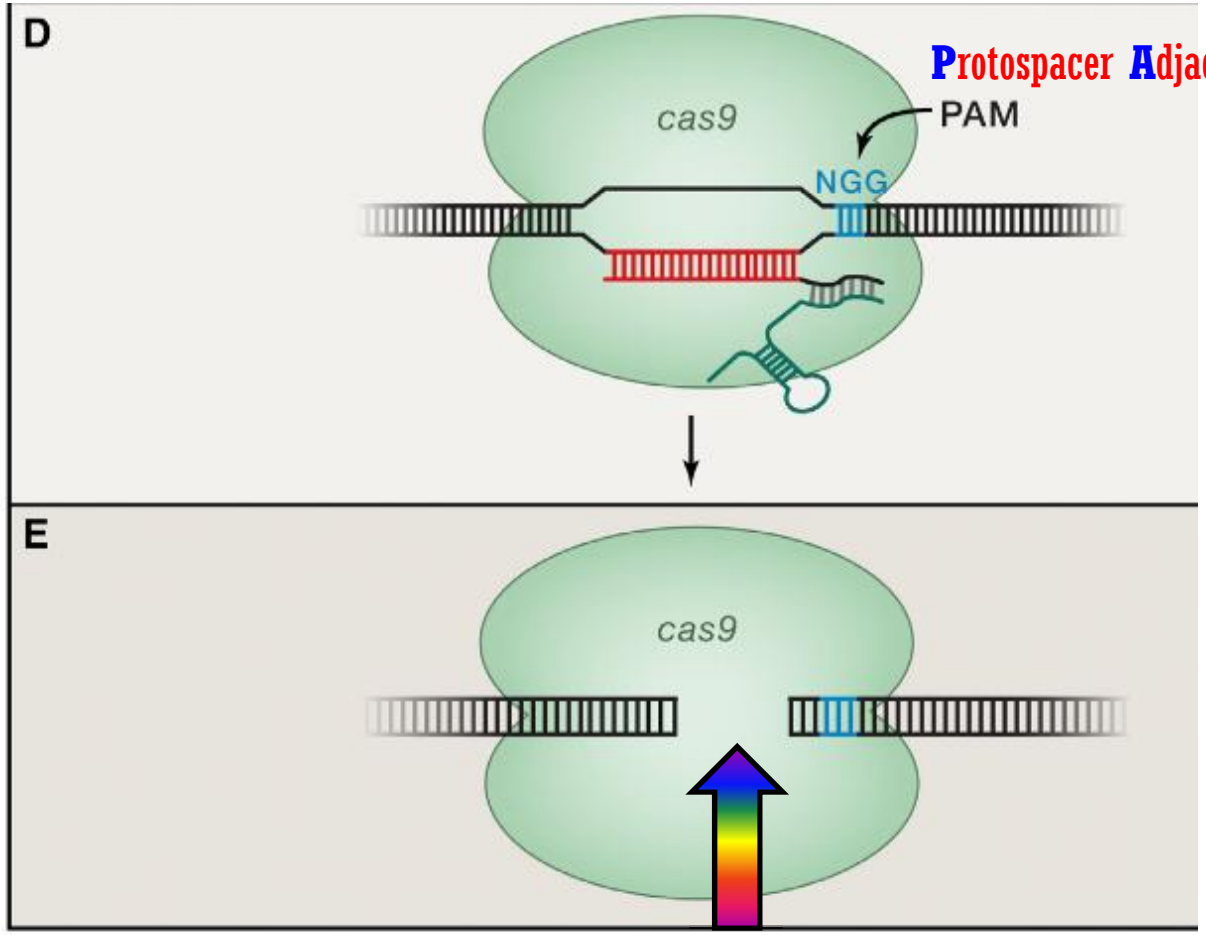


The complementarity suggested that this tracrRNA and the precursor of the crRNAs hybridized together and were processed into mature products by RNaseIII cleavage.

crRNA is also essential for the Cas9 nuclease complex to cleave DNA

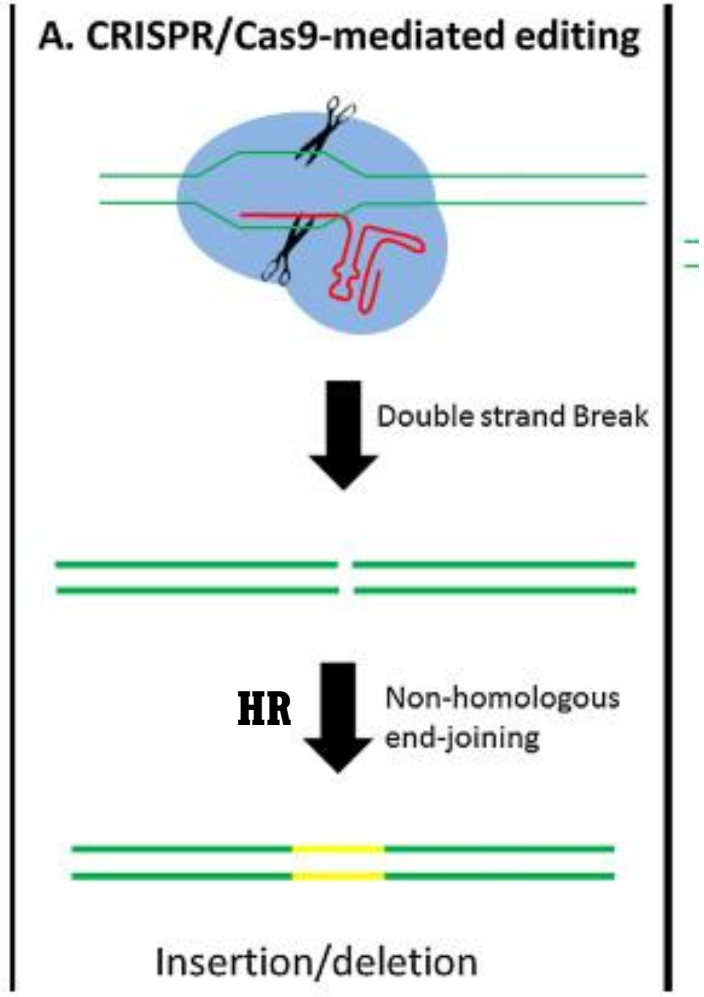
Lander E. S., The Heroes of CRISPR, Cell, 164(1-2): 18-28, (2016).

Class 2, Type II CRISPR-Cas9 System from *S. thermophilus*

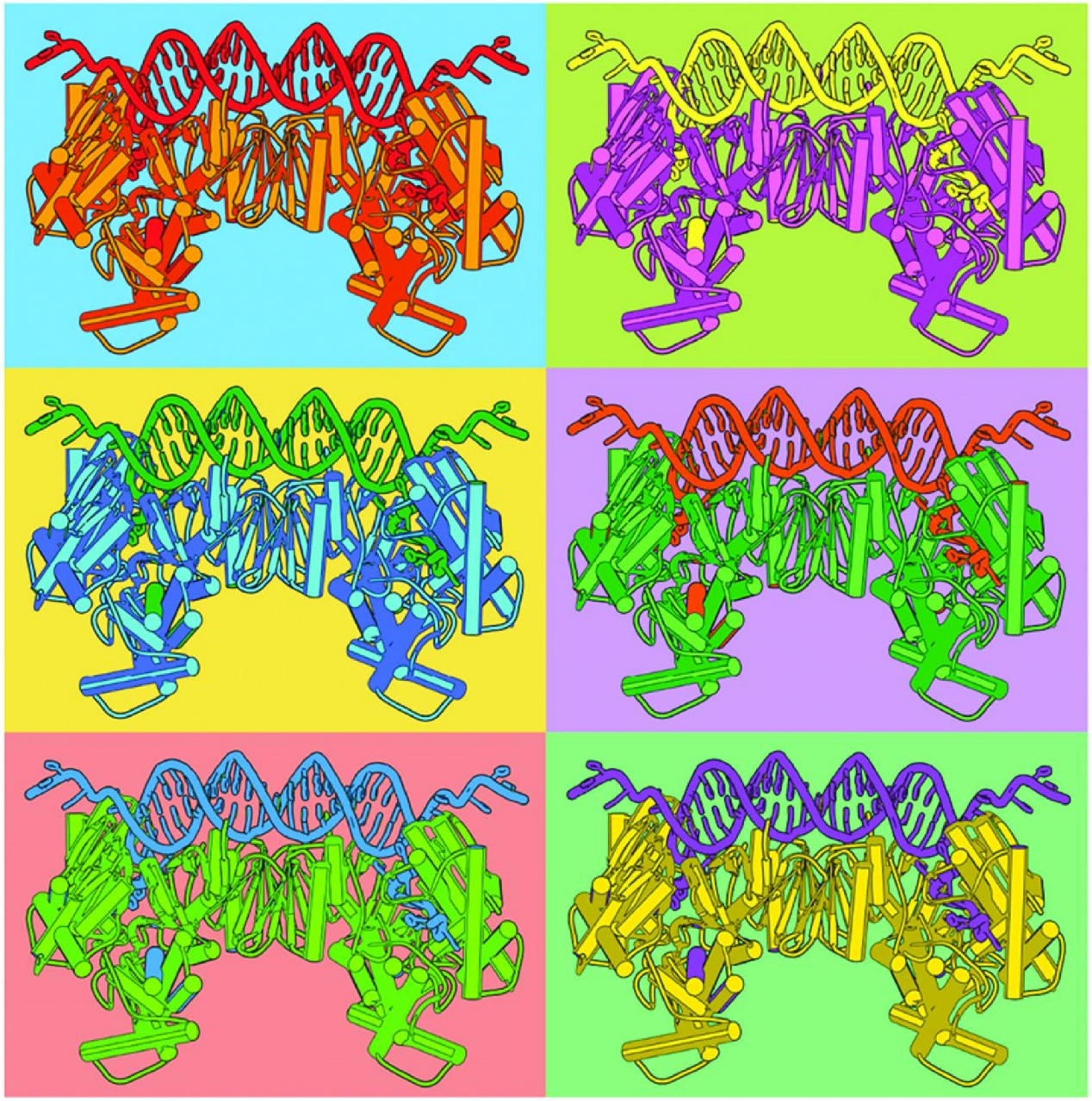


Protospacer **A**djacent **M**otif

Lander E. S., The Heroes of CRISPR, Cell, 164(1-2): 18-28, (2016).



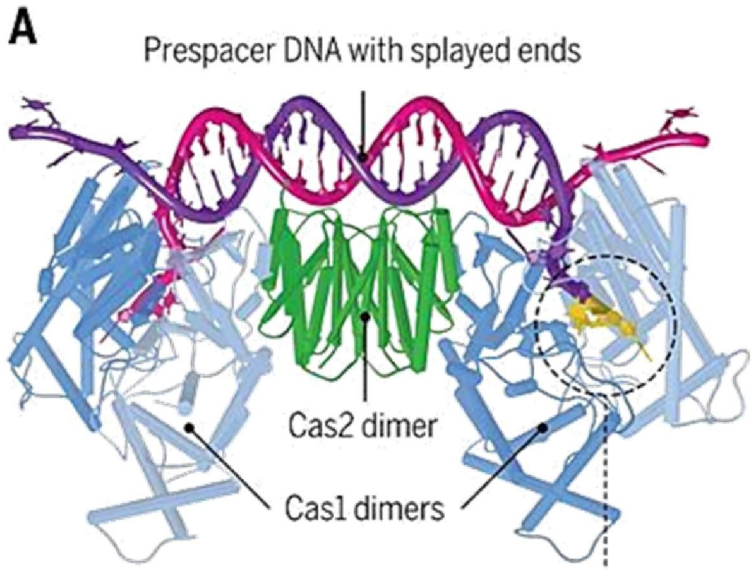
Schaeffer S. M. y P. A. Nakata, CRISPR/Cas9-mediated genome editing and gene replacement in plants: Transitioning from lab to field, Plant Sci., 240: 130-142, (2015).



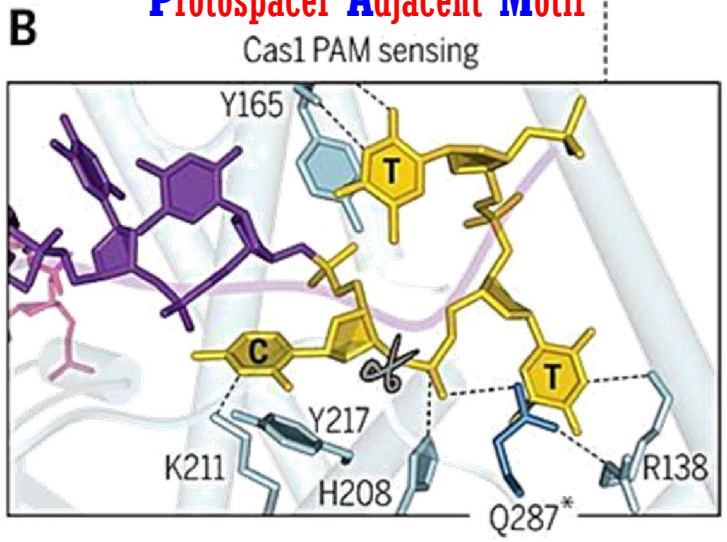
Many hues of the CRISPR-Cas adaptation machinery

Jackson S. A. et al., CRISPR-Cas: Adapting to change, Science, 356(6333): eaal5056, (2017).

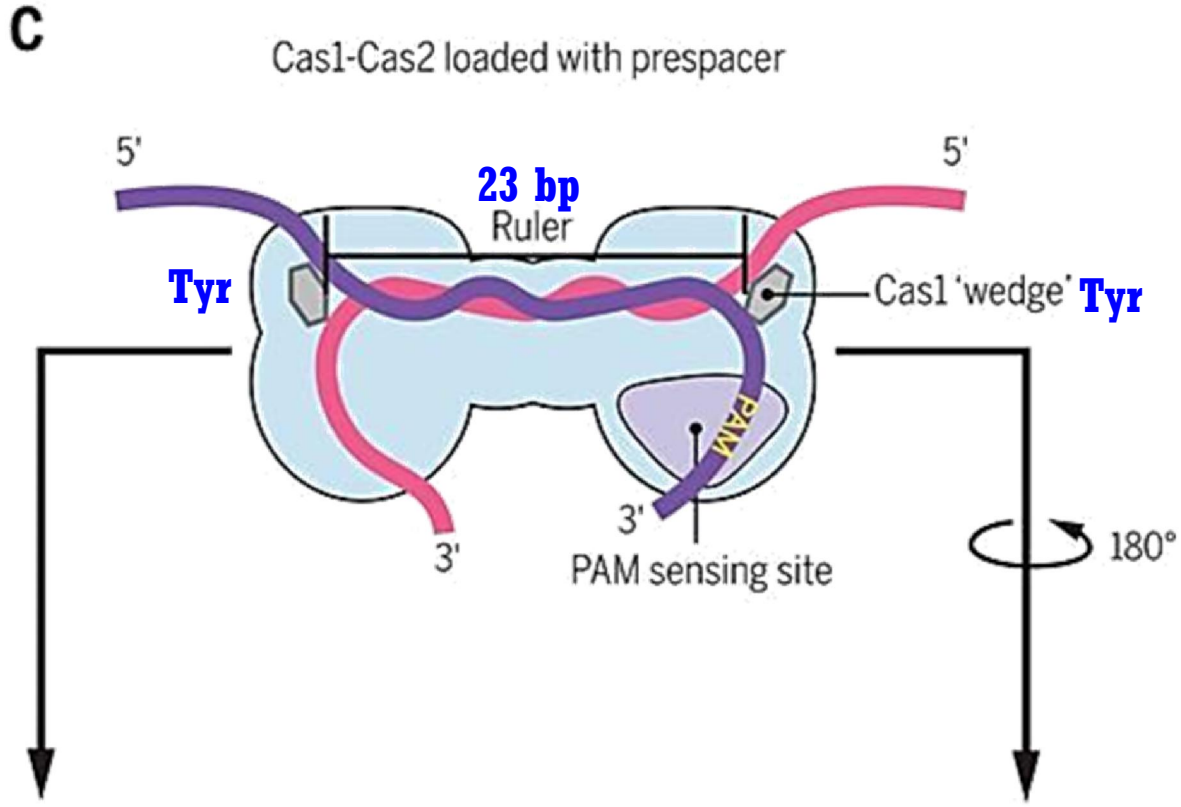
Cas1-Cas2–mediated spacer acquisition



Protospacer Adjacent Motif



2 Thr
1 Cys
2 Tyr
1 Lys
1 His
1 Arg
1 Gln



Jackson S. A. et al., CRISPR-Cas: Adapting to change, Science, 356(6333): eaal5056, (2017).

CRISPR and genome modifications

CRISPR pioneers

George Church. Harvard

Emmanuelle Charpentier. Umeå

Jennifer Doudna. Berkeley

Feng Zhang. Broad Institute

Cohen J., The birth of CRISPR Inc, Science, 355(6326): 680-684, (2017).

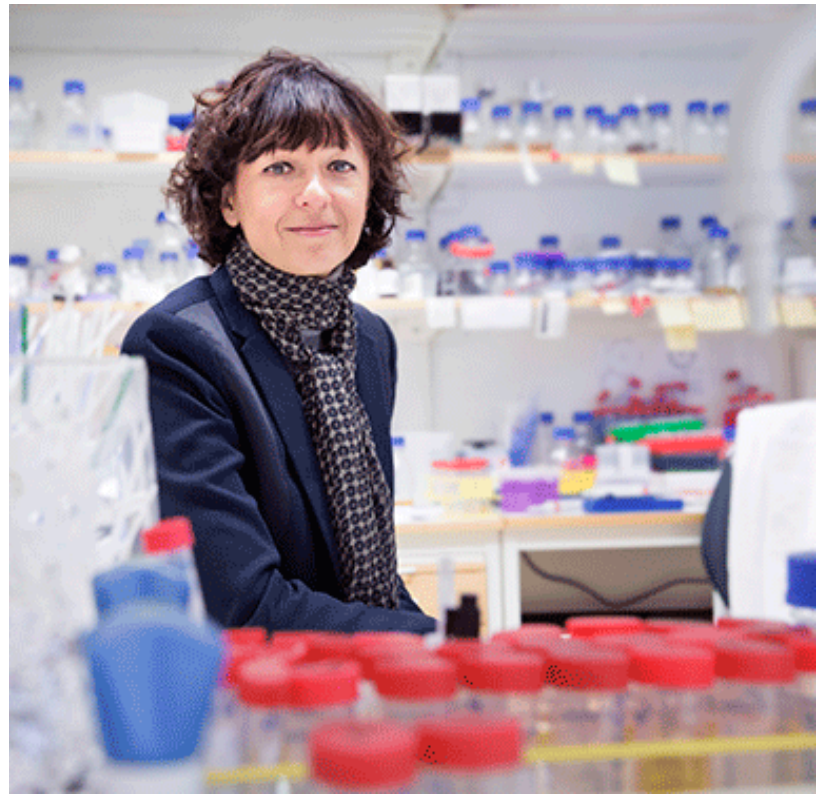
The pioneers

Emmanuelle Charpentier

Max Planck Institute for Infection Biology
Helmholtz Centre for Infection Research in Braunschweig
Umeå University in Sweden,

Jennifer Doudna

University of California (Berkeley)



For 25 years, she was a scientific nomad, working at nine institutions in five countries, scrambling for research funds, paid so little she barely scraped by. Now, at 48, with her gene editing discovery, her life has changed. director of the Max Planck Institute for Infection Biology in Berlin.

"Excellence is never an accident. It is always the result of high intention, sincere effort, and intelligent execution; it represents the wise choice of many alternatives – choice, not chance, and determines your destiny."

Aristotle (384-322 BC)

https://www.mpg.de/9343753/infektionsbiologie-charpentier

http://www.medfak.umu.se/english/about-the-faculty/news/newsdetailpage/umea-university-ec-jubilee-award-2015.cid253978

Emmanuelle Charpentier

2009 – Prize of the City of Vienna: Theodor Körner Prize for Science and Culture.
2011 – The Fernström Prize for young and promising scientists.
2013 – Alexander von Humboldt Professorship.
2014 – The Göran Gustafsson Prize for Molecular Biology (Royal Swedish Academy of Sciences).
2014 – Dr. Paul Janssen Award for Biomedical Research (shared with Jennifer Doudna).
2014 – The Jacob Heskel Gabbay Award (shared with Feng Zhang and Jennifer Doudna).
2014 – Grand Prix Jean-Pierre Lecocq from the French Academy of Sciences.
2015 – The Breakthrough Prize in Life Sciences (shared with Jennifer Doudna).
2015 – International Society for Transgenic Technologies prize (shared with Jennifer Doudna).
2015 – Louis-Jeantet Prize for Medicine.
2015 – Time 100: Pioneers (shared with Jennifer Doudna).
2015 – The Ernst Jung Prize in Medicine.
2015 – The Hansen Family Award.
2015 – **Princess of Asturias Awards (shared with Jennifer Doudna).**

https://en.wikipedia.org/wiki/Emmanuelle_Charpentier

Emmanuelle Charpentier

2015 – Gruber Foundation International Prize in Genetics (shared with Jennifer Doudna).
2015 – Umeå University Jubilee Award: The MIMS Excellence by Choice Programme.
2015 – Carus Medal (de), from German National Academy of Science, Leopoldina.
2015 – Massry Prize.
2016 – Otto Warburg Medal.
2016 – L'Oréal-UNESCO "For Women in Science" Award (jointly with Jennifer Doudna).
2016 – Leibniz Prize from the German Research Foundation.
2016 – Canada Gairdner International Award (shared with Jennifer Doudna and Feng Zhang).
2016 – Warren Alpert Foundation Prize.
2016 – Paul Ehrlich and Ludwig Darmstaedter Prize (jointly with Jennifer Doudna).
2016 – Tang Prize.
2016 – HFSP Nakasone Award (jointly with Jennifer Doudna).
2016 – Meyenburg Prize.
2016 – BBVA Foundation Frontiers of Knowledge Award (jointly with Jennifer Doudna and Francisco M. Mojica)
2016 – Wilhelm Exner Medal.
2017 – **Japan Prize (jointly with Jennifer Doudna).**

DNA is the target

- Luciano Marraffini was finishing his Ph.D., working on Staphylococcus, at the University of Chicago, when he learned about CRISPR from Malcolm Casadaban.
- Even before moving to Northwestern, Marraffini started working on CRISPR even as he completed his graduate work—exploring whether the Staphylococcus CRISPR system could block plasmid conjugation.
- Clearly, CRISPR blocked the plasmids, just as it blocked viruses.

Lander E. S., The Heroes of CRISPR, Cell, 164(1-2): 18-28, (2016).

CRISPR as genome-editing tool. 19 December 2008

CRISPR Interference Limits Horizontal Gene Transfer in Staphylococci by Targeting DNA

Luciano A. Marraffini and Erik J. Sontheimer*

Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, 2205 Tech Drive, Evanston, IL 60208, USA.

Horizontal gene transfer (HGT) in bacteria and archaea occurs through phage transduction, transformation, or conjugation, and the latter is particularly important for the spread of antibiotic resistance. Clustered, regularly interspaced, short palindromic repeat (CRISPR) loci confer sequence-directed immunity against phages. A clinical isolate of *Staphylococcus epidermidis* harbors a CRISPR spacer that matches the *nickase* gene present in nearly all staphylococcal conjugative plasmids. Here we show that CRISPR interference prevents conjugation and plasmid transformation in *S. epidermidis*. Insertion of a self-splicing intron into *nickase* blocks interference despite the reconstitution of the target sequence in the spliced mRNA, which indicates that the interference machinery targets DNA directly. We conclude that CRISPR loci counteract multiple routes of HGT and can limit the spread of antibiotic resistance in pathogenic bacteria.

From a practical standpoint, the ability to direct the specific addressable destruction of DNA that contains any given 24- to 48-nucleotide target sequence could have considerable functional utility, especially if the system can function outside of its native bacterial or archaeal context.

Marraffini L. A. y E. J. Sontheimer, CRISPR interference limits horizontal gene transfer in Staphylococci by targeting DNA, Science, 322(5909): 1843-1845, (2008).

First seat down

USPTO, however, rejected their patent application. "The vision and idea were out there, but we hadn't reduced it to practice," says Sontheimer, who is now at the University of Massachusetts Medical School in Worcester. "When we filed our patent in 2008 there were a million mechanistic questions."

Cohen J., The birth of CRISPR Inc, Science, 355(6326): 680-684, (2017).

The beginning

In early 2012, Emmanuelle Charpentier, a little-known French microbiologist who would soon meet worldwide fame, contacted her old friend Rodger Novak to tell him about her recent studies at Umeå University in Sweden of the mechanisms behind a novel bacterial immune system. "She said, 'Hey, what do you think about CRISPR?'" recalls Novak, a biotech executive who more than a decade earlier had worked with Charpentier in academic labs studying antibiotic resistance. "I had no clue what she was talking about."

- Charpentier, in collaboration with Jennifer Doudna (UC, Berkeley) transformed the bacteria CRISPR immune system into a tool that could edit genomes with great ease that had "considerable potential".

Cohen J., The birth of CRISPR Inc, Science, 355(6326): 680-684, (2017).

Emmanuelle Charpentier

I was in Umeå in Sweden, and my students were in Vienna. That evening, one wrote me an email. I was alone in my office, but at some point, I walked out and there was a colleague of mine there.

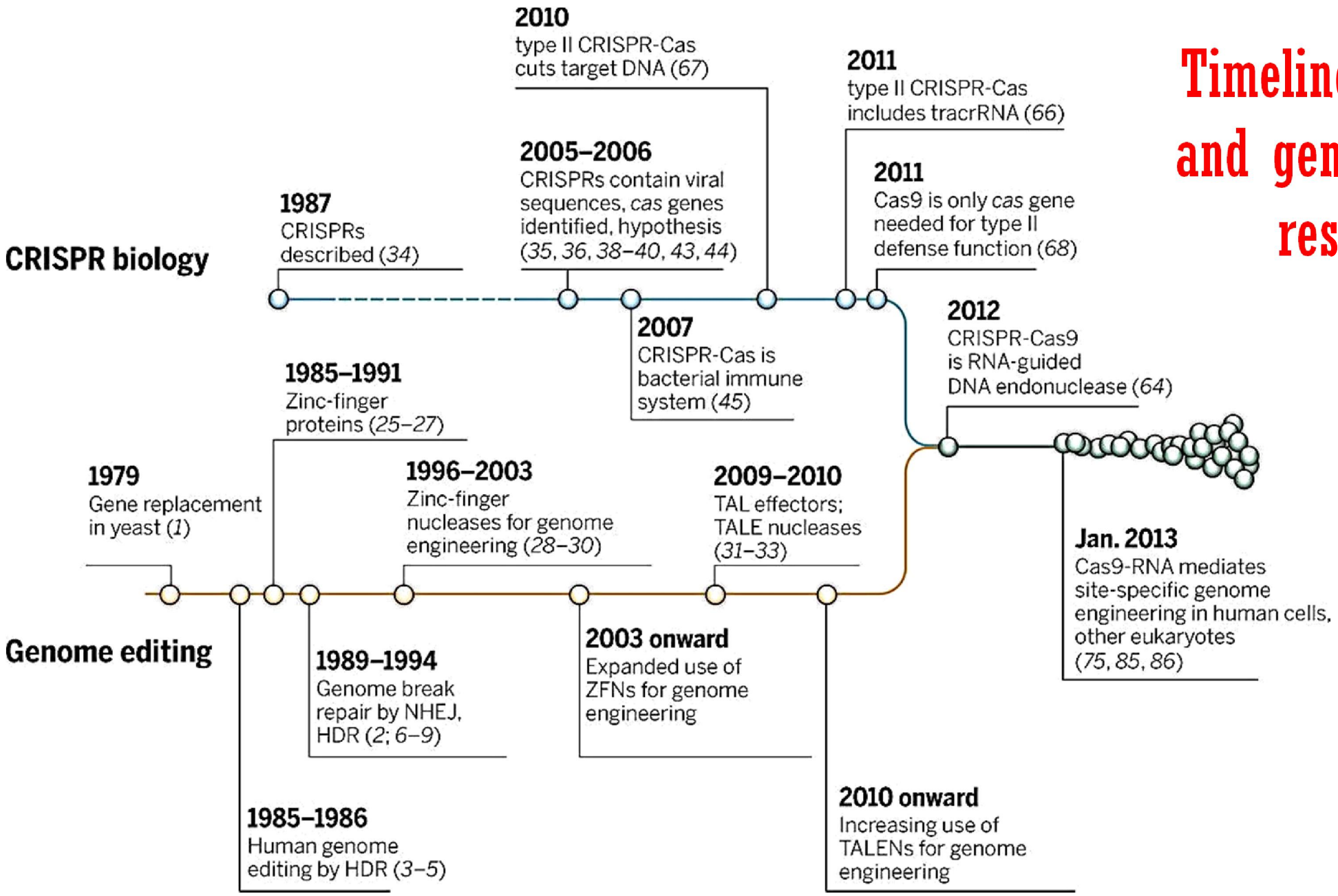
I said,"I have very good news and I am very happy." Then I went back and spent a lot of time writing an email to my students with the series of experiments that had to be done next.

The second moment was even more exciting. We did an experiment that showed Crispr/cas9 was cleaving DNA. This was really, really critical. It was the same story. My student wrote me an email. I called him right away.

This time, it was again in the evening and I was in my office, but there were other people in the lab. I ran out and told the others in the lab. Then I sat down and wrote down what to do next.

https://www.nytimes.com/2016/05/31/health/emmanuelle-charpentiers-crispr-dna-gene-editing.html

Timeline of CRISPR-Cas and genome engineering research fields



5 October 2012
12 December 2012
3 January 2013

15 December 2012
03 January 2013
29 January 2013

26 October 2012
12 December 2012
3 January 2013

Doudna J. A. y E. Charpentier, The new frontier of genome engineering with CRISPR-Cas9, Science, 346(6213): 1258096-1, (2014).

A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity

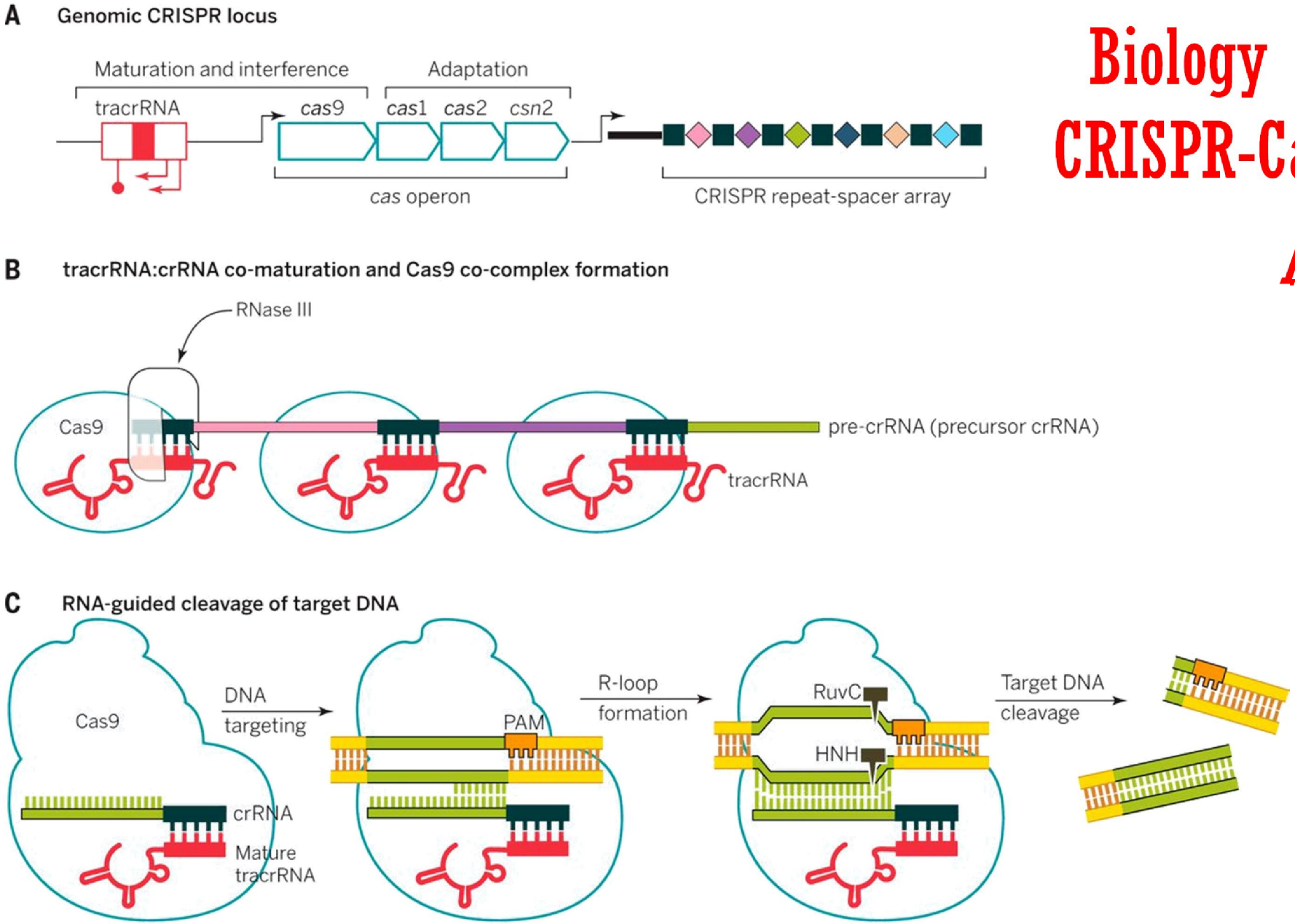
Martin Jinek,[1,2]* Krzysztof Chylinski,[3,4]* Ines Fonfara,[4] Michael Hauer,[2]†
Jennifer A. Doudna,[1,2,5,6]‡ Emmanuelle Charpentier[4]‡

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems provide bacteria and archaea with adaptive immunity against viruses and plasmids by using CRISPR RNAs (crRNAs) to guide the silencing of invading nucleic acids. We show here that in a subset of these systems, the mature crRNA that is base-paired to trans-activating crRNA (tracrRNA) forms a two-RNA structure that directs the CRISPR-associated protein Cas9 to introduce double-stranded (ds) breaks in target DNA. At sites complementary to the crRNA-guide sequence, the Cas9 HNH nuclease domain cleaves the complementary strand, whereas the Cas9 RuvC-like domain cleaves the noncomplementary strand. The dual-tracrRNA:crRNA, when engineered as a single RNA chimera, also directs sequence-specific Cas9 dsDNA cleavage. Our study reveals a family of endonucleases that use dual-RNAs for site-specific DNA cleavage and highlights the potential to exploit the system for RNA-programmable genome editing.

17 AUGUST 2012 VOL 337

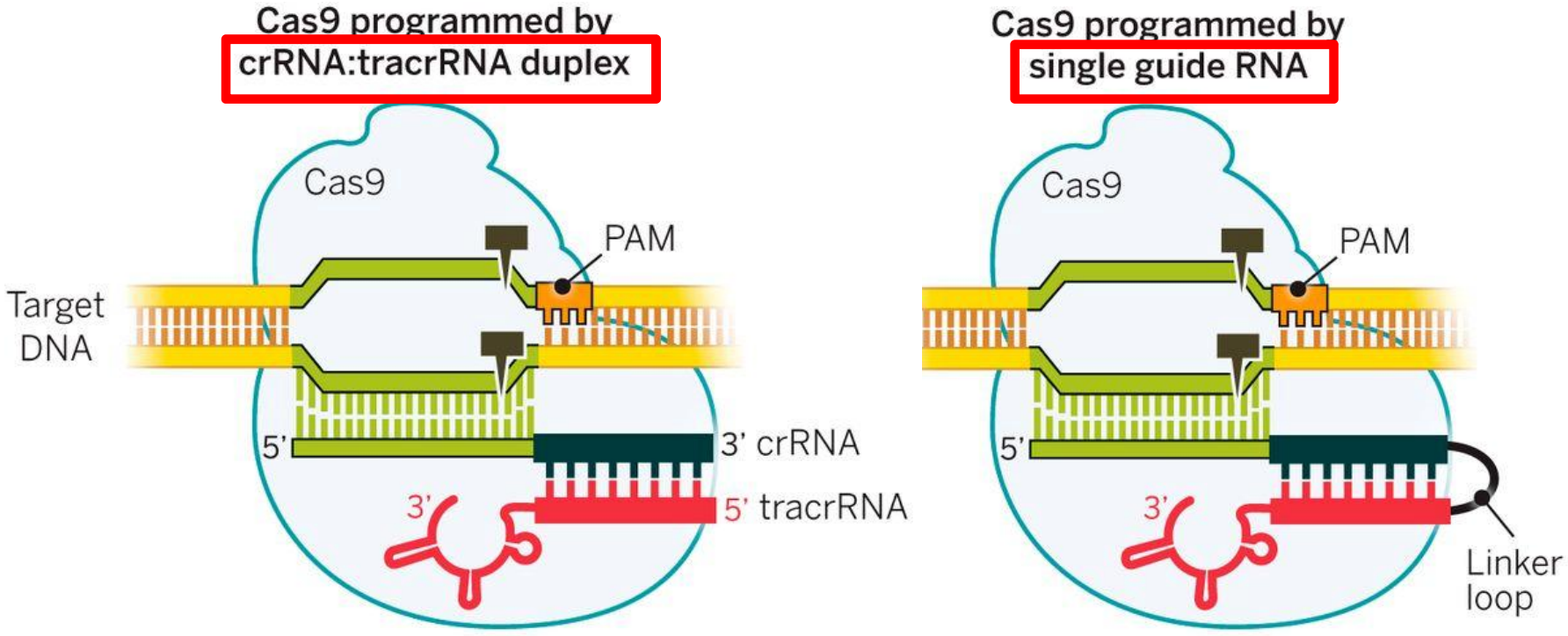
Jinek M., K. Chylinski, I. Fonfara, M. Hauer, J. A. Doudna y E. Charpentier, A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity, Science, 337(6096): 816-821, (2012).

Biology of the type II-A CRISPR-Cas system from *S. pyogenes*



Doudna J. A. y E. Charpentier, The new frontier of genome engineering with CRISPR-Cas9, Science, 346(6213): 1258096-1, (2014).

Evolution and structure of Cas9. The structure of *S. pyogenes* Cas9 in the unliganded and RNA-DNA–bound forms



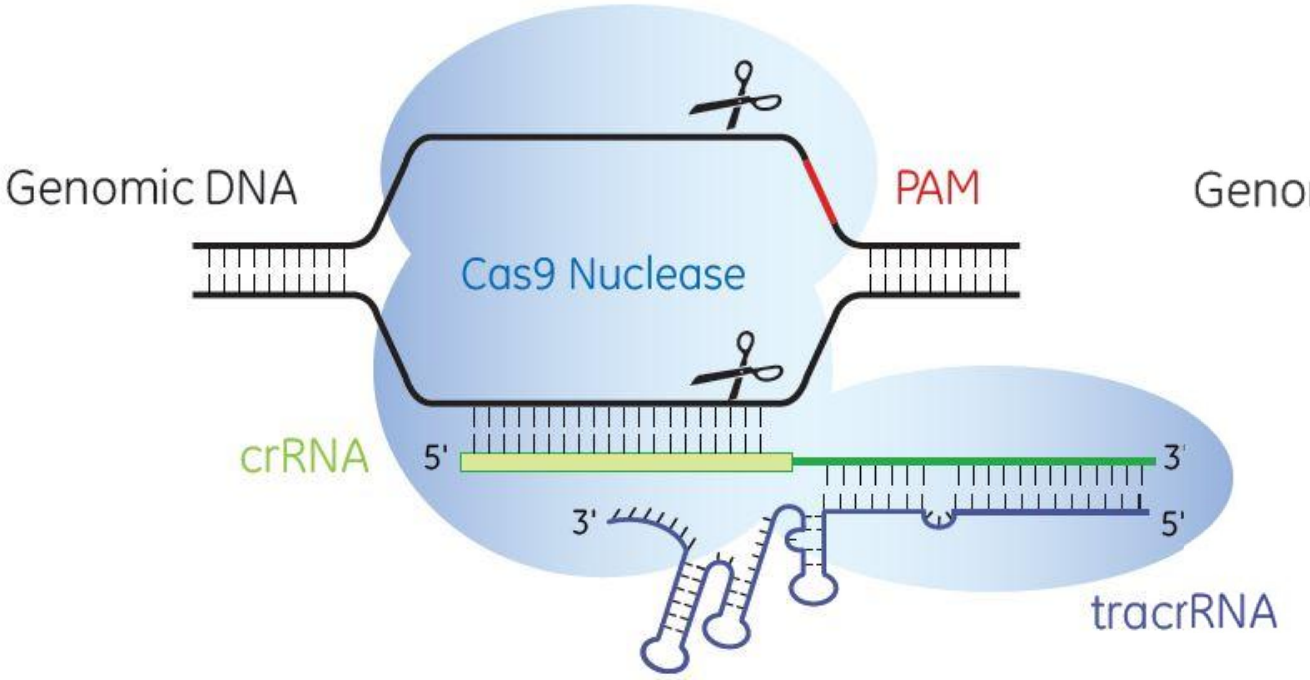
A sequence at the 5' side that determines the DNA target site by Watson-Crick base-pairing and a duplex RNA structure at the 3' side that binds to Cas9. This finding created a simple two-component system in which changes in the guide sequence of the sgRNA program Cas9 to target any DNA sequence of interest.

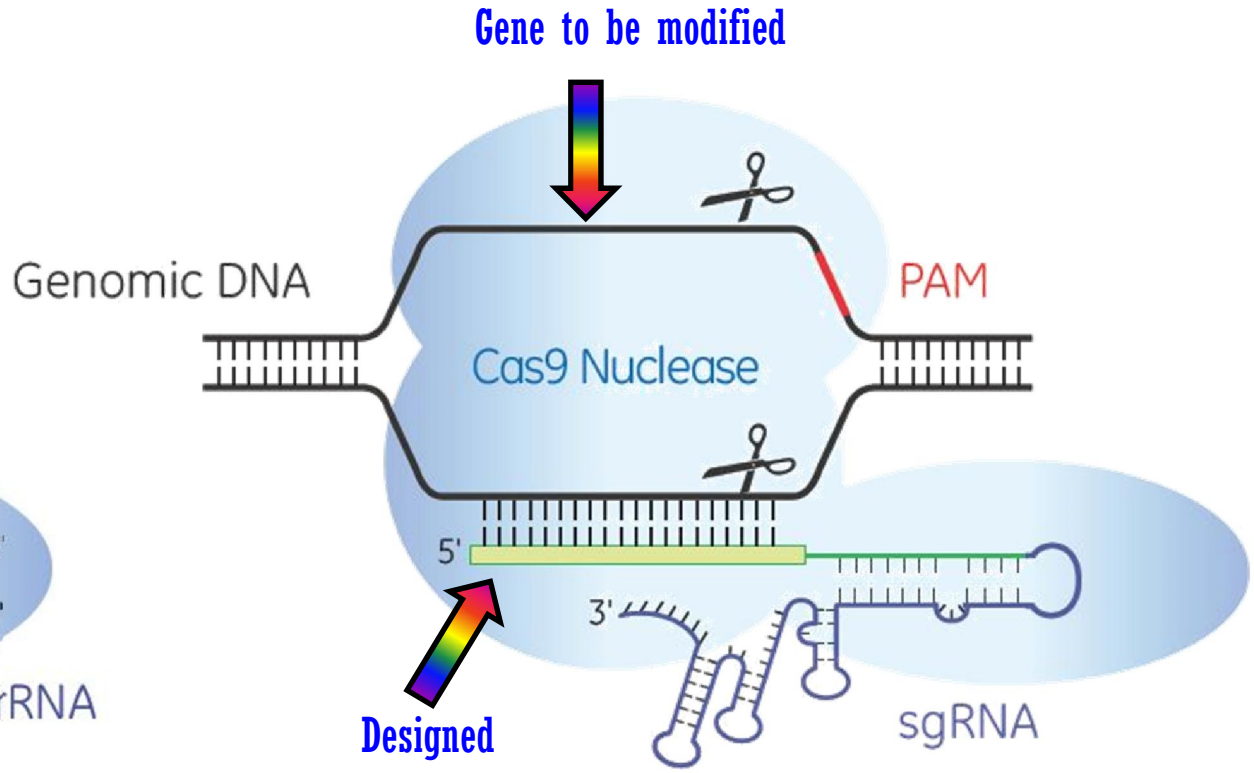
Doudna J. A. y E. Charpentier, The new frontier of genome engineering with CRISPR-Cas9, Science, 346(6213): 1258096-1, (2014).

Dharmacon

RNAi, Gene Expression & Gene Editing

This is for sale





Cas9 nuclease programmed by the **crRNA:tracr** complex cutting both strands of genomic DNA 5' of the PAM.

Cas9 nuclease programmed by the **sgRNA** complex cutting both strands of genomic DNA 5' of the PAM

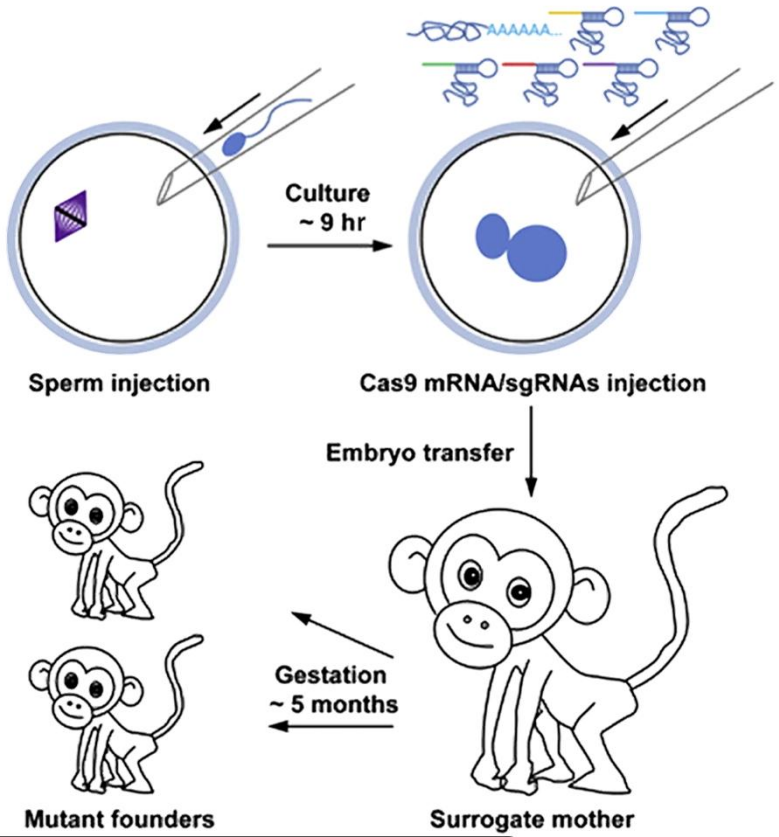
http://dharmacon.gelifesciences.com/gene-editing/crispr-cas9/crispr-guide-rna/

Breakthrough of the year 2015

Cheap, widely available, and easy to use, the genome editing system called CRISPR earned *Science*'s 2015 Breakthrough of the Year laurels for many great feats and some controversial ones—including the alteration of DNA in human embryos. Illustration: Davide Bonazzi/@SalzmanArt

CRISPR in animals

Genetically altered twin cynomolgus monkeys were created in China using the Crispr-Cas9 genome editing technique.



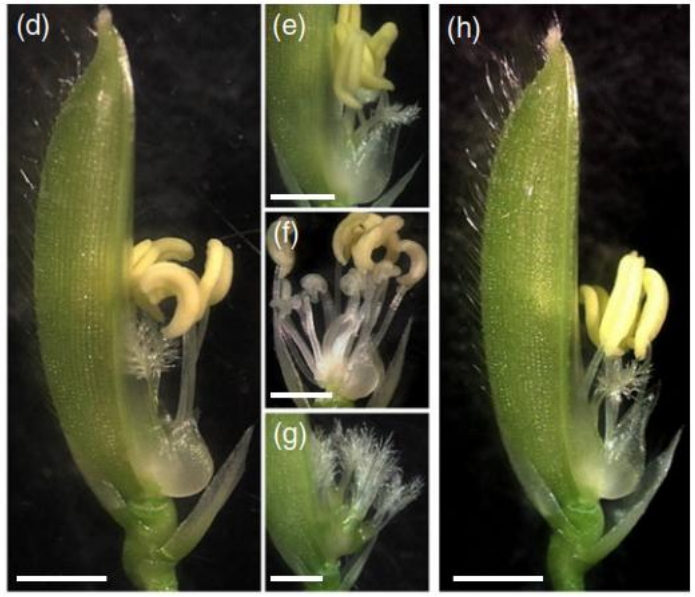
Niu Y. et al., Generation of gene-modified cynomolgus monkey via Cas9/RNA-mediated gene targeting in one-cell embryos, Cell, 156(4): 836-843, (2014).

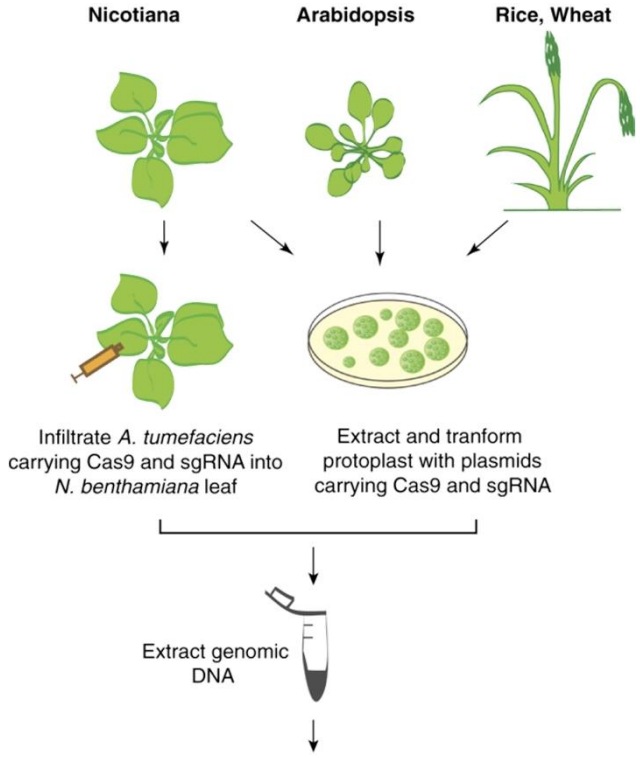
Alterations in coat color pigmentation in founder animals and their progeny

A strong reduction of coat color pigmentation is observed in mice carrying the deletion of the *Tyr 5* boundary element. All F1 progeny obtained from founder TYRINS5#18 (shown in B) show uniformly reduced pigmentation, indicating that both copies of the *Tyr 5* boundary element were deleted in this founder.

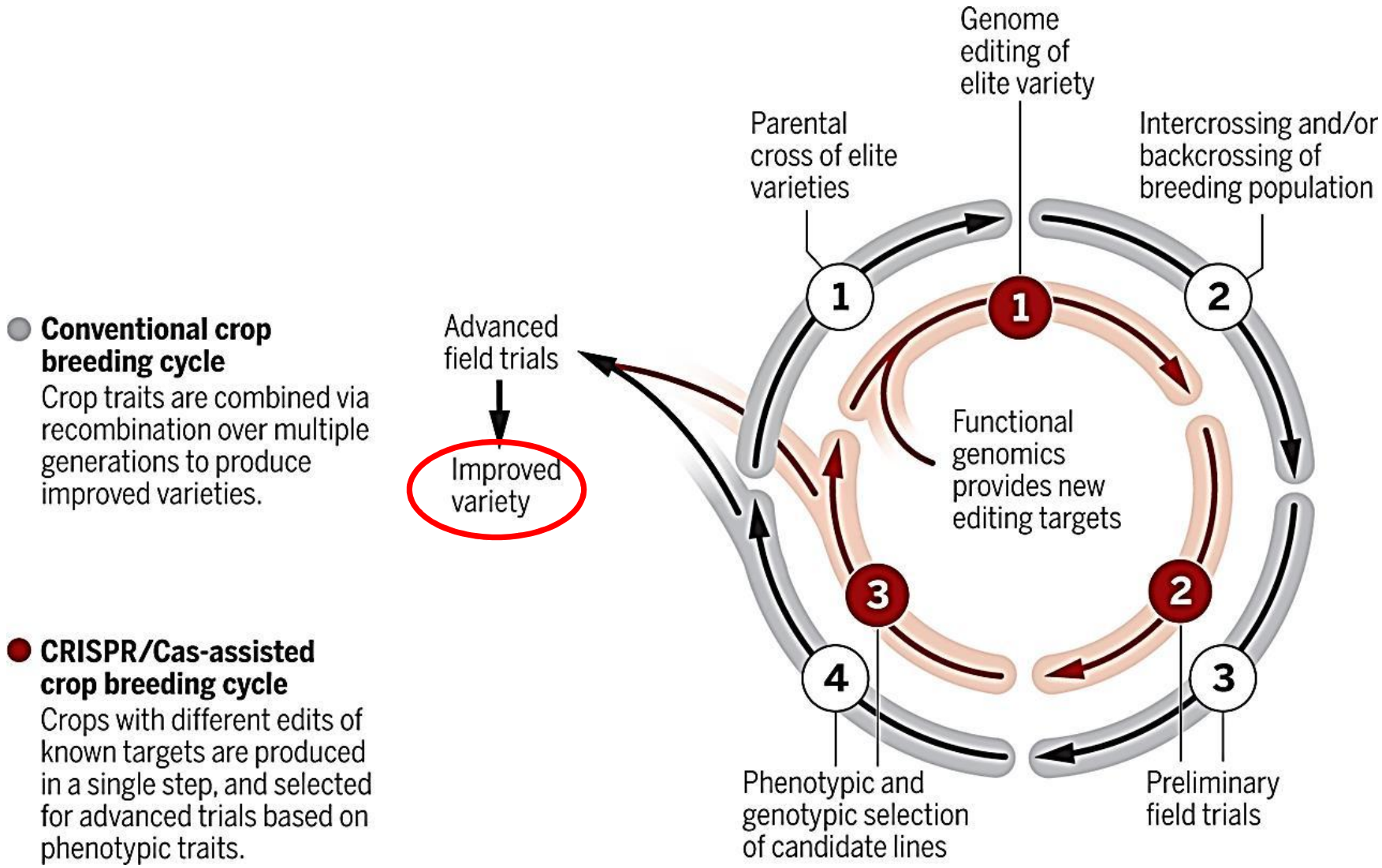
Seruggia D., A. Fernández, M. Cantero, P. Pelczar y L. Montoliu, Functional validation of mouse tyrosinase non-coding regulatory DNA elements by CRISPR-Cas9-mediated mutagenesis, Nucleic Acids Res., 43(10): 4855-4867, (2015).

CRISPR in plants





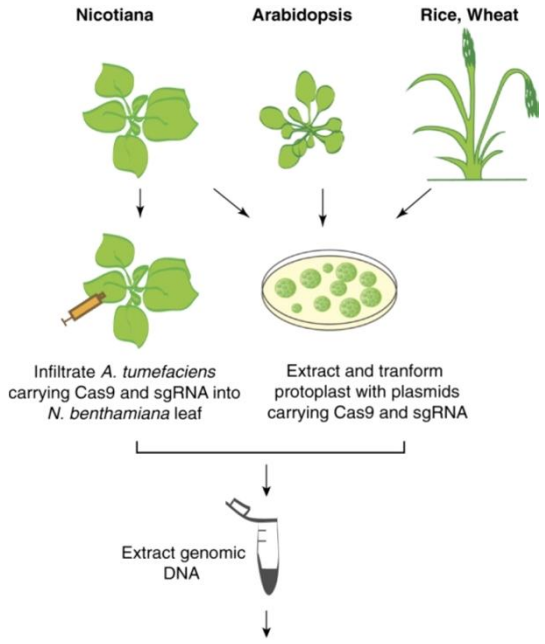
Crop improvement schemes



A. Scheben, D. Edwards, Science 355: 1122, (2017).

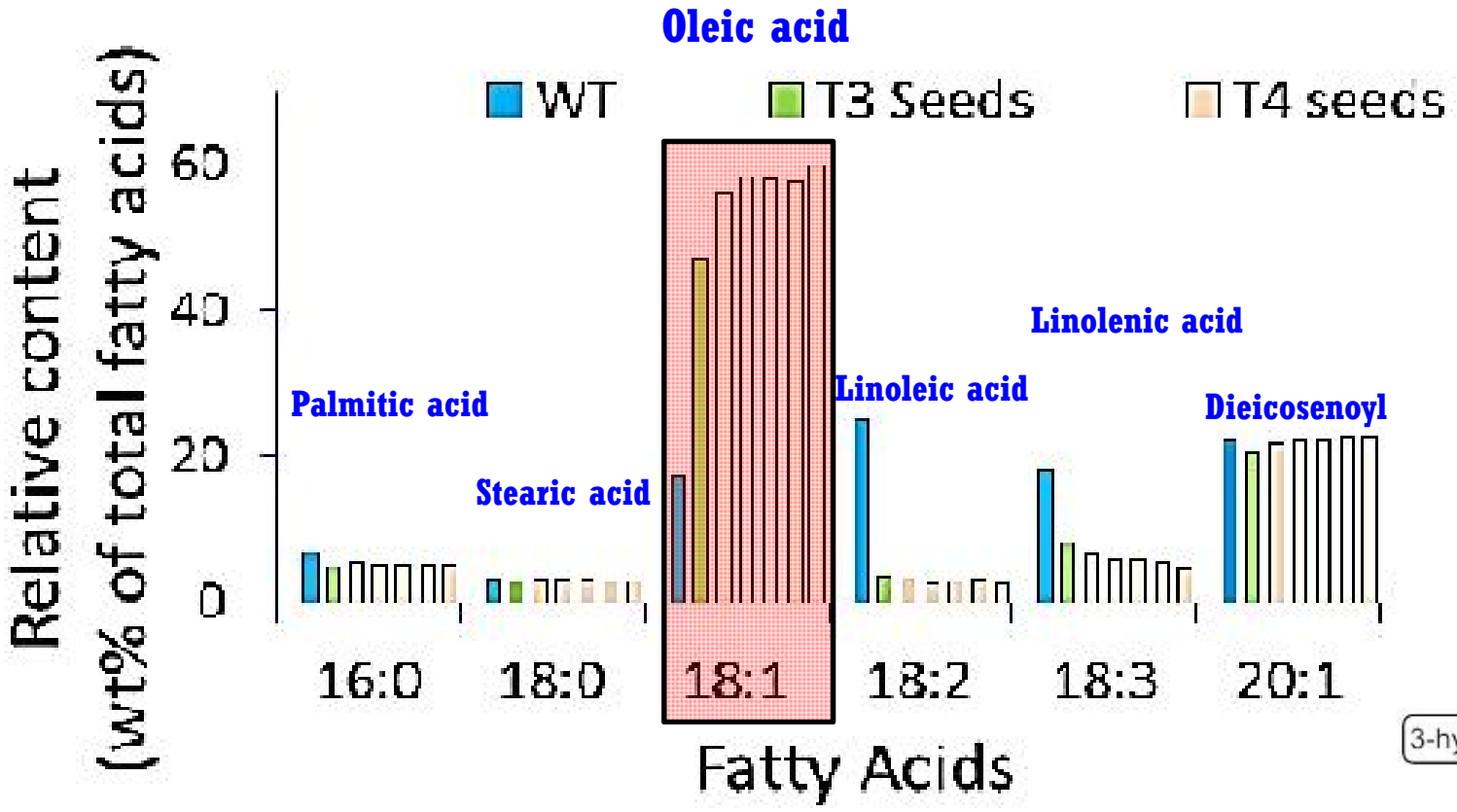
First papers in plants

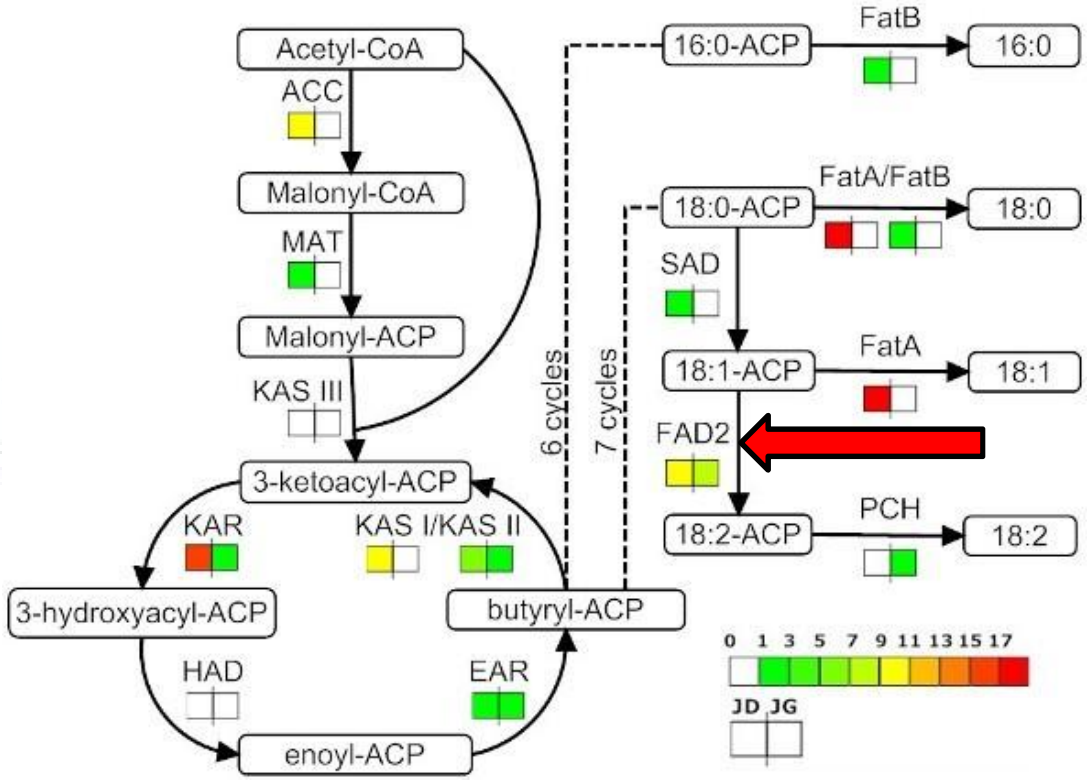
Six different plants



DoL	Species	Tissues	Ref
8 August	*A. thaliana*	Protoplasts	Li J. F. et al., Nat. Biotechnol., 31(8): 688-691, (2013).
	N. benthamiana	Protoplasts	
8 August	*N. benthamiana*	Leaves	Nekrasov V. et al., Nat. Biotechnol., 31(8): 691-693, (2013).
8 August	*O. sativa*	Protoplasts	Shan Q. et al., Nat. Biotechnol., 31(8): 686-688, (2013).
	T. aestivum	Protoplasts	
12 August	*A. thaliana*	Protoplasts	Mao Y. et al., Mol. Plant, 6(6): 2008-2011, (2013).
	O. sativa	Plantlets	
17 August	*O. sativa*	Protoplasts	Xie K. y Y. Yang, Mol. Plant, 6(6): 1975-1983, (2013).
20 August	*A. thaliana*	Protoplasts	Feng Z. et al., Cell Res., 23(10): 1229-1232, (2013).
	O. sativa	Plants	
2 September	*A. thaliana*	Leaves	Jiang W. et al., Nucleic Acids Res., 41(20): e188, (2013).
	N. tabacum	Leaves	
	S. bicolor	Zygotic E.	
	O. sativa	Protoplasts	
3 September	*O. sativa*	Calli	Miao J. et al., Cell Res., 23(10): 1233-1236, (2013).
11 October	*T. aestivum*	SC	Upadhyay S. K. et al., G3, 3(12): 2233-2238, (2013).
	N. benthamiana	Leaves	

Seed oil profile of seeds from Arabidopsis plants transformed using Cas9/sgRNA targeting the R2 site of the AtFAD2 gene



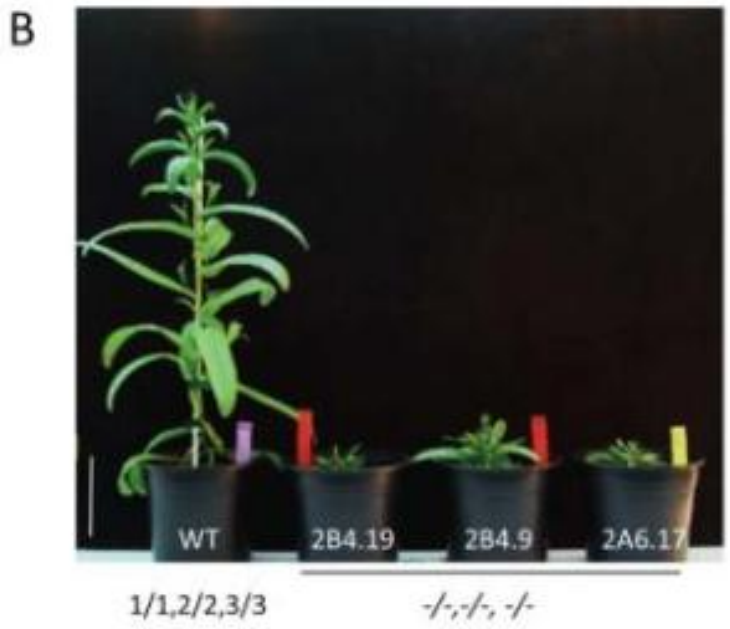


Jiang W. Z. et al., Significant enhancement of fatty acid composition in seeds of the allohexaploid, *Camelina sativa*, using CRISPR/Cas9 gene editing, Plant Biotechnol. J., (2017). (In press).

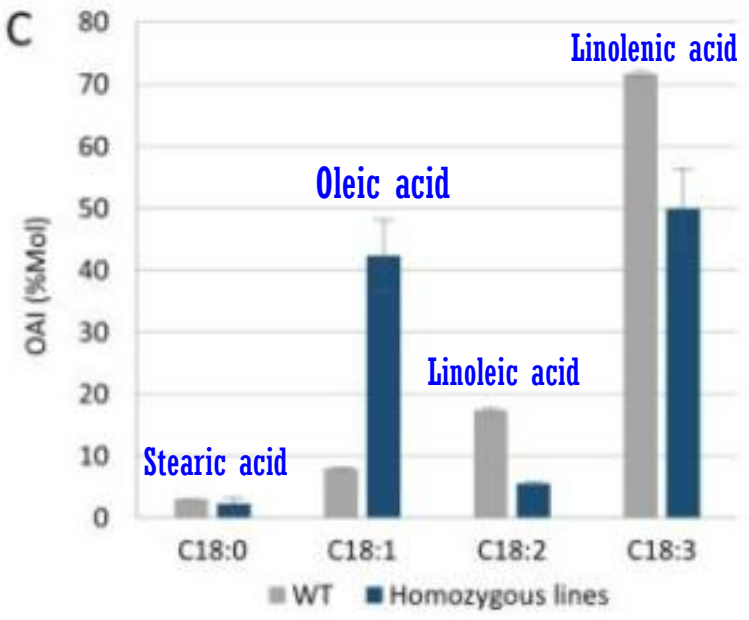
Costa G. et al., Transcriptome analysis of the oil-rich seed of the bioenergy crop *Jatropha curcas* L, BMC Genomics, 11(1): 462, (2010).

csfad2 mutants

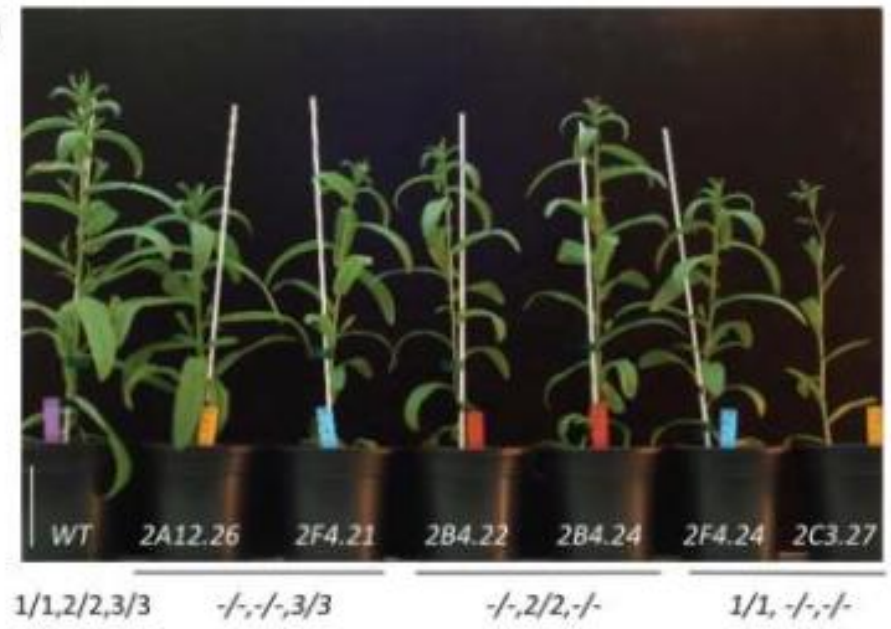
Three Δ-12-desaturase (*FAD2) genes*



Phenotype of two months-old triple homozygous *csfad2* mutants



OAI of leaves from WT and triple *csfad2* mutants



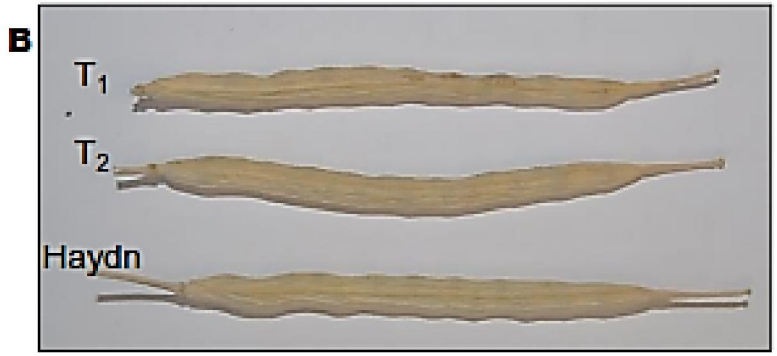
Phenotype of two months-old double homozygous *csfad2* mutants

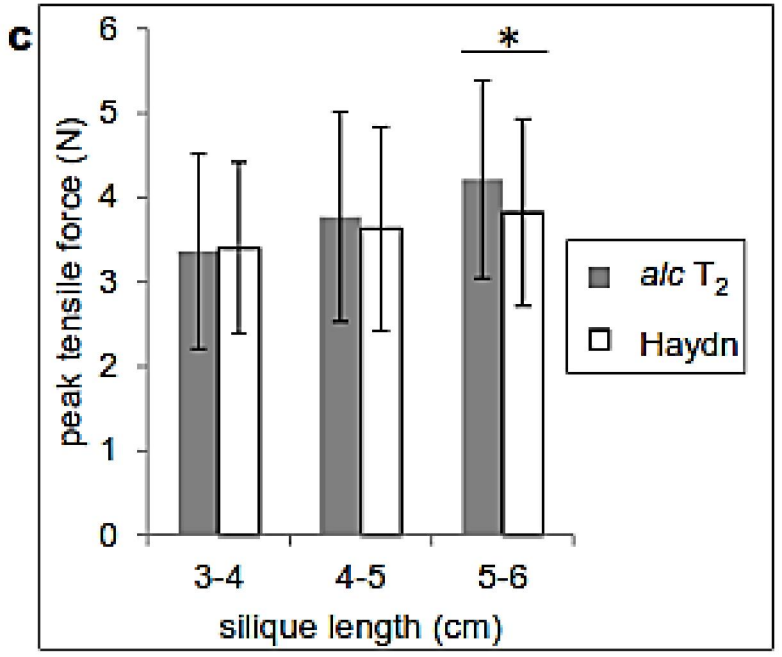
Analysis of mutations over four generations demonstrated the presence of a large variety of heritable mutations in the three isologous *CsFAD2 genes*

Morineau C. et al., Selective gene dosage by CRISPR-Cas9 genome editing in hexaploid *Camelina sativa*, Plant Biotechnol. J., (2017). (In press).

Growth types of CRISPR-Cas9 *alc* mutants

ALCATRAZ (ALC) is involved in valve-margin development and thus contributes to seed shattering from mature fruits





Shatter resistance

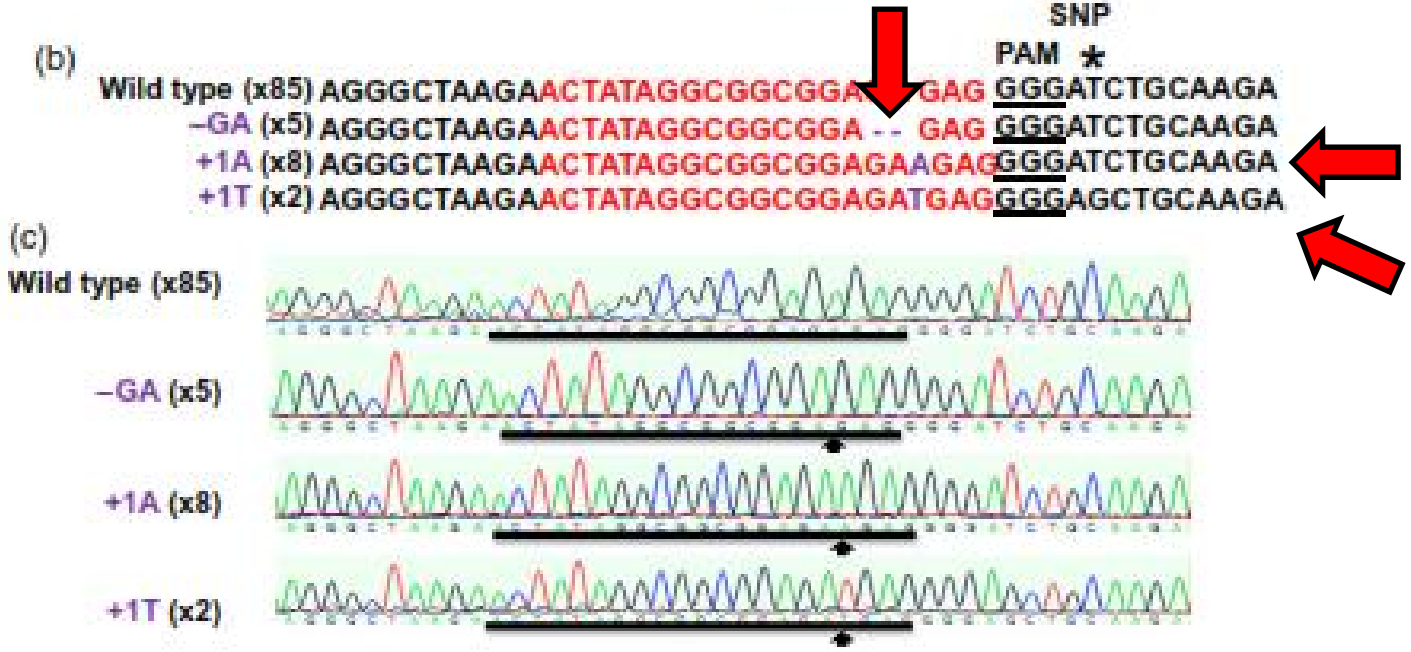
Braatz J. et al., CRISPR-Cas9 targeted mutagenesis leads to simultaneous modification of different homoeologous gene copies in polyploid oilseed rape (*Brassica napus* L.), Plant Physiol., (2017). (In press).

Function analysis of GFP-p1380N-Cas9/sgRNA:cslob1 in Duncan leaves with the aid of GFP

Four days after agroinfiltration with Agrobacterium cells harbouring

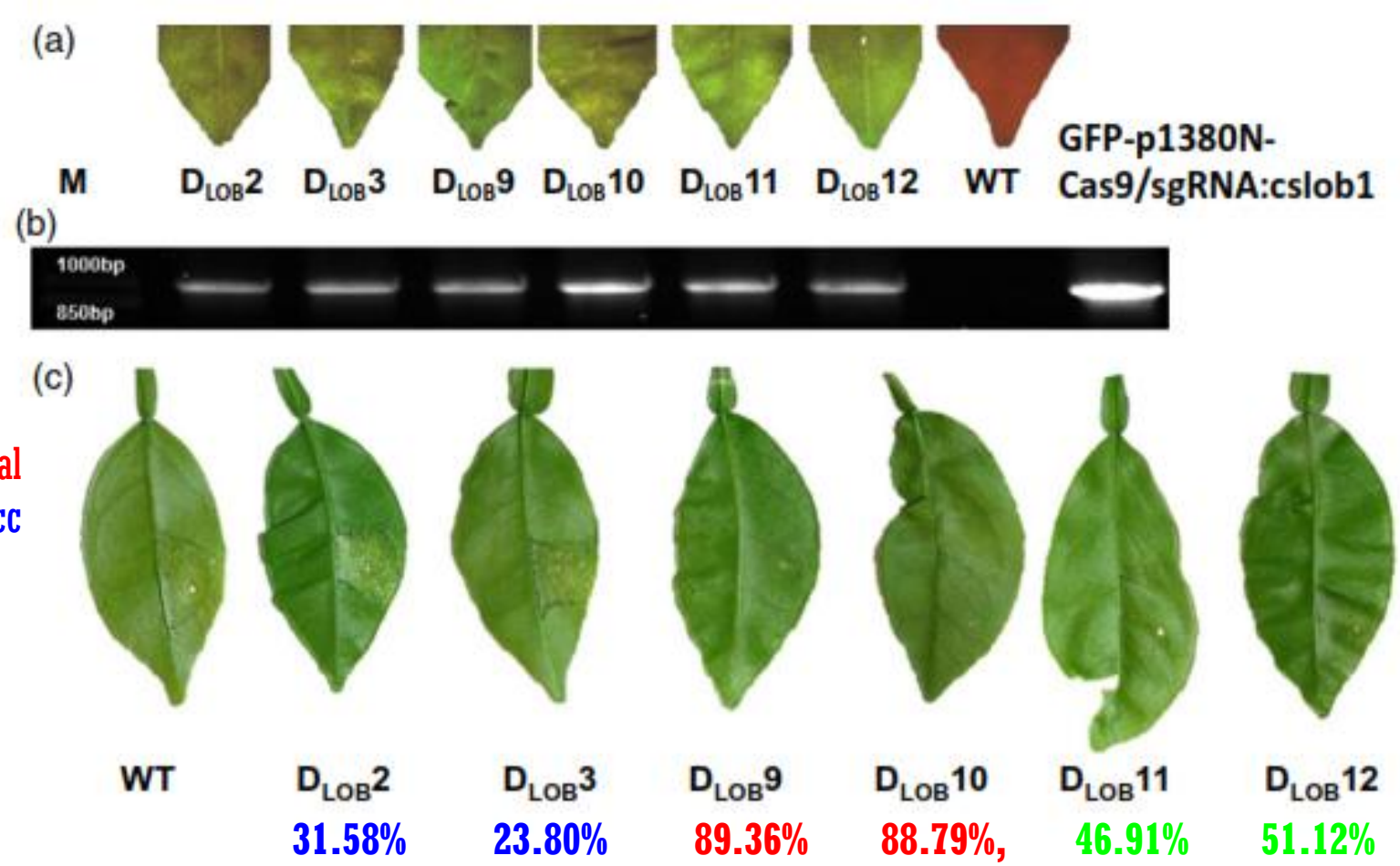
Negative control

Used of CRISPR/Cas9/sgRNA technology to modify the canker susceptibility gene CsLOB1.



Jia H. et al., Genome editing of the disease susceptibility gene CsLOB1 in citrus confers resistance to citrus canker, Plant Biotechnol. J., (2017). (In press).

Analysis of GFP-p1380N-Cas9/sgRNA:cslob1-transformed Duncan grapefruit



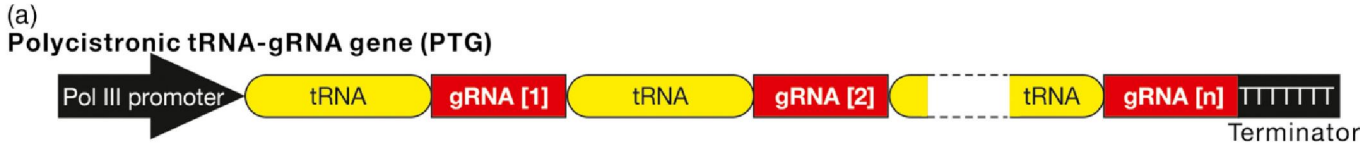
The six CsLOB1-modified lines showed differential resistance to Xcc

Mutation rate	**31.58%**	**23.80%**	**89.36%**	**88.79%,**	**46.91%**	**51.12%**

Canker symptoms (*Xanthomonas citri* subsp. Citri) were observed on normal grapefruit, LOB2 and DLOBD3, but absent or reduced on DLOB9, DLOB10, DLOB11 and DLOB12.

Jia H. et al., Genome editing of the disease susceptibility gene CsLOB1 in citrus confers resistance to citrus canker, Plant Biotechnol. J., (2017). (In press).

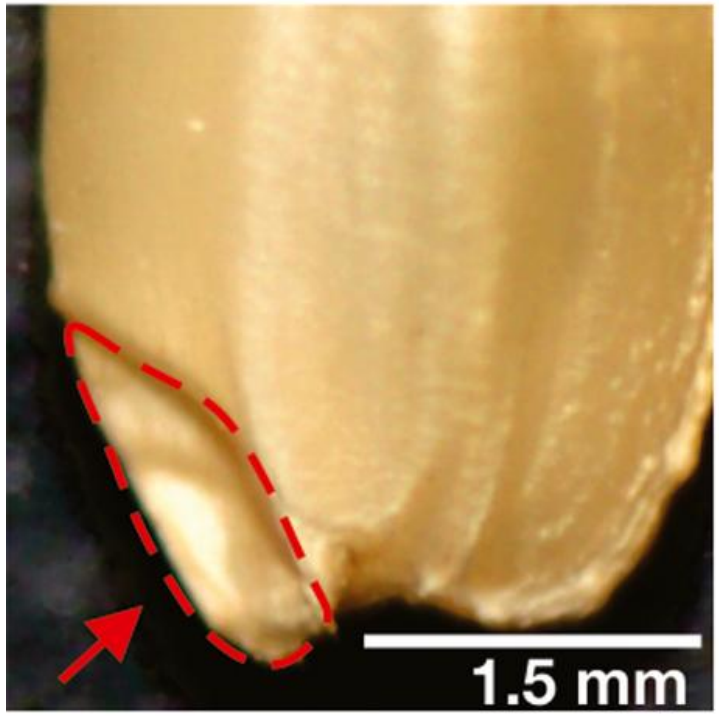
Discovery of rice essential genes by characterizing a CRISPR-edited mutation of closely related rice MAP kinase genes



The polycistronic tRNA-gRNA gene (PTG) processing system and design of PTGs to target four *MPKs*. (a) A PTG consists of tRNA-gRNA repeats transcribed by a *Po*AIII promoter.

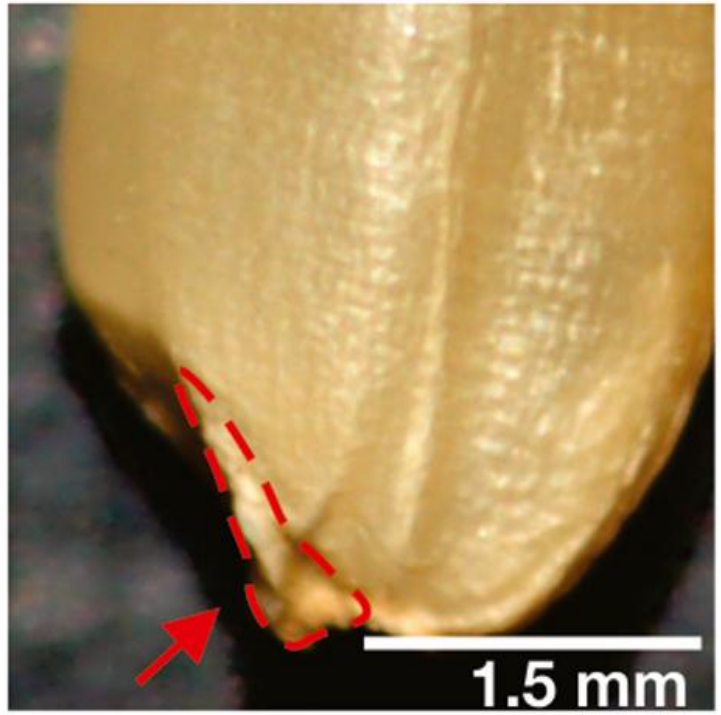
Minkenberg B., K. Xie y Y. Yang, Discovery of rice essential genes by characterizing a CRISPR-edited mutation of closely related rice MAP kinase genes, Plant J., 89(3): 636-648, (2017).

Phenotypes of true mpk1 knock-out plants and seeds from heterozygous parents



Normal embryo

84

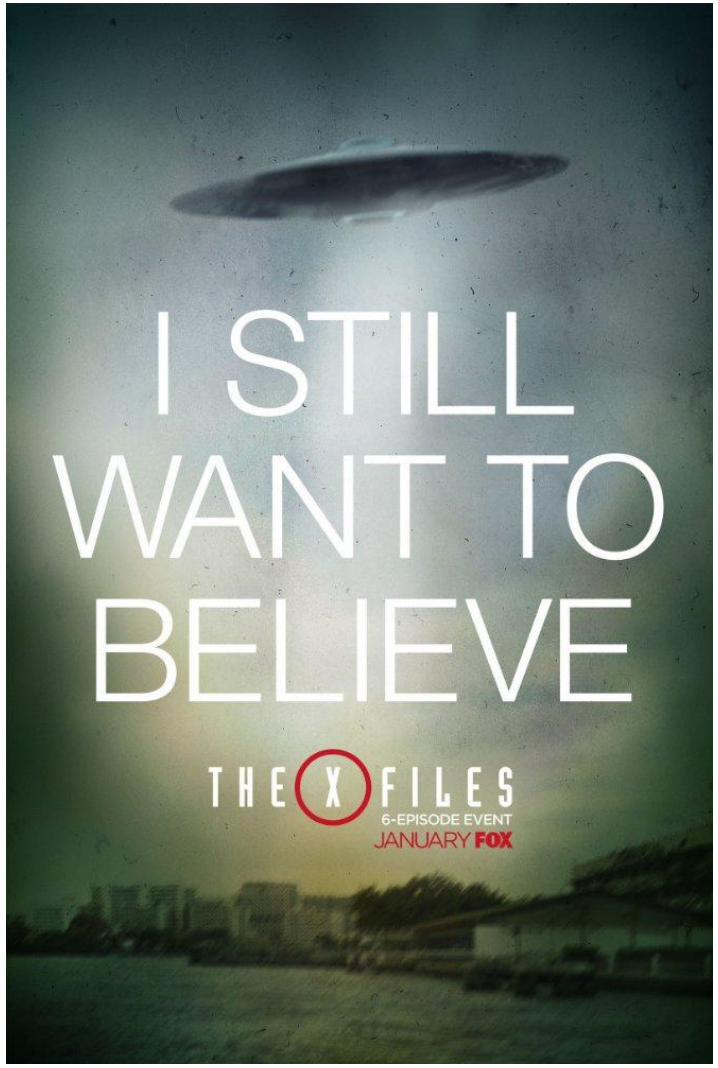


Abnormal small embryo

26

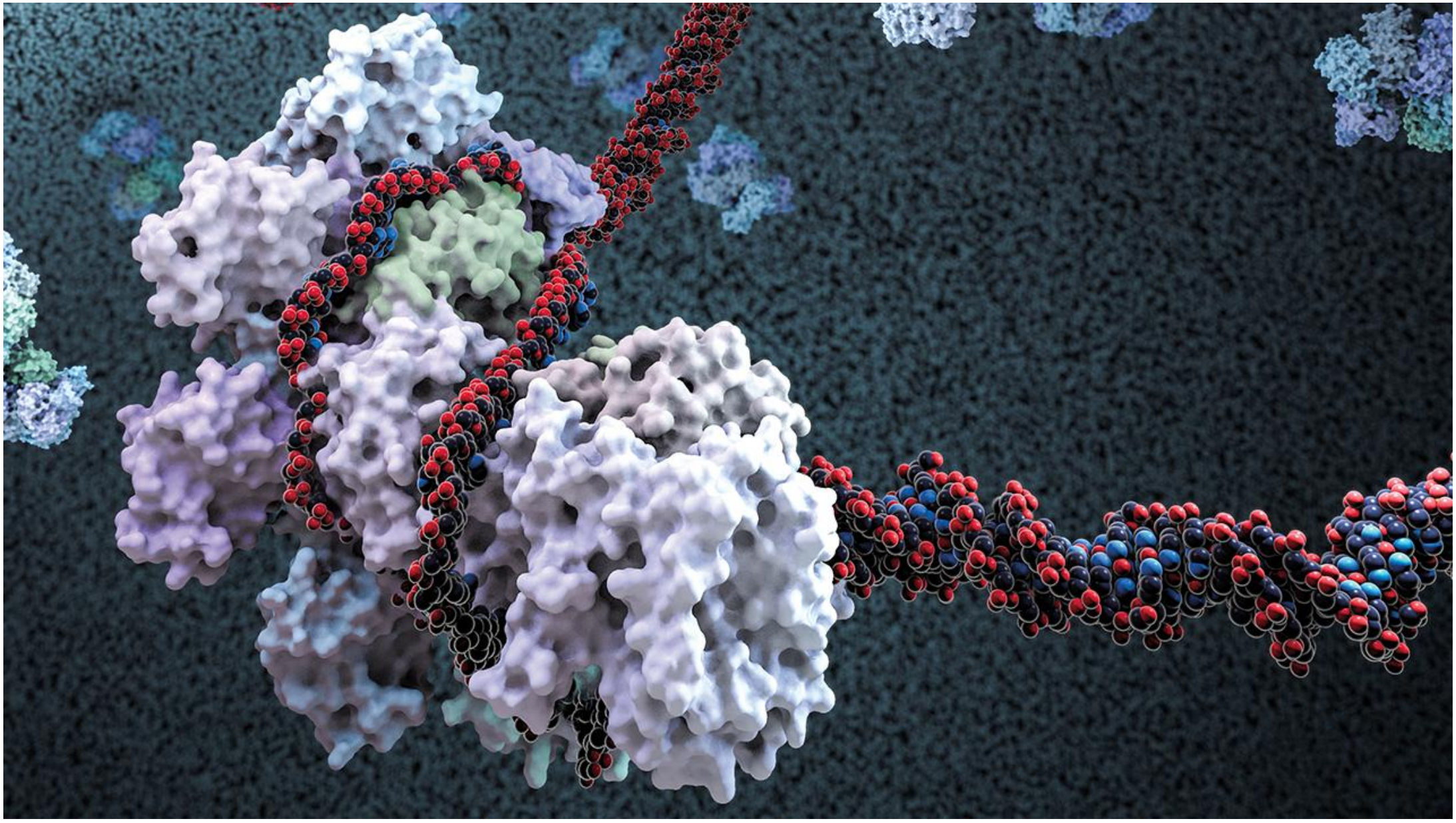
Minkenberg B., K. Xie y Y. Yang, Discovery of rice essential genes by characterizing a CRISPR-edited mutation of closely related rice MAP kinase genes, Plant J., 89(3): 636-648, (2017).

The Red Queen strike back



- In an episode of the resurrected X-Files TV show that ran earlier 2016, aliens attack Earth with a bioweapon based on **CRISPR**. Agent Dana Scully resists the attack because her genome earlier had incorporated some alien DNA that had anti-CRISPR defenses. Now, the art that imitated the science is being followed by the science imitating the art: Researchers have found for the first time anti-CRISPR proteins that shut off the genome editor and shown they can use them to control the cutting of DNA in human cells.

http://www.sciencemag.org/news/2016/12/stopping-crispr-s-genome-editing-scissors-snipping-out-control



There are several proteins that stop the Cas9 enzyme (white) from cutting DNA.

http://www.sciencemag.org/news/2016/12/stopping-crispr-s-genome-editing-scissors-snipping-out-control

LETTER

Bondy-Denomy J. et al., Bacteriophage genes that inactivate the CRISPR/Cas bacterial immune system, Nature, 493(7432): 429-432, (2013).

doi:10.1038/nature11723

Bacteriophage genes that inactivate the CRISPR/Cas bacterial immune system

Joe Bondy-Denomy[1], April Pawluk[2], Karen L. Maxwell[3] & Alan R. Davidson[1,2]

LETTER

Bondy-Denomy J. et al., Multiple mechanisms for CRISPR-Cas inhibition by anti-CRISPR proteins, Nature, 526(7571): 136-139, (2015).

doi:10.1038/nature15254

Multiple mechanisms for CRISPR–Cas inhibition by anti-CRISPR proteins

Joseph Bondy-Denomy[1]†, Bianca Garcia[1], Scott Strum[2], Mingjian Du[1], MaryClare F. Rollins[3], Yurima Hidalgo-Reyes[1], Blake Wiedenheft[3], Karen L. Maxwell[4] & Alan R. Davidson[1,2]

nature microbiology

LETTERS

PUBLISHED: 13 JUNE 2016 | ARTICLE NUMBER: 16085 | DOI: 10.1038/NMICROBIOL.2016.85

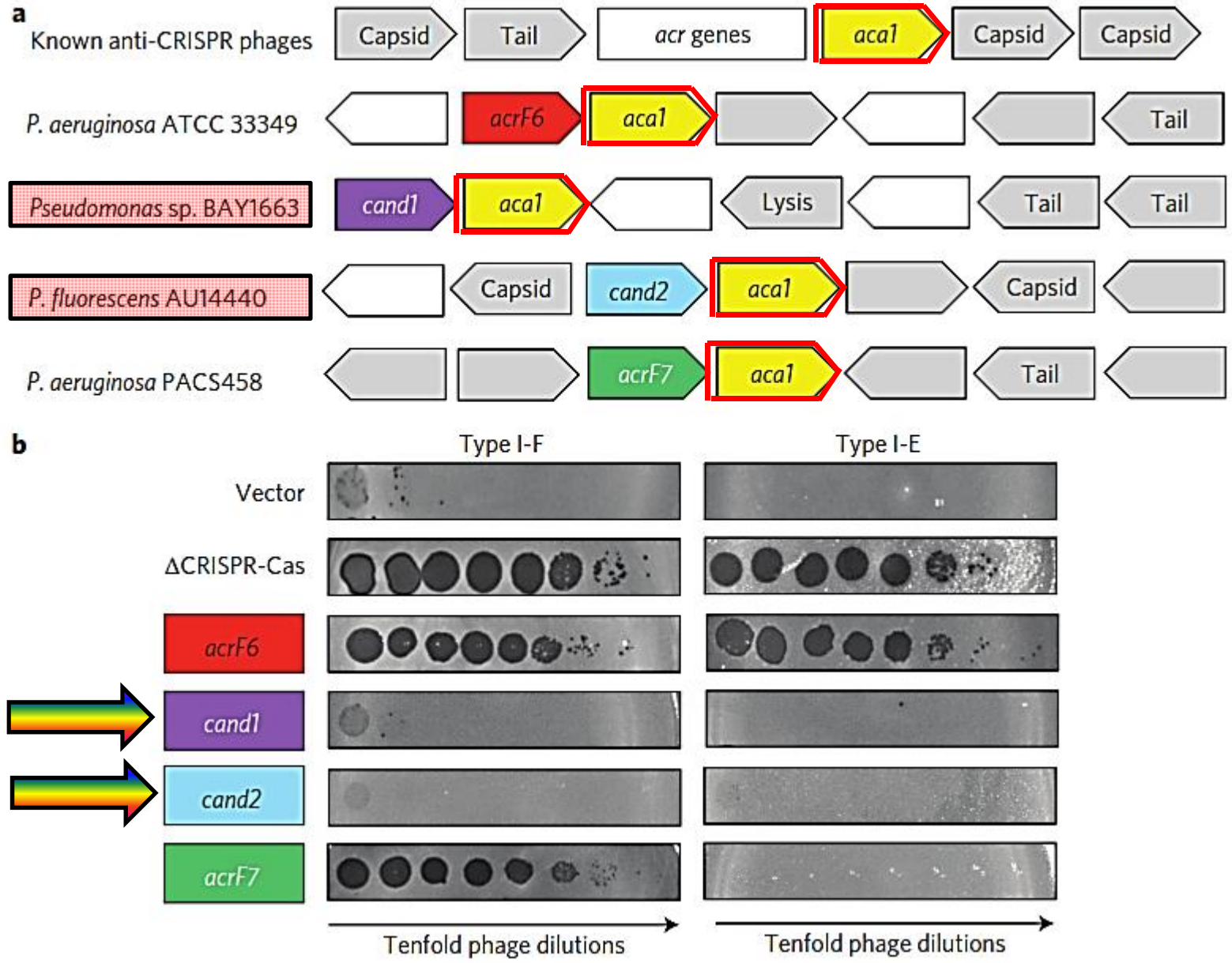
Inactivation of CRISPR-Cas systems by anti-CRISPR proteins in diverse bacterial species

April Pawluk[1], Raymond H.J. Staals[2], Corinda Taylor[2], Bridget N.J. Watson[2], Senjuti Saha[3], Peter C. Fineran[2], Karen L. Maxwell[4]* and Alan R. Davidson[1,3]*

Pawluk A. et al., Inactivation of CRISPR-Cas systems by anti-CRISPR proteins in diverse bacterial species, Nature Microbiology, 11: 6085, (2016).

Discovery and characterization of aca1-associated anti-CRISPR genes

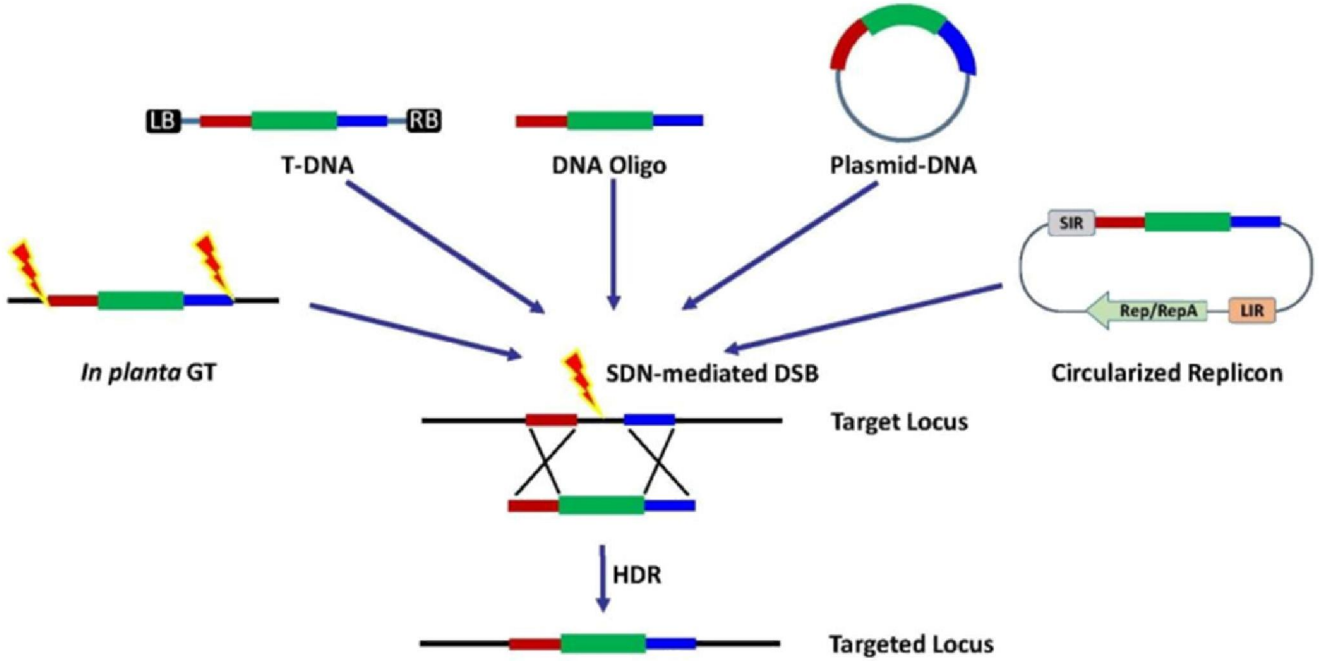
Given the widespread occurrence and promiscuous activity of the anti-CRISPRs described here, we propose that anti-CRISPRs play an influential role in facilitating the movement of DNA between prokaryotes by breaching the barrier imposed by CRISPR-Cas systems.

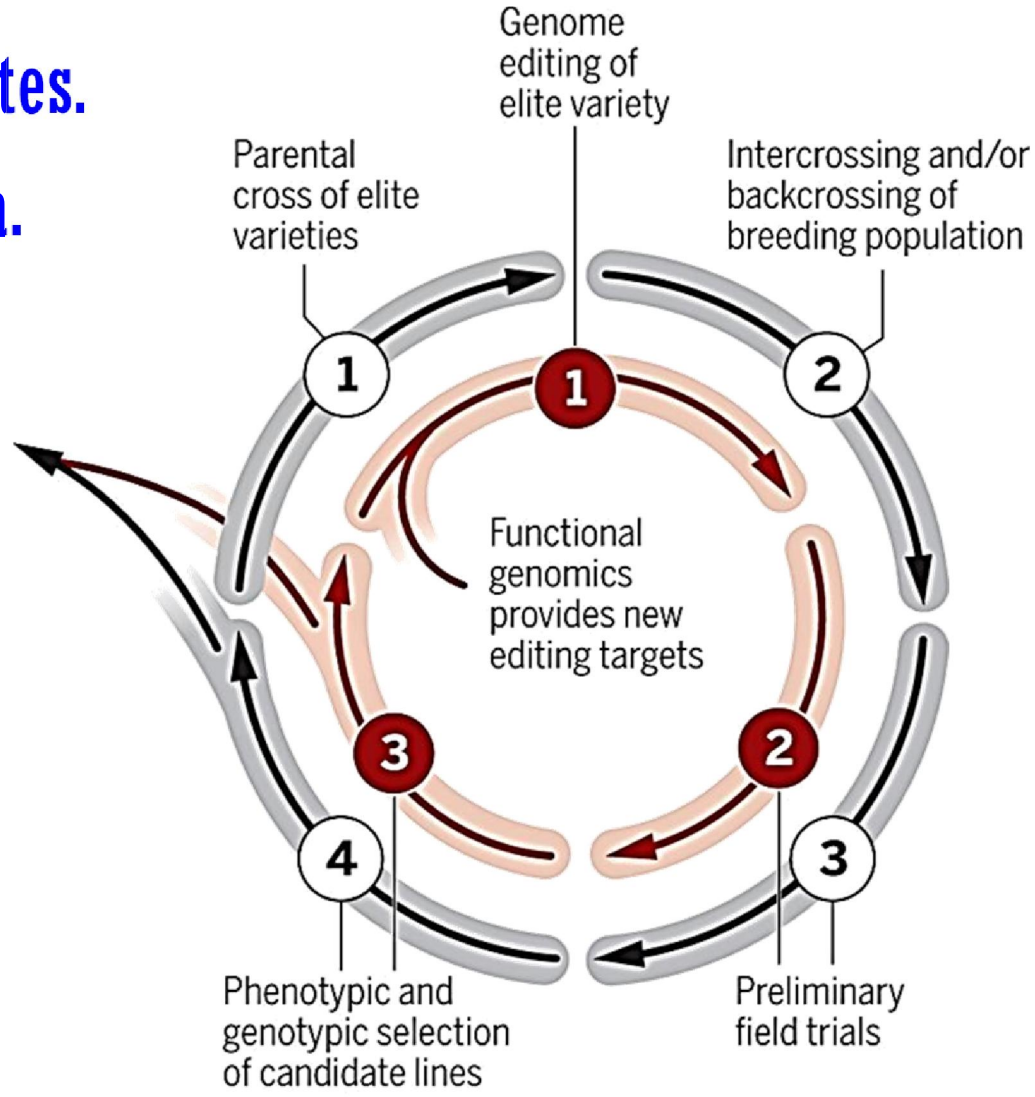


Pawluk A. et al., Inactivation of CRISPR-Cas systems by anti-CRISPR proteins in diverse bacterial species, Nature Microbiology, 11: 6085, (2016).

Conclusions

- CRISPR are present in most of the prokaryotes.
- The CRISPR array can be used with BT aim.
- Plants can be manipulated using CRISPR.





Pacher M. y H. Puchta, From classical mutagenesis to nuclease-based breeding - directing natural DNA repair for a natural end-product, Plant J., 90(4): 819-833, (2017).

¡¡¡GRACIAS!!!