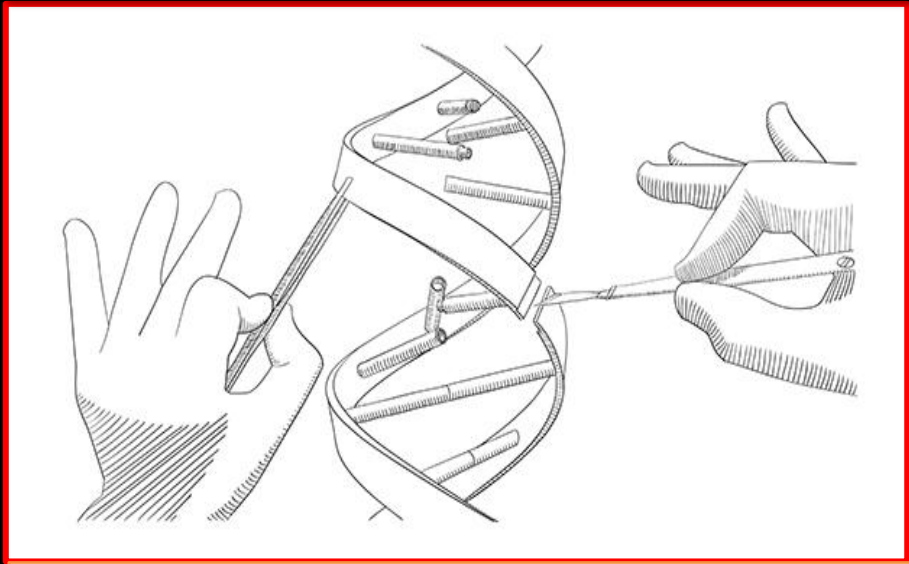
Seventh Asian Cotton Research and Development Network (ACRDN) Meeting

September 15-17,2017

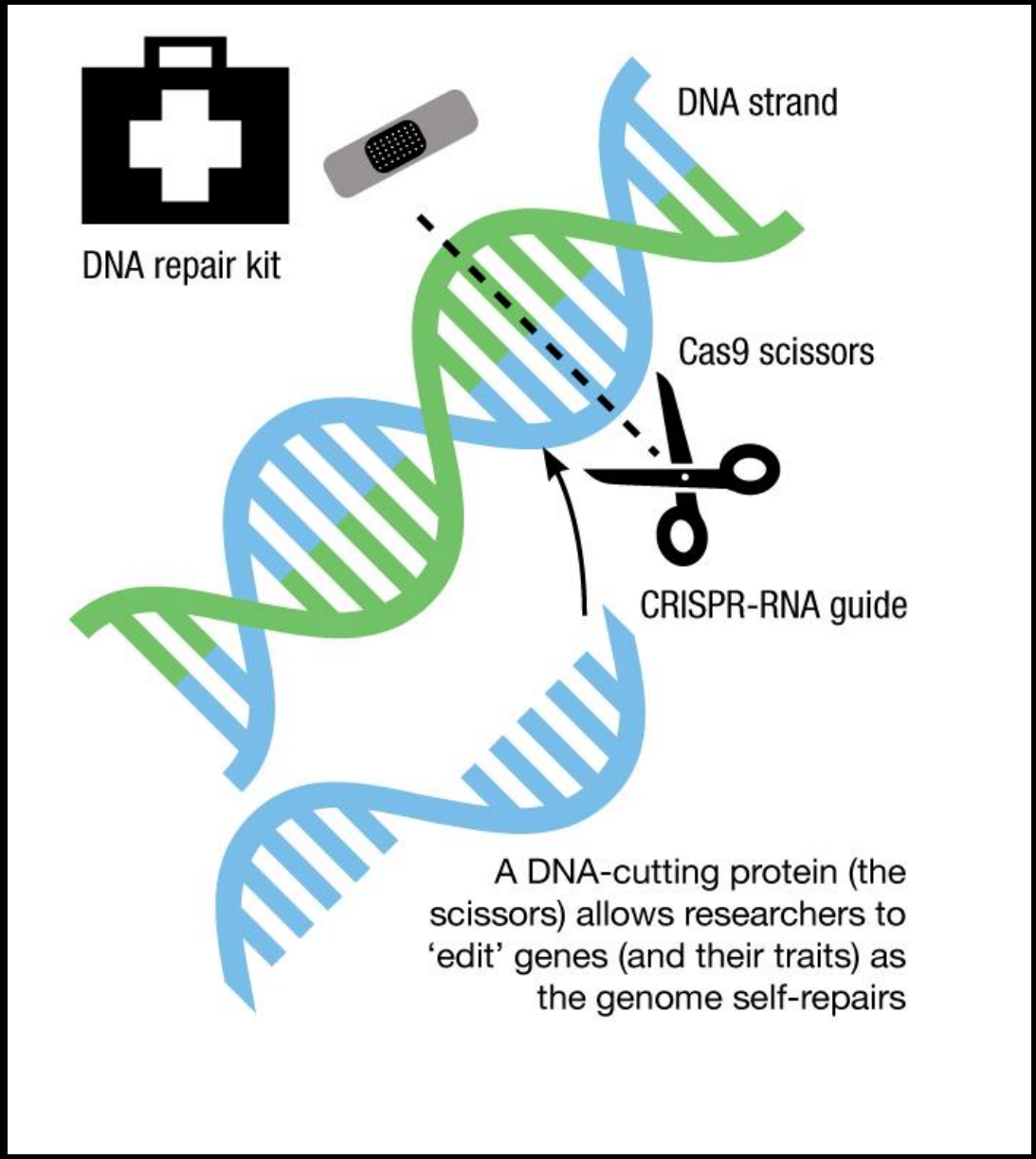
CRISPR/Cas technology for next-generation cotton: an overview

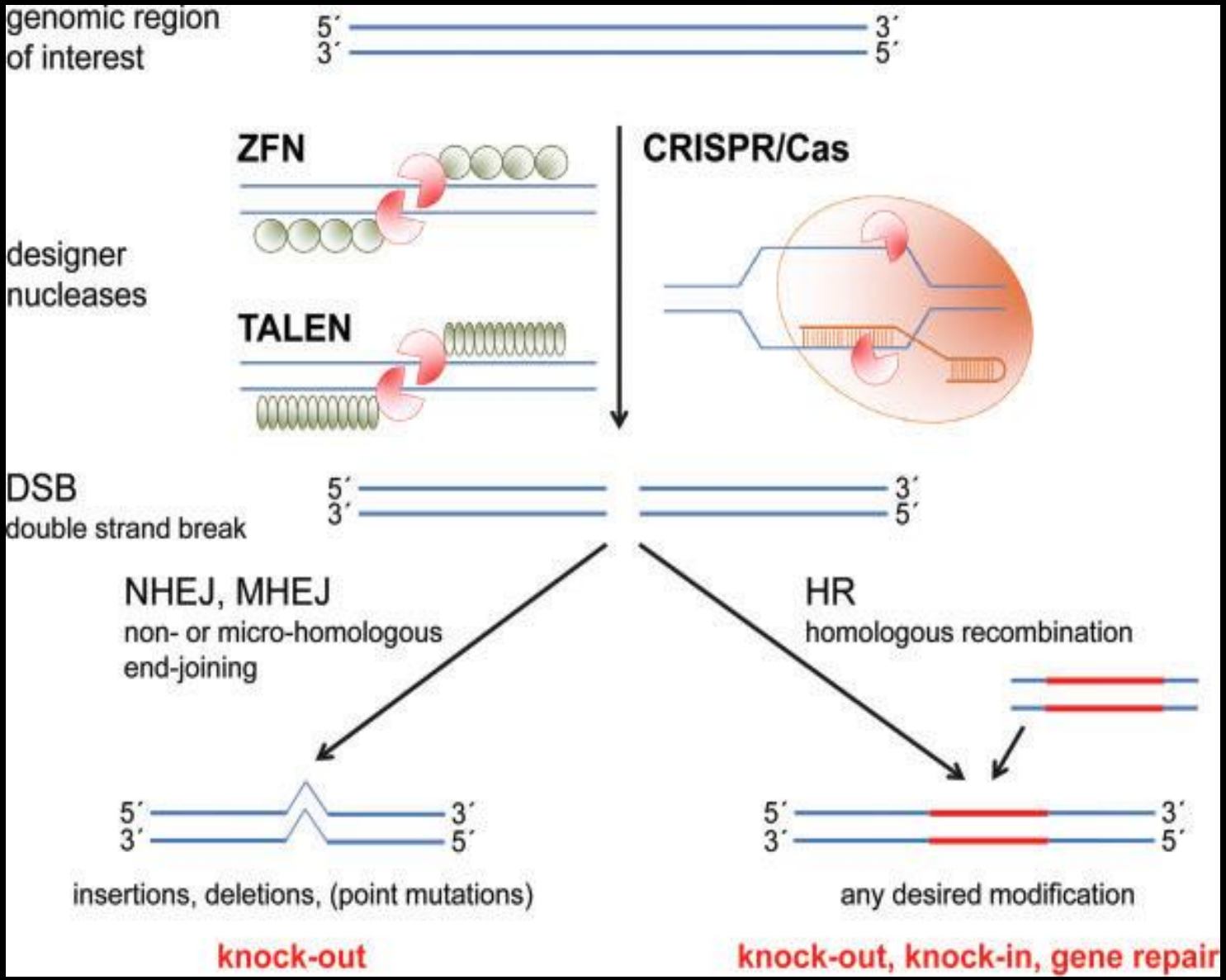


A Presentation By:

JOY DAS

Scientist, ICAR-CICR


DNA repair kit
DNA strand
Cas9 scissors
CRISPR-RNA guide
A DNA-cutting protein (the scissors) allows researchers to 'edit' genes (and their traits) as the genome self-repairs



Klymiuk *et al.* 2015, *Toxic. Pathol.*

The Discovery.....

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

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

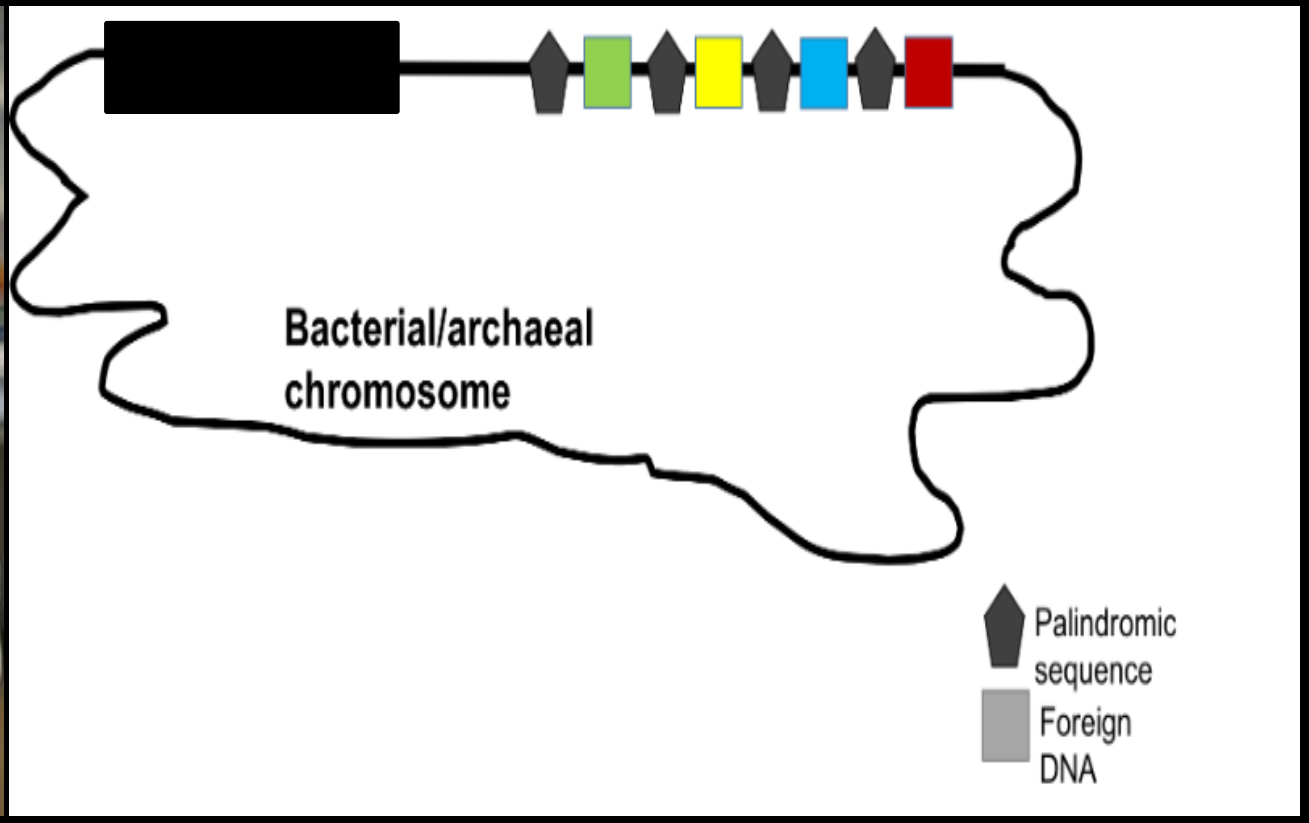
```
TGAAAATGGGAGGGAGTTCTACCGCAGAGGCGGGGGAACTCCAAGTGATATCCATCATCGCATCCAGTGCGCC (1,451)
(1,452) CGGTTTATCCCCGCTGATGCGGGGAACACCAGCGTCAGGCGTGAAATCTCACCGTCGTTGC (1,512)
(1,513) CGGTTTATCCCTGCTGGCGCGGGGAACTCTCGGTTCAGGCGTTGCAAACCTGGCTACCGGG (1,573)
(1,574) CGGTTTATCCCCGCTAACGCGGGGAACTCGTAGTCCATCATTCCACCTATGTCTGAACTCC (1,634)
(1,635) CGGTTTATCCCCGCTGGCGCGGGGAACTCG (1,664)

consensus: CGGTTTATCCCCGCT GG/AA CGCGGGGAACTC
```

An unusual structure was found in the 3′-end flanking region of *iap* (Fig. 5). Five highly homologous sequences of 29 nucleotides were arranged as direct repeats with 32 nucleotides as spacing. The first sequence was included in the putative transcriptional termination site and had less homology than the others. Well-conserved nucleotide sequences containing a dyad symmetry, named REP sequences, have been found in *E. coli* and *Salmonella typhimurium* (28) and may act to stabilize mRNA (18). A dyad symmetry with 14 nucleotide pairs was also found in the middle of these sequences (underlining, Fig. 5), but no homology was found between these sequences and the REP sequence. So far, no sequence homologous to these has been found elsewhere in procaryotes, and the biological significance of these sequences is not known.

1990-2002

“pedestrian zebra crossing-like”

Francisco Mojica



https://www.linkedin.com/pulse/crispr-discovery-century-power-money-nobel-dreams-macarena-fritz

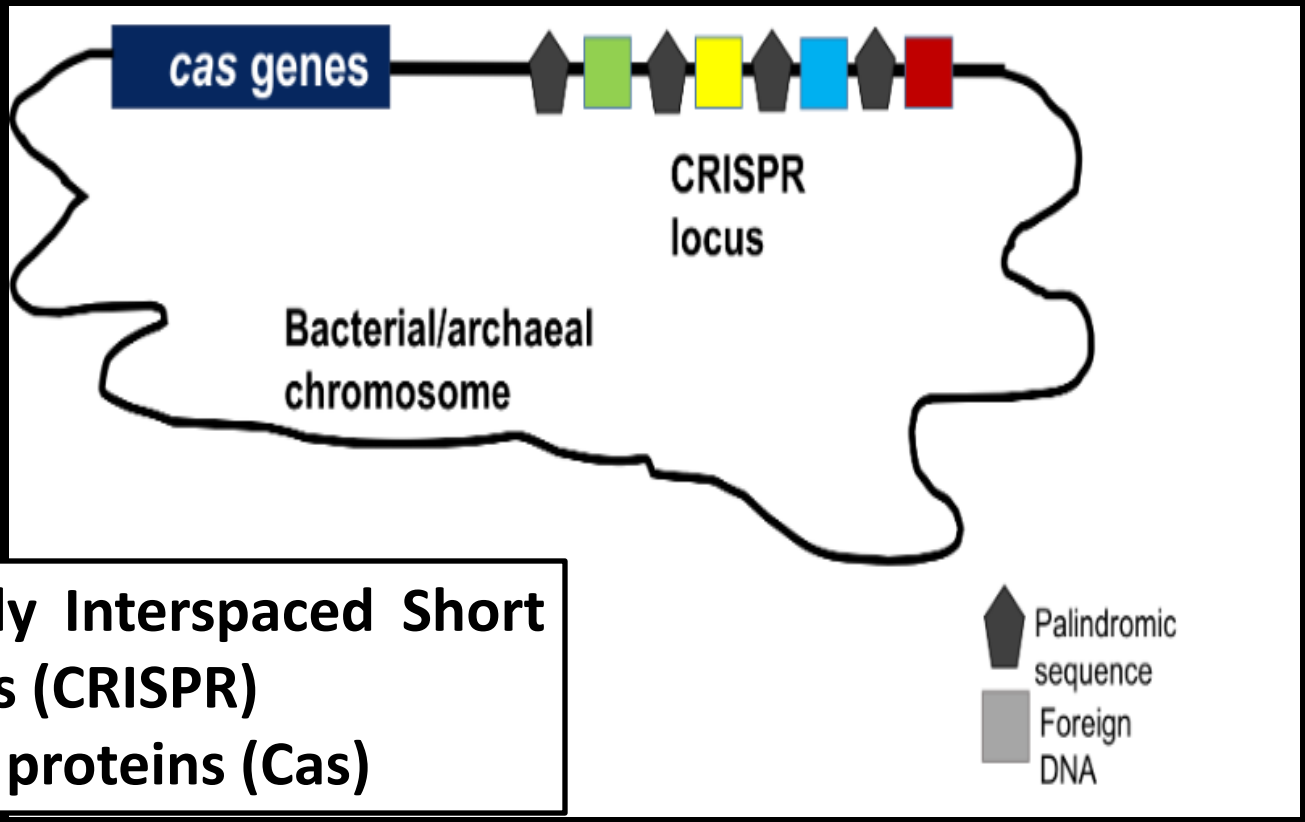
molecular microbiology

Identification of genes that are associated with DNA repeats in prokaryotes

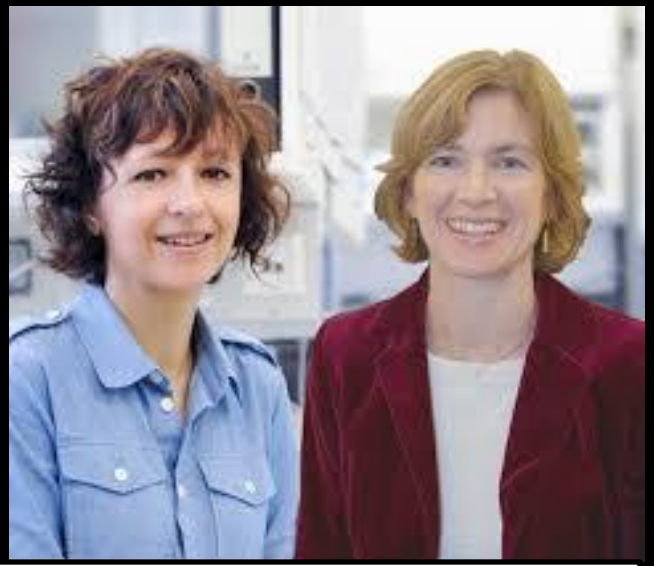
First published: March 2002

Ruud. Jansen ✉, Jan. D. A. van Embden, Wim. Gaastra, Leo. M. Schouls

Unique to certain prokaryotes and not viruses nor eukaryotes



- **Clustered Regularly Interspaced Short Palindormic Repeats (CRISPR)**
- **CRISPR associated proteins (Cas)**



Emmanuelle Charpentier & Jennifer Doudna

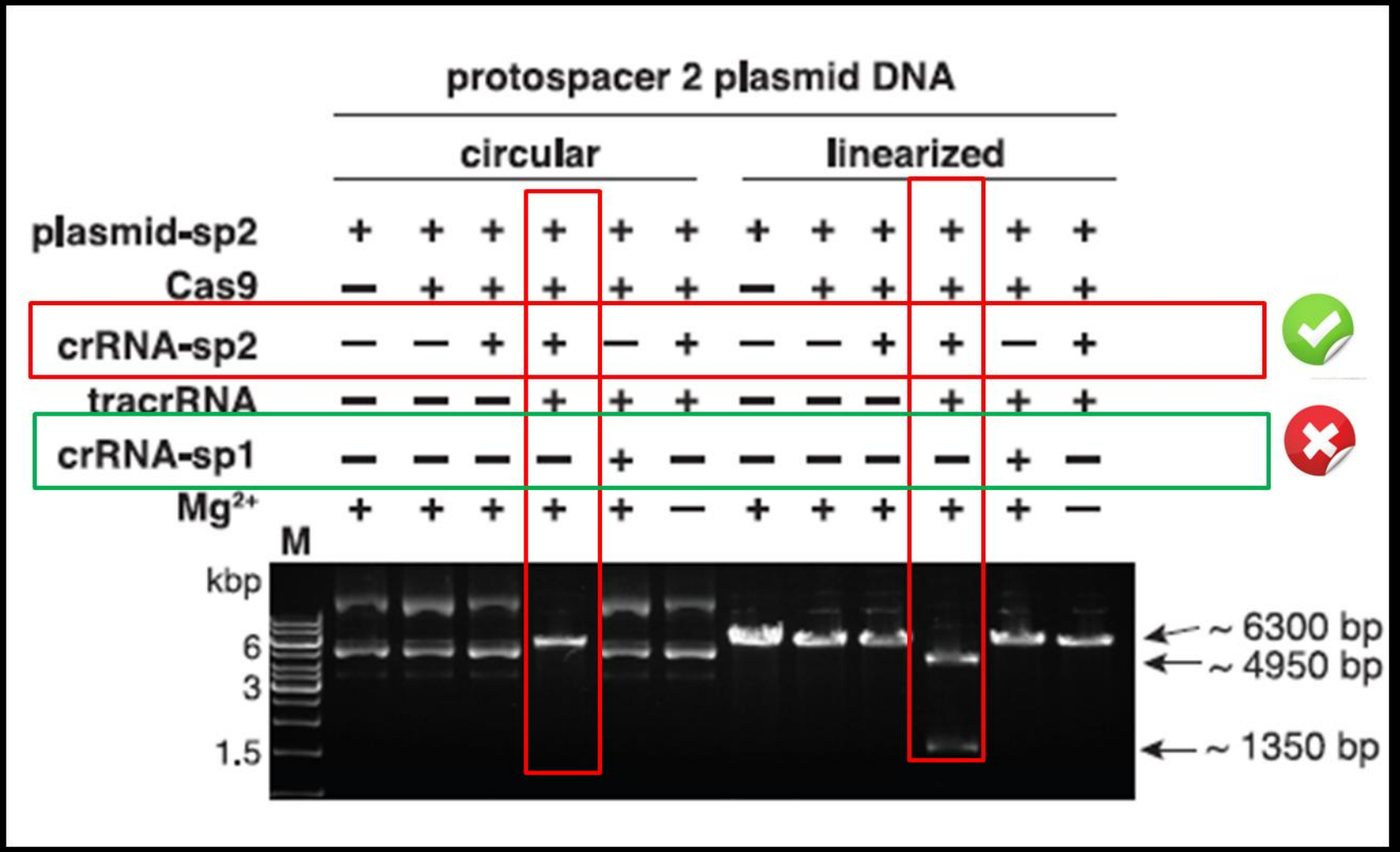
Science

17 AUGUST 2012 VOL 337

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]‡

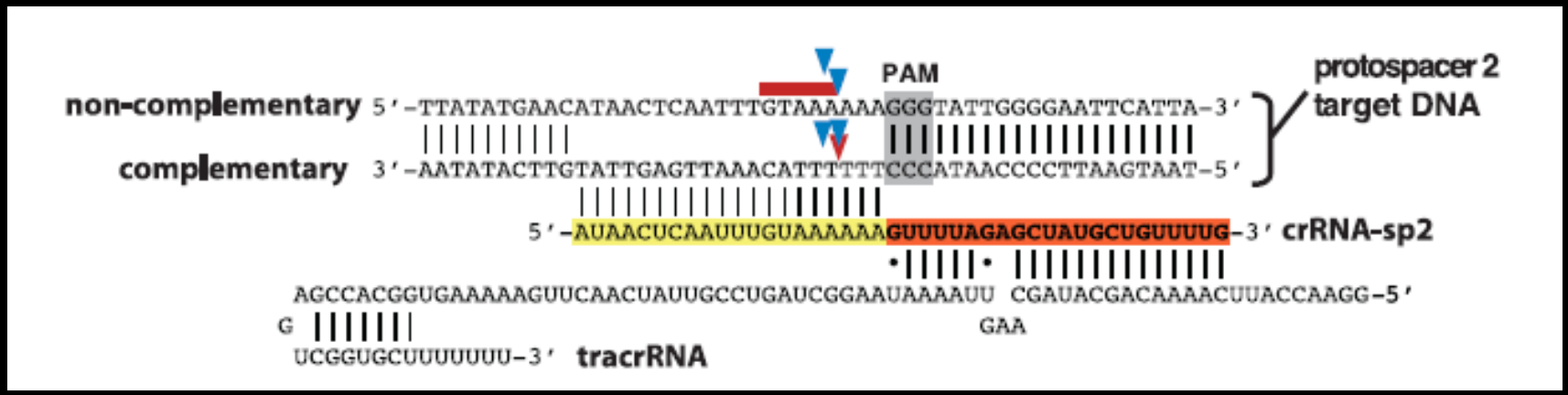
Biochemical studies of Cas 9 and CRISPR linked RNAs



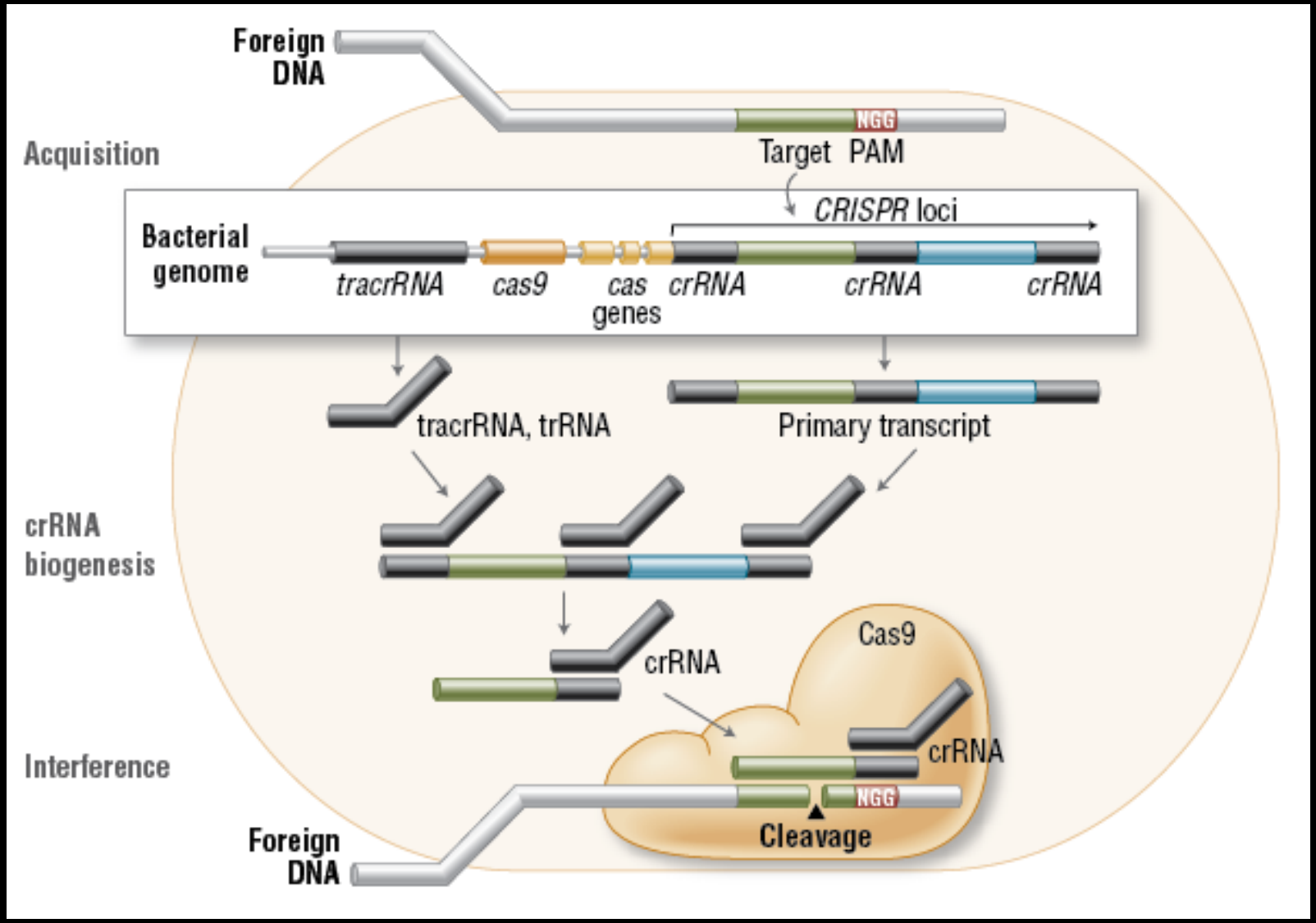
Jinek *et al.* 2012, *Science* 337: 816-821

Biochemical studies of Cas 9 and CRISPR linked RNAs

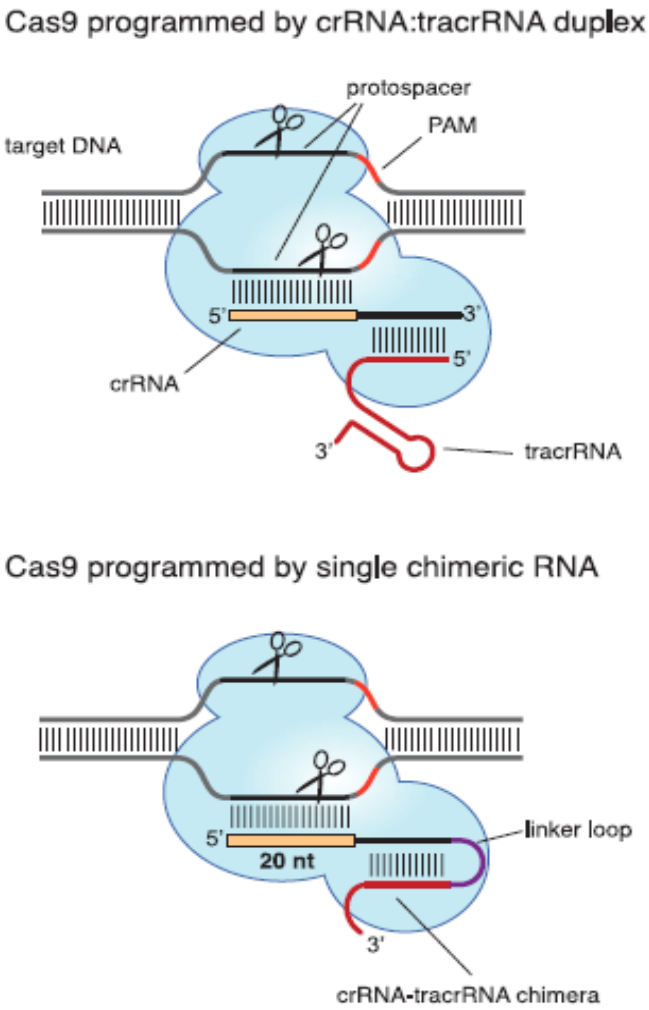
Cas9 cuts 3’ upstream of protospacer adjacent motif



Jinek *et al.* 2012, *Science* 337: 816-821


Foreign DNA
NGG
Target PAM
Acquisition
CRISPR loci
Bacterial genome
tracrRNA
cas9
cas genes
crRNA
crRNA
crRNA
tracrRNA, trRNA
Primary transcript
crRNA biogenesis
Cas9
crRNA
crRNA
Interference
NGG
Cleavage
Foreign DNA

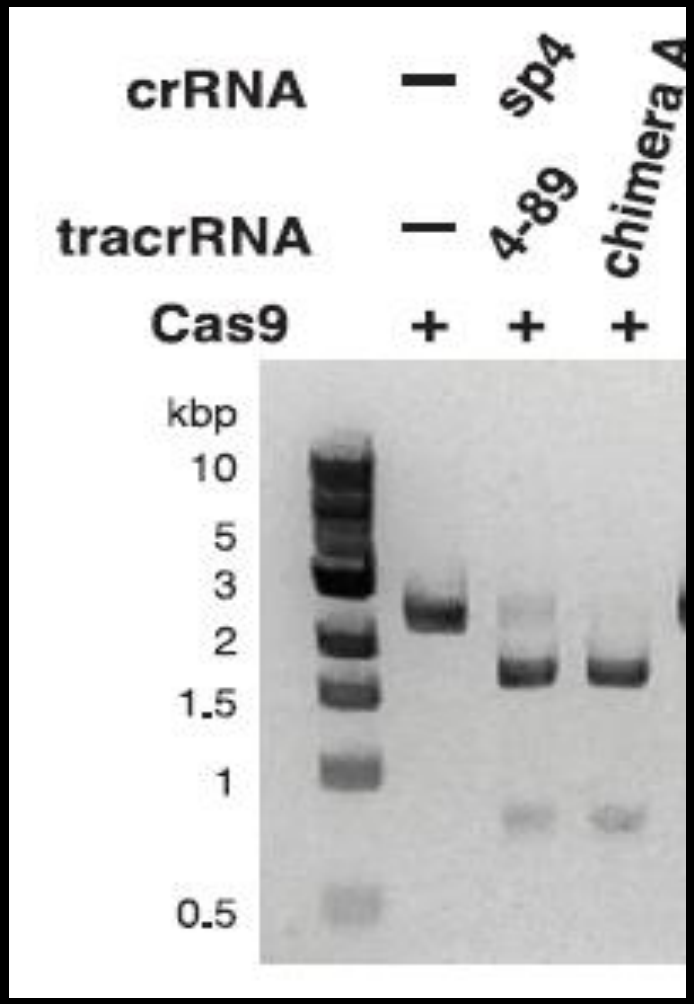
Biochemical studies of Cas 9 and CRISPR linked RNAs



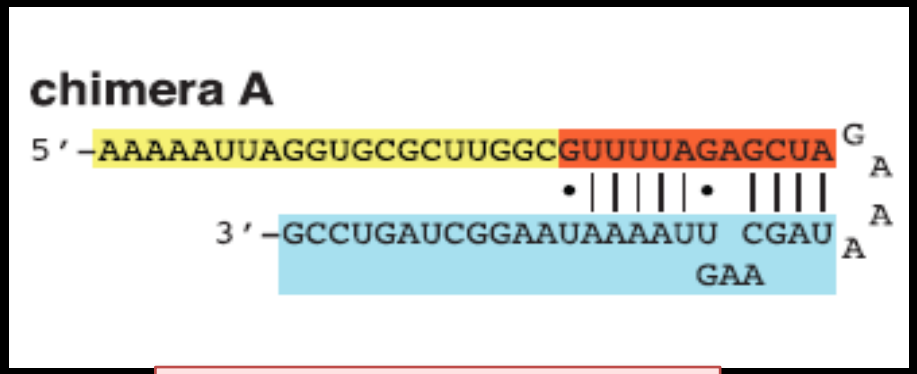
crRNA and tracrRNA can be merged into one

Jinek *et al.* 2012, *Science* 337: 816-821

Biochemical studies of Cas 9 and CRISPR linked RNAs



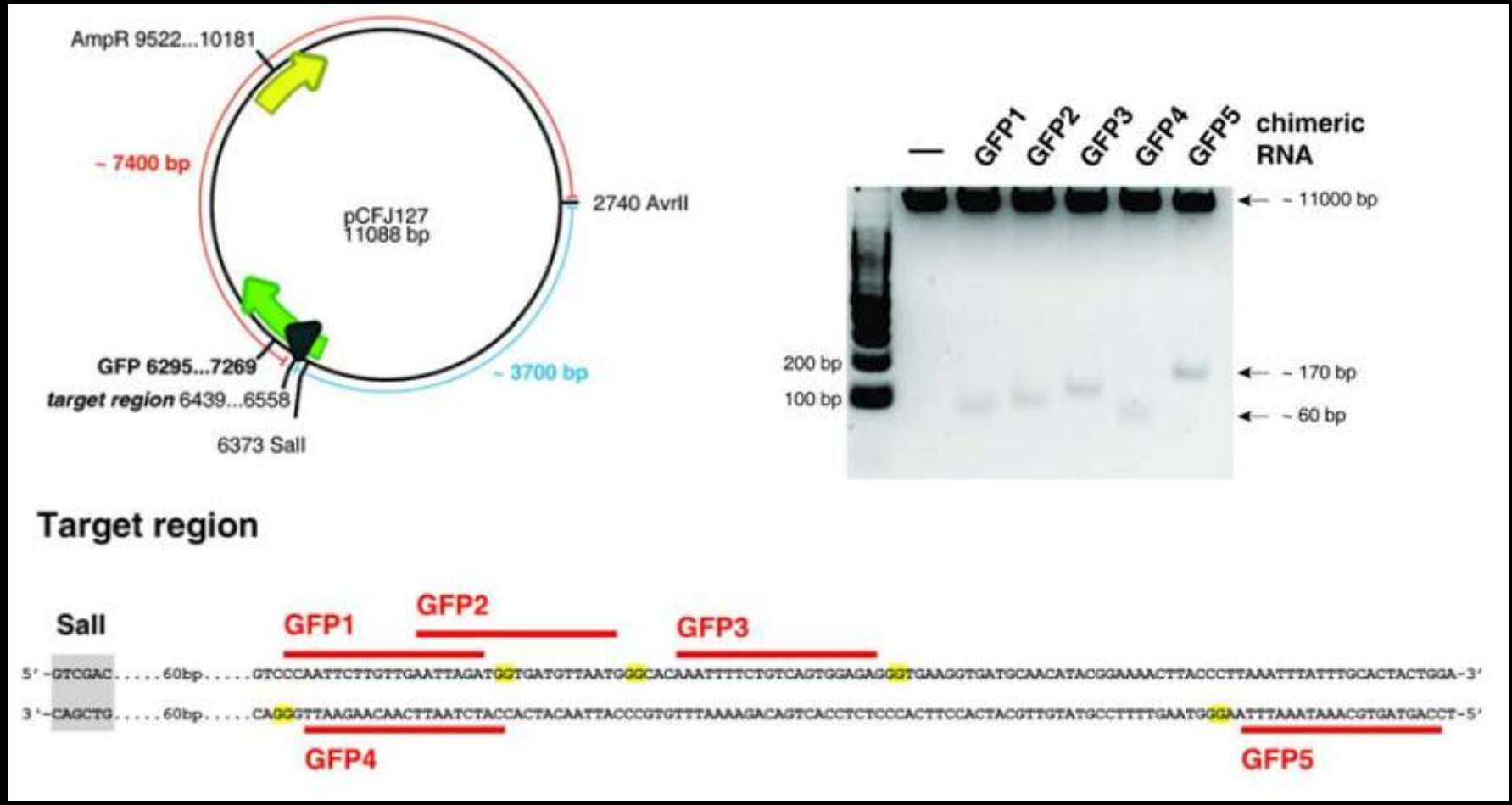
"A substantially truncated version of the tracrRNA retaining nucleotides 23-48 of the native sequence was capable of supporting robust dual-RNA–guided Cas9-catalyzed DNA Cleavage."



Truncated tracrRNA

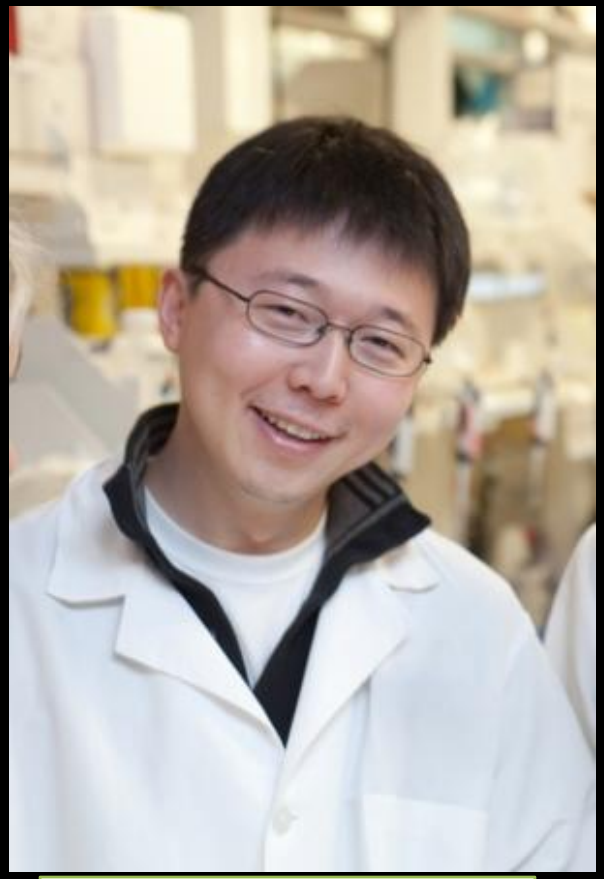
Jinek *et al.* 2012, *Science* 337: 816-821

In vitro test



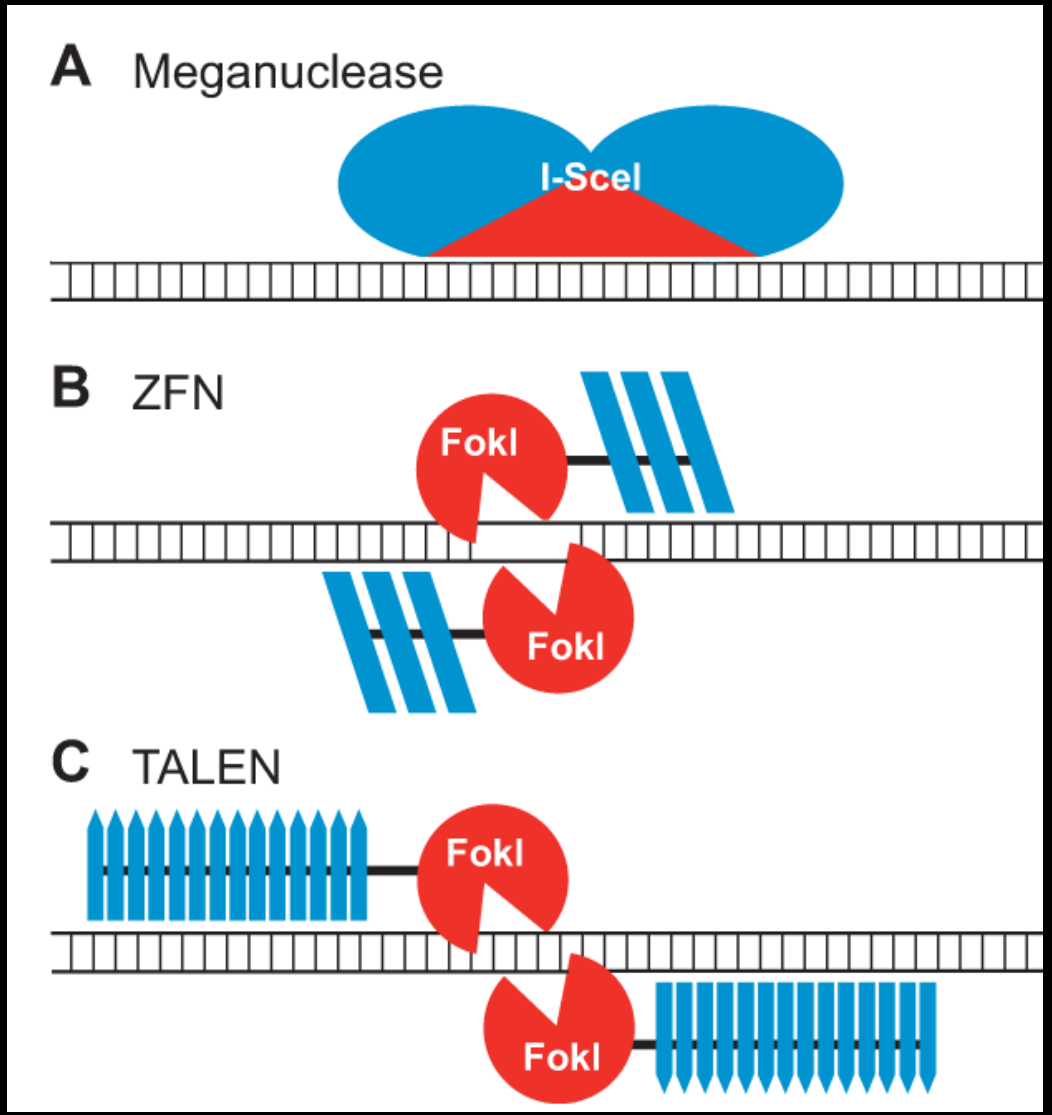
Jinek *et al.* 2012, *Science* 337: 816-821

Streptococcus pyogenes Cas9 (SpCas9) = 1368 amino acid nuclease



Feng Zhang
Broad Institute of MIT and Harvard

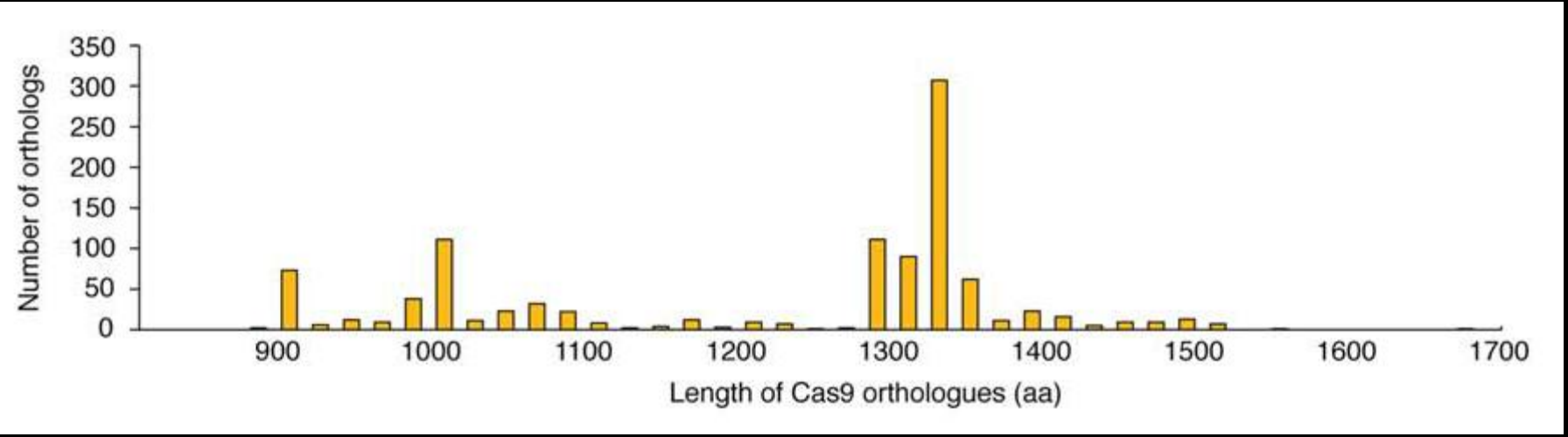
Programmable DNA binding domains



Voytas and Gao, 2014, *Plos*

Are there other smaller orthologues of Cas9 present in nature?

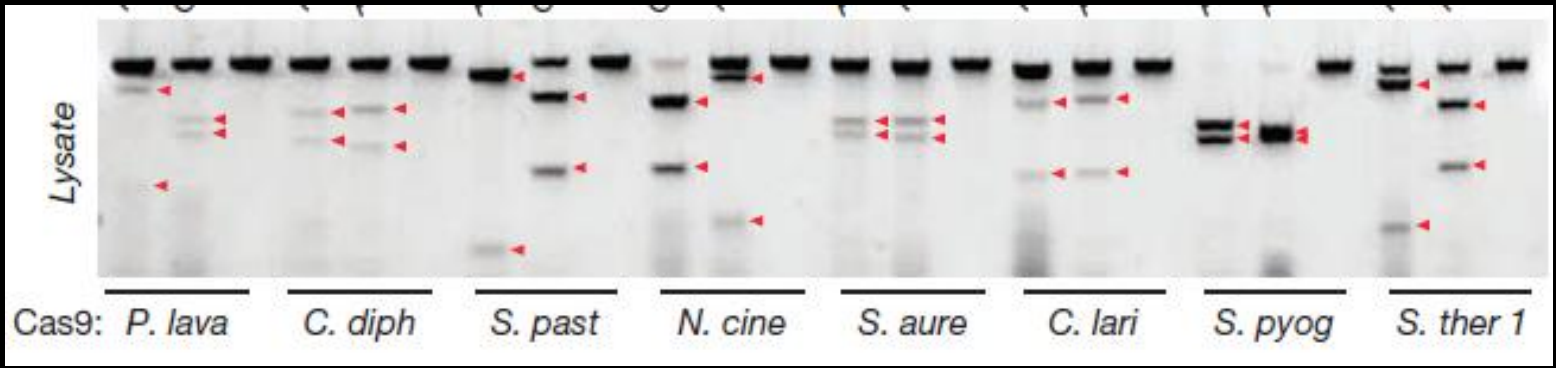
Streptococcus pyogenes Cas9 (SpCas9) = 1368 amino acid nuclease



Ran *et. al.* 2015, *Nature*

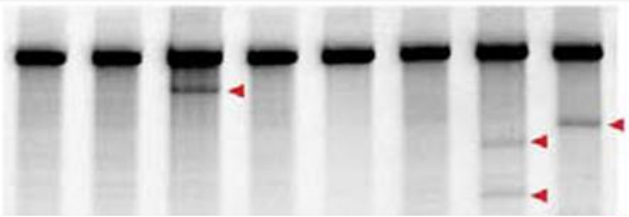
All Cas9 works *in vitro* while only a few performs *in vivo*

In vitro



Staphylococcus aureus Cas9 (SaCas9) = 1053 amino acid nuclease

In vivo



prokaryotic CRISPR system can be harnessed into eukaryotic cells

Ran *et. al.* 2015, *Nature*

Barrangou & Doudna Nature Biotechnology, 2015

MIT Technology Review

Topics+

Rewriting Life

First Monkeys with Autism Created in China

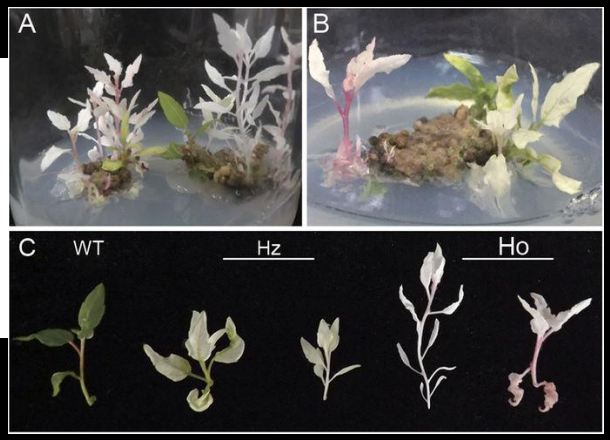
They spin in their cages and don't interact. The scientists who created autistic monkeys say they'll now try to cure them.

by Antonio Regalado January 25, 2016

Article | OPEN

Efficient CRISPR/Cas9-mediated Targeted Mutagenesis in Populus in the First Generation

Di Fan, Tingting Liu, Chaofeng Li, Bo Jiao, Shuang Li, Yishu Hou & Keming Luo



Off-targets!!

nature biotechnology

Home | Current issue | News & comment | Research | Archive ▾ | Authors & referees ▾ | About the journa

home ▸ archive ▸ issue ▸ research ▸ letter ▸ full text

NATURE BIOTECHNOLOGY | RESEARCH | LETTER

日本語要約

DNA targeting specificity of RNA-guided Cas9 nucleases

Patrick D Hsu, David A Scott, Joshua A Weinstein, F Ann Ran, Silvana Konermann, Vineeta Agarwala, Yinqing Li, Eli J Fine, Xuebing Wu, Ophir Shalem, Thomas J Cradick, Luciano A Marraffini, Gang Bao & Feng Zhang

Affiliations | Contributions | Corresponding author

Molecular Therapy
Nucleic Acids
Volume 4, 2015, Article e264
open access

Review

Off-target Effects in CRISPR/Cas9-mediated Genome Engineering

Xiao-Hui Zhang [1, 2], Louis Y Tee [3], Xiao-Gang Wang [4], Qun-Shan Huang [1], Shi-Hua Yang [1]

Show more

https://doi.org/10.1038/mtna.2015.37

GENOME RESEARCH

CSHL Press | Journal Home | Subscriptions | eTOC Alerts | BioSupplyNet

Genome Res. 2014 Jan; 24(1): 132–141.
doi: 10.1101/gr.162339.113

PMCID: PMC3875854

Analysis of off-target effects of CRISPR/Cas-derived RNA-guided endonucleases and nickases

Seung Woo Cho,[1] Sojung Kim,[1] Yongsub Kim,[1] Jiyeon Kweon, Heon Seok Kim, Sangsu Bae, and Jin-S

Author information ▸ Article notes ▸ Copyright and License information ▸

COSMOS
THE SCIENCE OF EVERYTHING
HOME LATEST TOPICS PRINT ISSUES EXPLORE

SUBSCRIBE

NEWS BIOLOGY 30 MAY 2017

CRISPR gene editing causes hundreds of unintended, off-target mutations

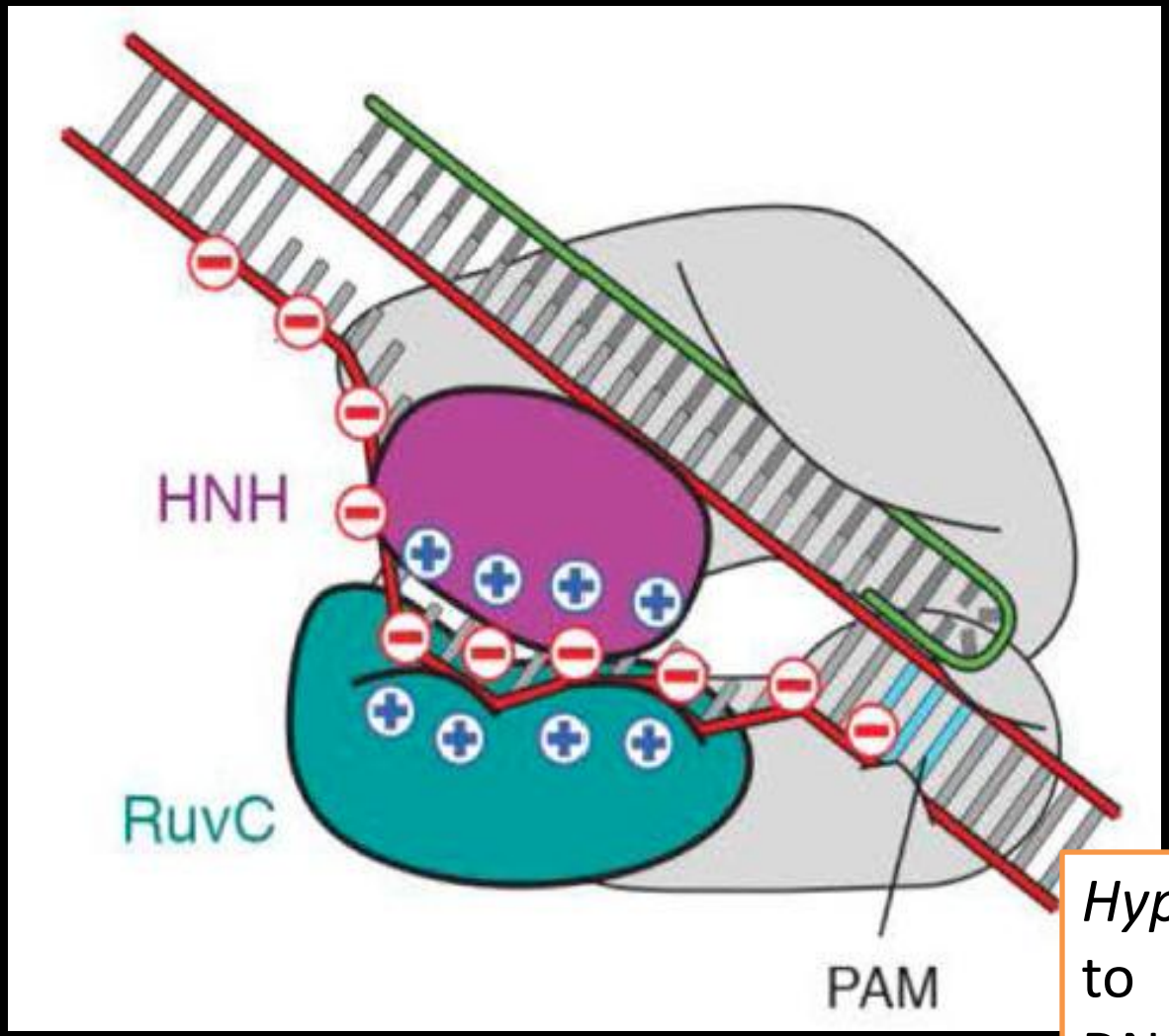
Can CRISR-Cas9 be more specific?

Ian M. Slaymaker

Linyi Gao

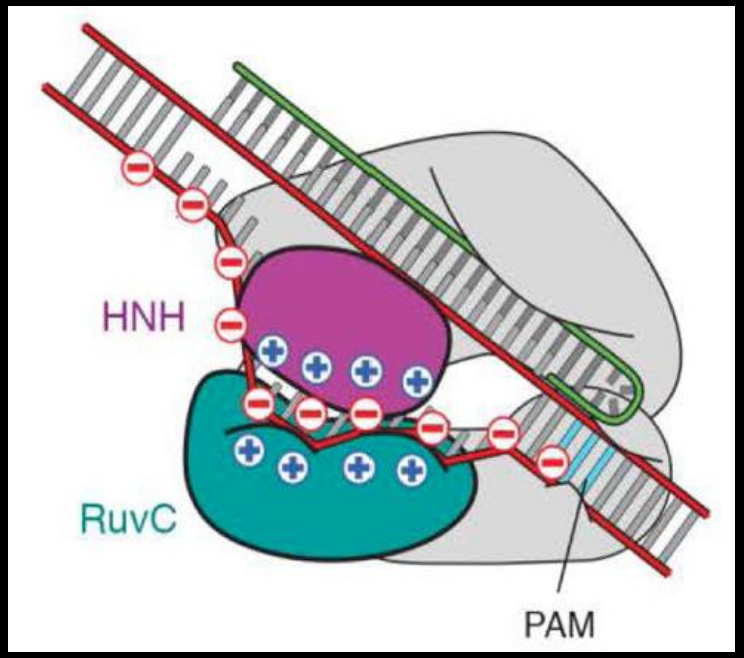
Feng Zhang

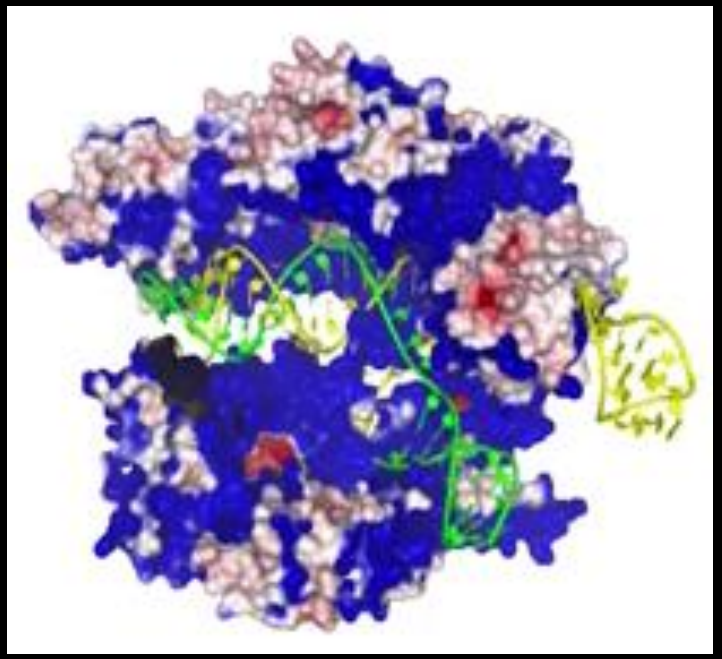
Bernd Zetsche



Hypothesis: Cas9 needs to stabilize denatured DNA strands

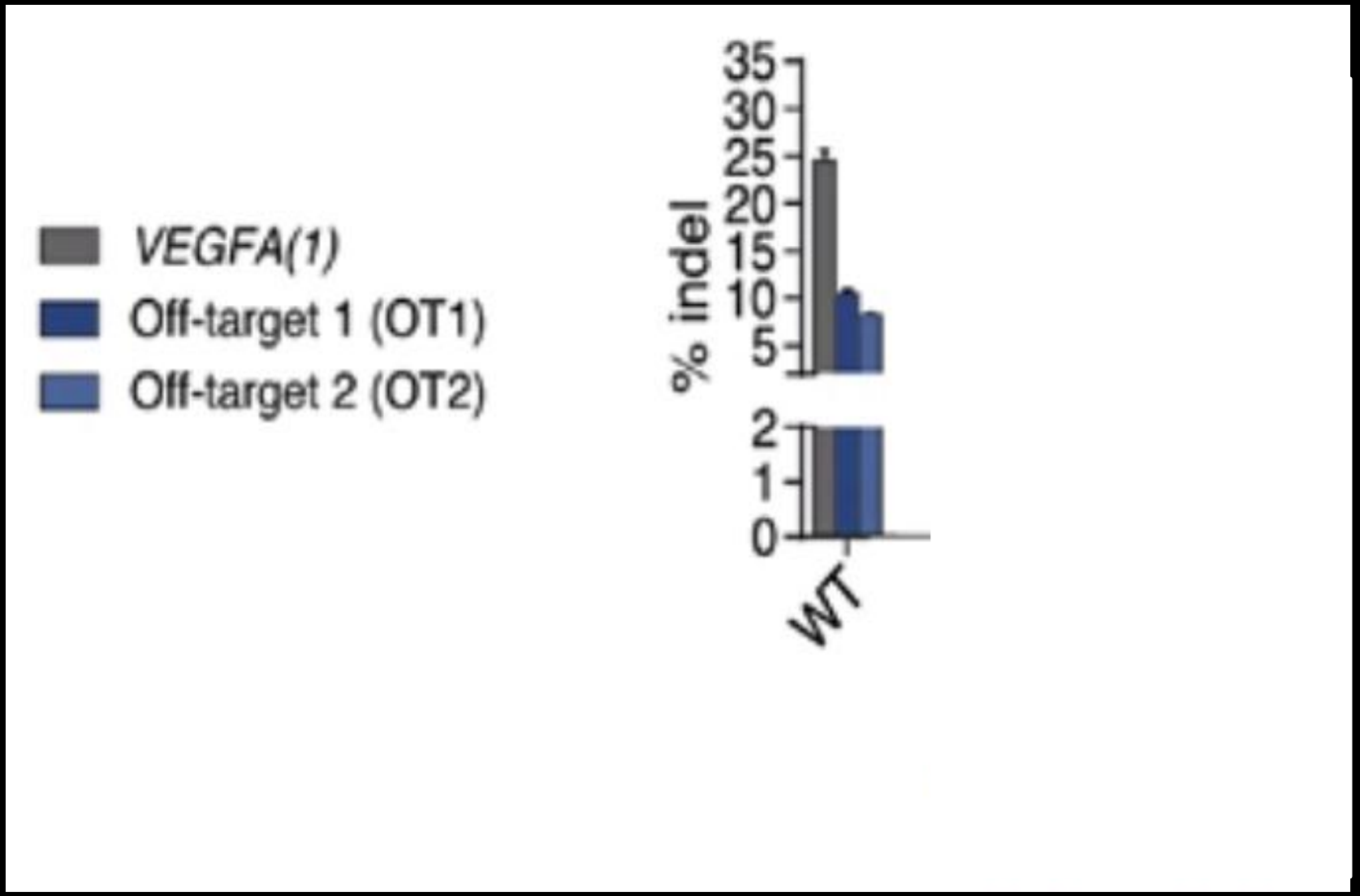
Slaymaker *et al.* 2016, *Science*





The positive amino acid residues were replaced with neutral ones
Eg. Arg, Lys replaced with Ala

Slaymaker *et al.* 2016, *Science*



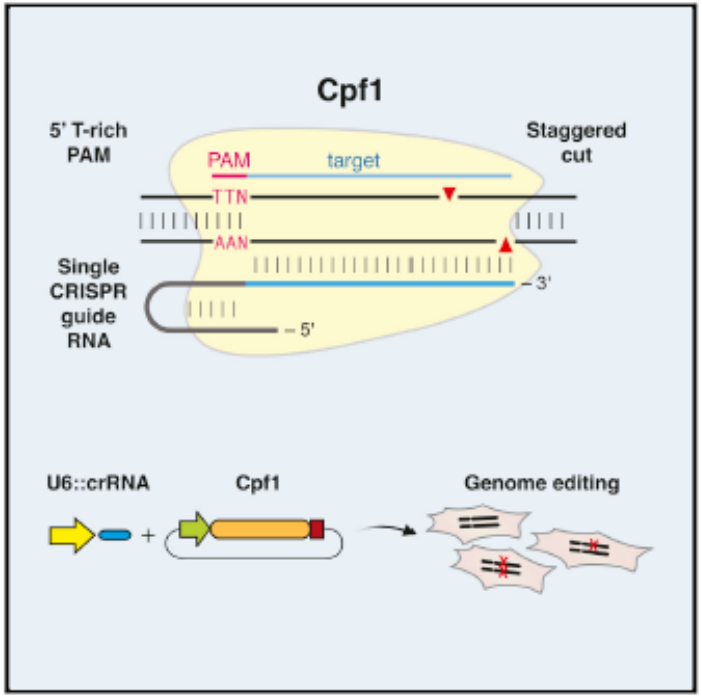
Slaymaker *et al.* 2016, *Science*

Do we have other alternatives to Cas9 ?!

Cell

Cpf1 Is a Single RNA-Guided Endonuclease of a Class 2 CRISPR-Cas System

Graphical Abstract



Authors

Bernd Zetsche, Jonathan S. Gootenberg, Omar O. Abudayyeh, ..., Aviv Regev, Eugene V. Koonin, Feng Zhang

Correspondence

zhang@broadinstitute.org

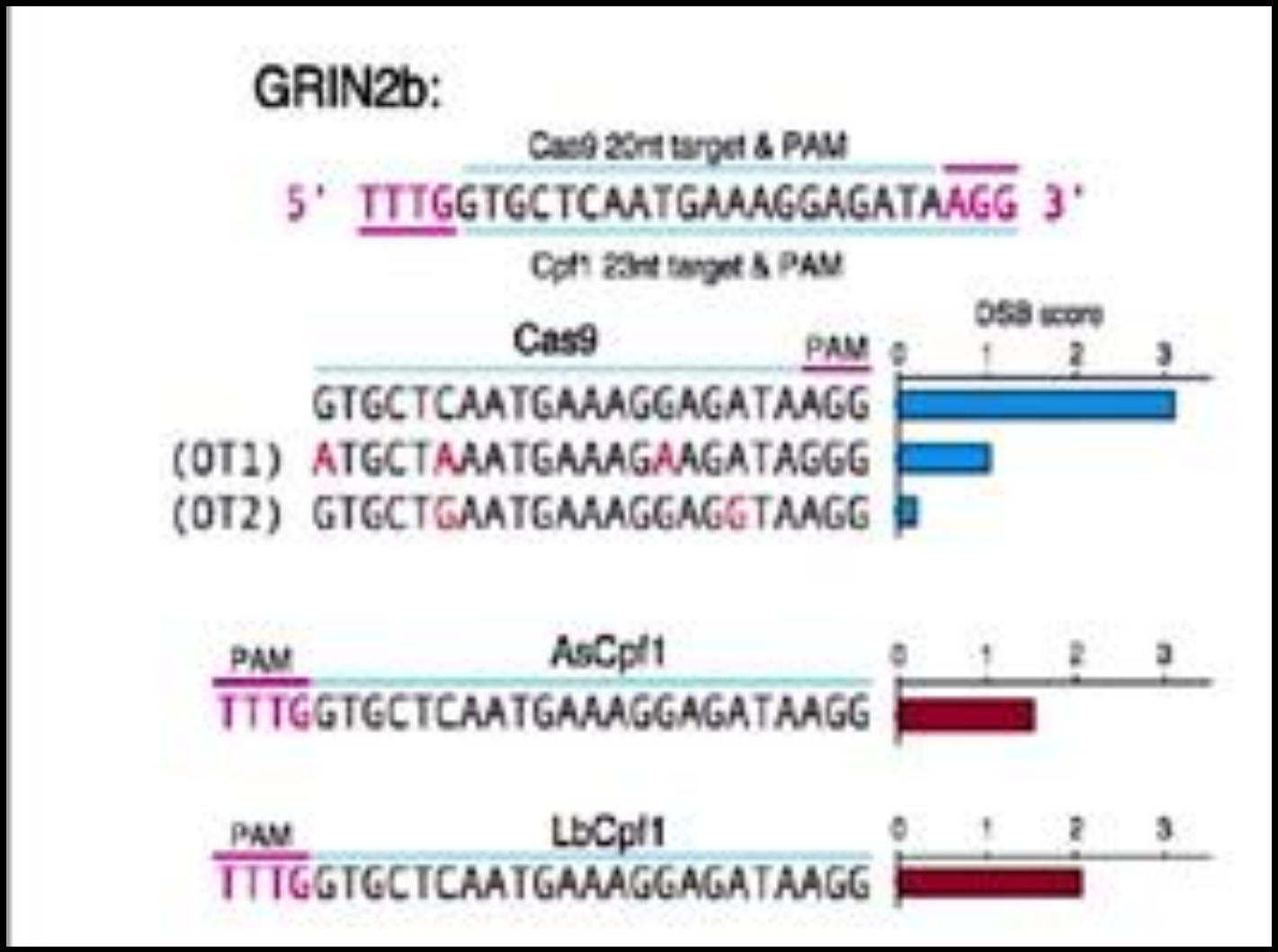
In Brief

Cpf1 is a RNA-guided DNA nuclease that provides immunity in bacteria and can be adapted for genome editing in mammalian cells.

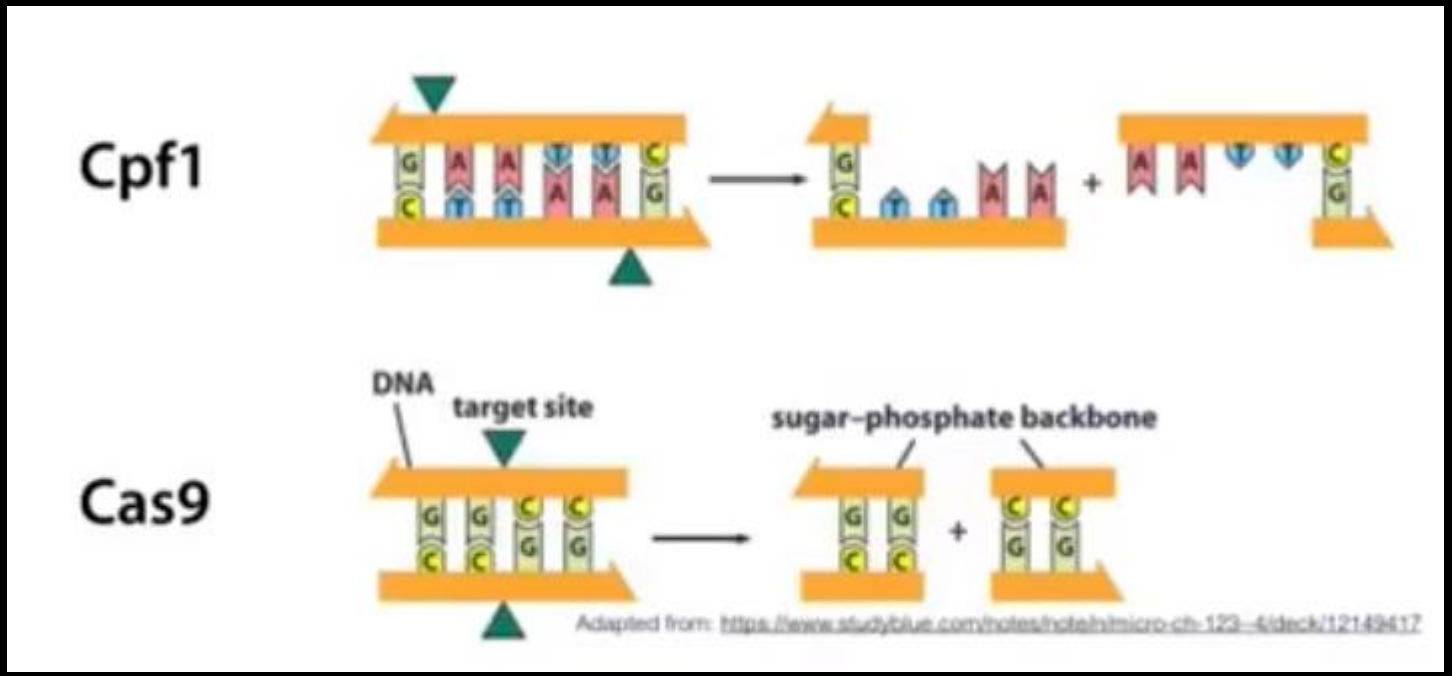
Highlights

- CRISPR-Cpf1 is a class 2 CRISPR system
- Cpf1 is a CRISPR-associated two-component RNA-programmable DNA nuclease
- Targeted DNA is cleaved as a 5-nt staggered cut distal to a 5′ T-rich PAM
- Two Cpf1 orthologs exhibit robust nuclease activity in human cells

Zetsche et al., 2015, *Cell*



Yan *et al*. (unpublished)



Zetsche et al., 2015, *Cell*

Discovery of New effectors

Shmakov*, Abudayyeh* et al., Molecular Cell 2015

Can CRISPR-Cas9 technology evade the stringent and time consuming scrutiny of Bio-safety regulation?!

nature International weekly journal of science

NATURE | NEWS

Gene-edited CRISPR mushroom escapes US regulation

A fungus engineered with the CRISPR–Cas9 technique can be cultivated and sold without further oversight

Emily Waltz

Edited polyphenol oxidase (PPO) — an enzyme that causes browning to stop browning

Penn State developer of gene-edited mushroom wins 'Best of What's New' award

October 19, 2016

Yinong Yang

The first CRISPR-Cas9 gene-edited organism deemed to require no regulatory review by USDA.

CRISPR-Cas9 for cotton genome editing

Positive test results of CRISPR-Cas9 technology in Cotton

SCIENTIFIC REPORTS
/ABOUT
/EDITORIAL BOARD
/FOR AUTHORS

Sci Rep. 2017; 7: 44304.
Published online 2017 Mar 13. doi: 10.1038/srep44304
PMCID: PMC534708

Targeted mutagenesis in cotton (*Gossypium hirsutum* L.) using the CRISPR/Cas9 system

Xiugui Chen,[1,2] Xuke Lu,[1] Na Shu,[1] Shuai Wang,[1] Junjuan Wang,[1] Delong Wang,[1] Lixue Guo,[1] and Wuwei Ye[a,1]

Author information ► Article notes ► Copyright and License information ►

Plant Molecular Biology
July 2017, Volume 94, Issue 4–5, pp 349–360

CRISPR/Cas9-mediated targeted mutagenesis in upland cotton (*Gossypium hirsutum* L.)

Authors Authors and affiliations

Madhusudhana R. Janga, LeAnne M. Campbell, Keerti S. Rathore

SCIENTIFIC REPORTS

Article | OPEN

A high-efficiency CRISPR/Cas9 system for targeted mutagenesis in Cotton (*Gossypium hirsutum* L.)

Chao Li, Turgay Unver & Baohong Zhang

Plant Biotechnology Journal Open Access aab SEB Society for Experimental Biology
Explore this journal >

Research Article

High efficient multisites genome editing in allotetraploid cotton (*Gossypium hirsutum*) using CRISPR/Cas9 system

Pengcheng Wang, Jun Zhang, Lin Sun, Yizan Ma, Jiao Xu, Sijia Liang, Jinwu Deng, Jiafu Tan, Qinghua Zhang, Lili Tu, Henry Daniell, Shuangxia Jin, Xianlong Zhang

Front Plant Sci. 2017; 8: 1364.
Published online 2017 Aug 3. doi: 10.3389/fpls.2017.01364
PMC

Genome Editing in Cotton with the CRISPR/Cas9 System

Wei Gao,[1,†] Lu Long,[1,†] Xinquan Tian,[1] Fuchun Xu,[1] Ji Liu,[2] Prashant K. Singh,[1] Jose R. Botella,[3] and Chunpeng Song[1,*]

Author information ► Article notes ► Copyright and License information ►

thank you!