巴基斯坦农业“千人计划”在华培训项目课程

基因编辑技术/Gene Editing Technology

基因编辑基础--基因剪刀手
Basic Principles of Gene Editing--Gene Scissors

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西北农林科技大学

基因剪刀手/Gene Scissors—

人工特异性核酸酶技术/Programmable specific endonuclease technology

What's next?

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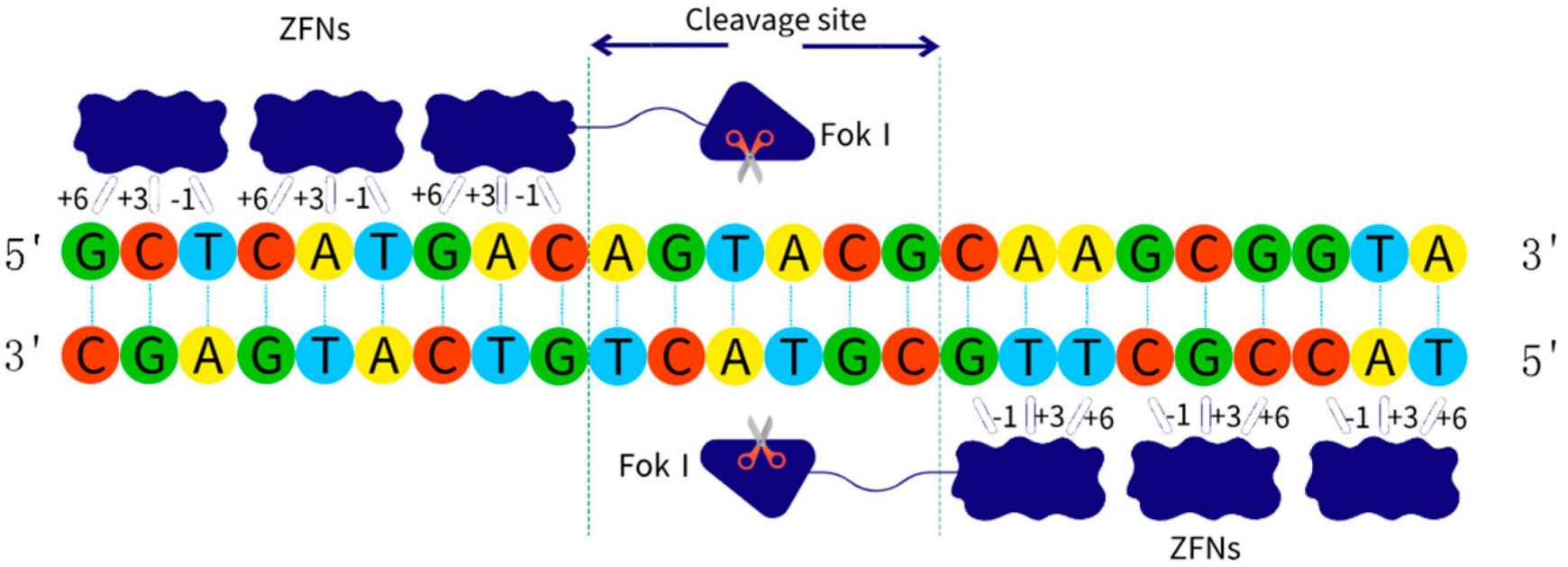
中国成语
Chinese idioms

西北农林科技大学

十年磨一剑/Ten years to sharpen：ZFNs

锌指核酸酶：Zinc-Finger Nucleases (ZFNs)



十年磨一剑：ZFNs

https://en.wikipedia.org/wiki/Zinc-finger_nuclease

Zinc-finger nuclease

12 languages

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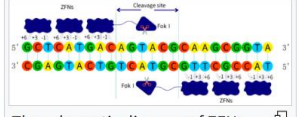
From Wikipedia, the free encyclopedia

Zinc-finger nucleases (**ZFNs**) are artificial restriction enzymes generated by fusing a zinc finger DNA-binding domain to a DNA-cleavage domain. Zinc finger domains can be engineered to target specific desired DNA sequences and this enables zinc-finger nucleases to target unique sequences within complex genomes. By taking advantage of endogenous DNA repair machinery, these reagents can be used to precisely alter the genomes of higher organisms. Alongside CRISPR/Cas9 and TALEN, ZFN is a prominent tool in the field of genome editing.



The schematic diagram of ZFNs

It was initially created by researcher Srinivasan Chandrasegaran.

Domains [edit]

DNA-binding domain [edit]

The DNA-binding domains of individual ZFNs typically contain between three and six individual zinc finger repeats and can each recognize between 9 and 18 basepairs. If the zinc finger domains perfectly recognize a 3 basepair DNA sequence, they can generate a 3-finger array that can recognize a 9 basepair target site. Other procedures can utilize either 1-finger or 2-finger modules to generate zinc-finger arrays with six or more individual zinc fingers. The main drawback with this procedure is the specificities of individual zinc fingers can overlap and can depend on the context of the surrounding zinc fingers and DNA. Without methods to account for this "context dependence", the standard modular assembly procedure often fails.[1]

Numerous selection methods have been used to generate zinc-finger arrays capable of targeting desired sequences. Initial

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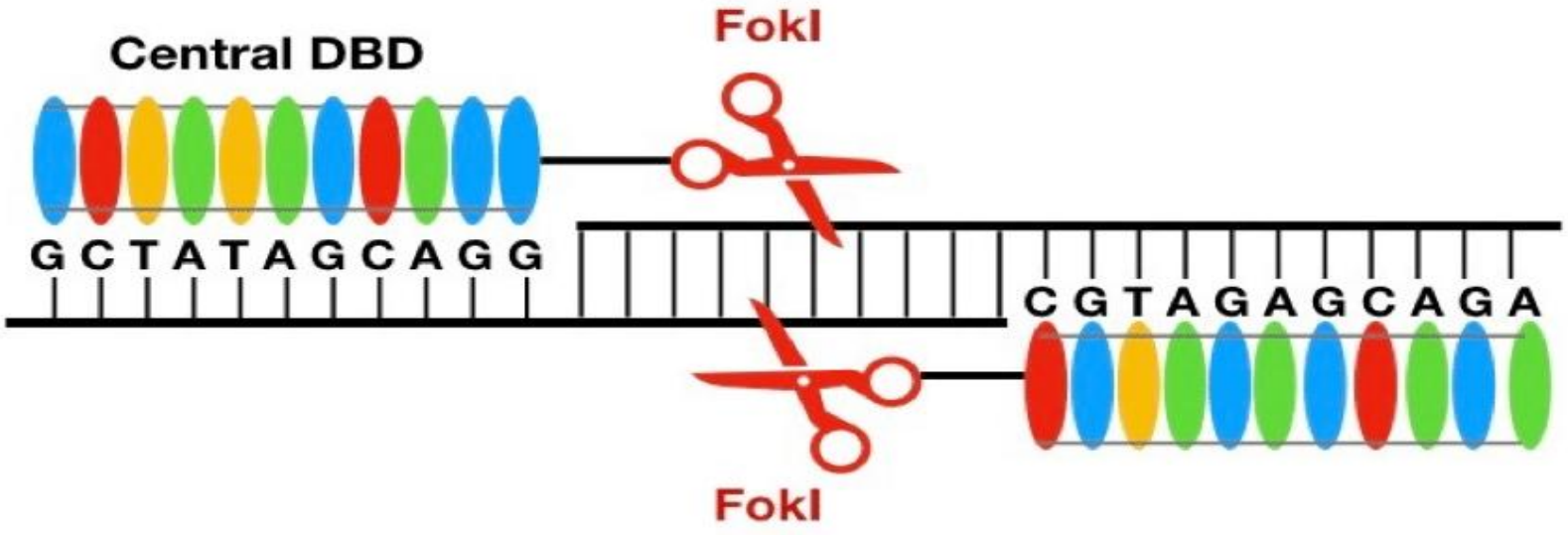
昙花终一现/Sudden bloom：TALENs

Transcription Activator-Like Effector Nucleases (TALEN)



昙花终一现：TALENs

https://en.wikipedia.org/wiki/Transcription_activator-like_effector_nuclease

Transcription activator-like effector nuclease

7 languages

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From Wikipedia, the free encyclopedia

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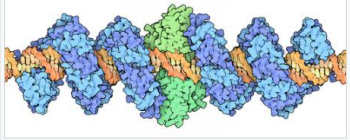
Transcription activator-like effector nucleases (**TALEN**) are restriction enzymes that can be engineered to cut specific sequences of DNA. They are made by fusing a TAL effector DNA-binding domain to a DNA cleavage domain (a nuclease which cuts DNA strands). Transcription activator-like effectors (TALEs) can be engineered to bind to practically any desired DNA sequence, so when combined with a nuclease, DNA can be cut at specific locations.[1] The restriction enzymes can be introduced into cells, for use in gene editing or for genome editing *in situ*, a technique known as genome editing with engineered nucleases. Alongside zinc finger nucleases and CRISPR/Cas9, TALEN is a prominent tool in the field of genome editing.



Spacefill drawing of dimeric TALE-FokI fusion (blue: TALE; green: FokI) bound to DNA (PDB: 1FOK, 3UGM), by David Goodsell

Part of a series on

Genetic engineering

Genetically modified organisms

Bacteria • Viruses
Animals (Mammals • Fish • Insects)
Plants (Maize/corn • Rice • Soybean •

TALE DNA-binding domain [edit]

TAL effectors are proteins that are secreted by *Xanthomonas* bacteria via their type III secretion system when they infect plants.[2] The DNA binding domain contains a repeated highly conserved 33–34 amino acid sequence with divergent 12th and 13th amino acids. These two positions, referred to as the Repeat Variable Diresidue (RVD), are highly variable and show a strong correlation with specific nucleotide recognition.[3][4] This straightforward relationship between amino acid sequence and DNA recognition has allowed for the engineering of specific DNA-binding domains by selecting a combination of repeat segments containing the appropriate RVDs.[1] Notably, slight changes in the RVD and the incorporation of

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The favored and the best

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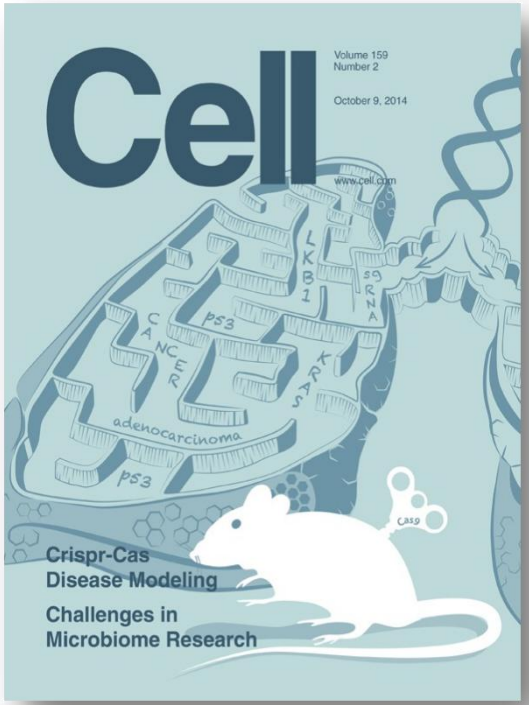
CRISPR/Cas9—
改变世界的技术/Technologies that changing the world



西北农林科技大学

原核生物的『获得性免疫』系统

The "acquired immunity" system of prokaryotes

CRISPR:

Clustered **R**egularly **I**nterspaced

Short **P**alindromic **R**epeats

规律间隔成簇短回文重复序列

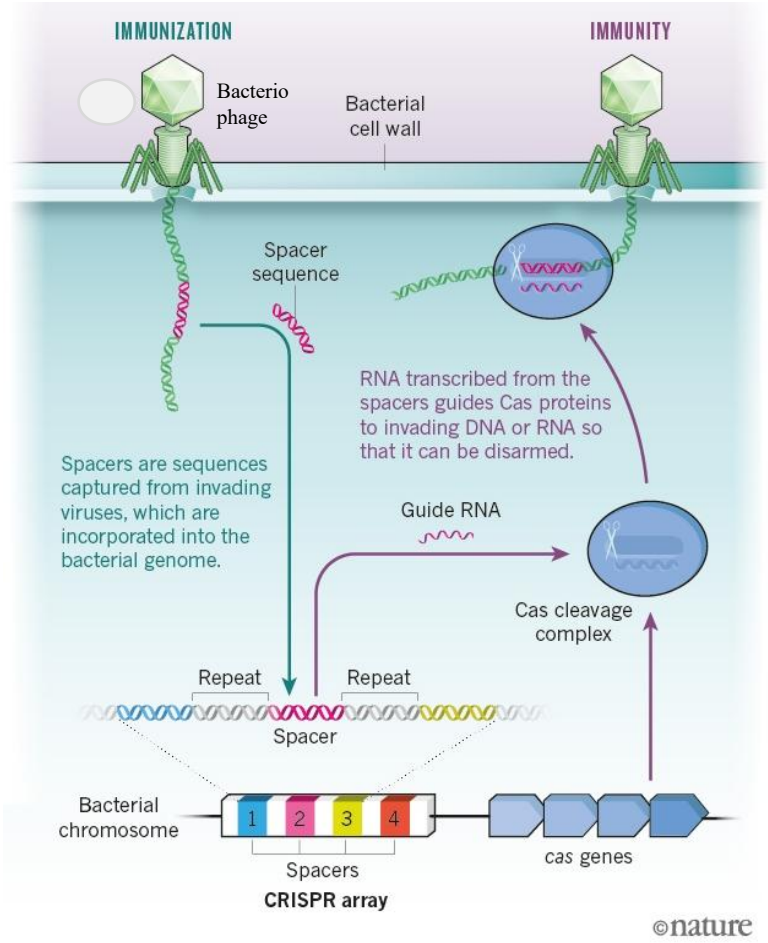
间隔序列（Spacer）

重复序列（Repeat）

Cas:

CRISPR关联基因（CRISPR associated）

核酸内切酶(eg. Cas9)



天选之骄子/Allah's Chosen One：CRISPR/Cas9

https://pubmed.ncbi.nlm.nih.gov/22745249/

CRISPR/Cas9技术

1. Cas9蛋白：

核酸内切酶,

SpCas9/SaCas9/StCas9,,,

2. gRNA：

sgRNA=crRNA+tracrRNA

3. 靶点带PAM：

NGG→NGN、NRN（G/A）

4. gRNA/Cas9

RGNs：RNA Guided Nucleases

天选之骄子：CRISPR/Cas9

https://en.wikipedia.org/wiki/Cas9#cite_note-P24529477-1

Cas9

20 languages

Article Talk | Read Edit View history Tools

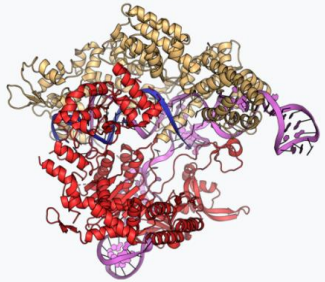
From Wikipedia, the free encyclopedia

This article **is missing information** about homologs including other Cas9s, other Class 2 Type II CRISPR systems. Please expand the article to include this information. Further details may exist on the talk page. *(September 2021)*

Cas9 (**CRISPR associated protein 9**, formerly called **Cas5**, **Csn1**, or **Csx12**) is a 160 kilodalton protein which plays a vital role in the immunological defense of certain bacteria against DNA viruses and plasmids, and is heavily utilized in genetic engineering applications. Its main function is to cut DNA and thereby alter a cell's genome. The CRISPR-Cas9 genome editing technique was a significant contributor to the Nobel Prize in Chemistry in 2020 being awarded to Emmanuelle Charpentier and Jennifer Doudna.[2]

More technically, Cas9 is a RNA-guided DNA endonuclease enzyme associated with the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) adaptive immune system in *Streptococcus pyogenes*.[3][4][5] *S. pyogenes* utilizes CRISPR to memorize and Cas9 to later interrogate and cleave foreign DNA, such as invading bacteriophage DNA or plasmid DNA.[4][6][7][8] Cas9 performs this interrogation by unwinding foreign DNA and checking for sites complementary to the 20 nucleotide spacer region of the guide RNA (gRNA). If the DNA substrate is complementary to the guide RNA, Cas9 cleaves the invading DNA. In this sense, the CRISPR-Cas9 mechanism has a number of parallels with the RNA interference (RNAi) mechanism

CRISPR-associated endonuclease Cas9



S. pyogenes Cas9 in complex with sgRNA and its target DNA. PDB: 4OO8 [1]

Identifiers	
Organism	Streptococcus pyogenes M1
Symbol	cas9

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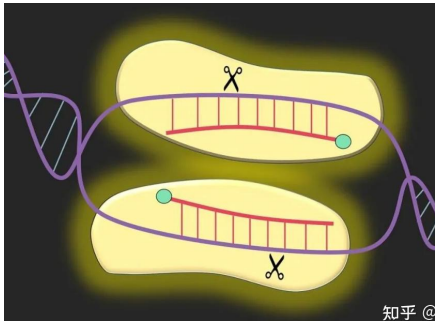
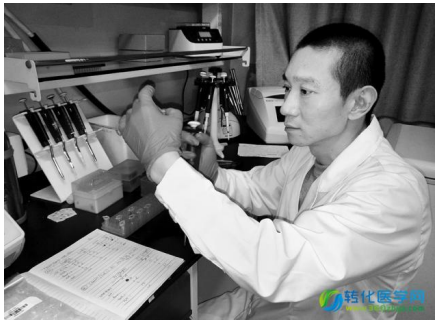
Good idea
but failed

西北农林科技大学

黄粱成一梦：gDNA/NgAgo





Article | Published: 02 May 2016

DNA-guided genome editing using the *Natronobacterium gregoryi* Argonaute

Feng Gao, Xiao Z Shen, Feng Jiang, Yongqiang Wu & Chunyu Han

Nature Biotechnology **34**, 768–773 (2016) | Cite this article

A Retraction to this article was published on 01 August 2017

A Retraction to this article was published on 01 August 2017

An Addendum to this article was published on 28 November 2016

This article has been updated

Associated content

Failure to detect DNA-guided genome editing using *Natronobacterium gregoryi* Argonaute

Seung Hwan Lee, Giandomenico Turchiano ... Jin-Soo Kim

Nature Biotechnology | **Correspondence** | 28 Nov 2016

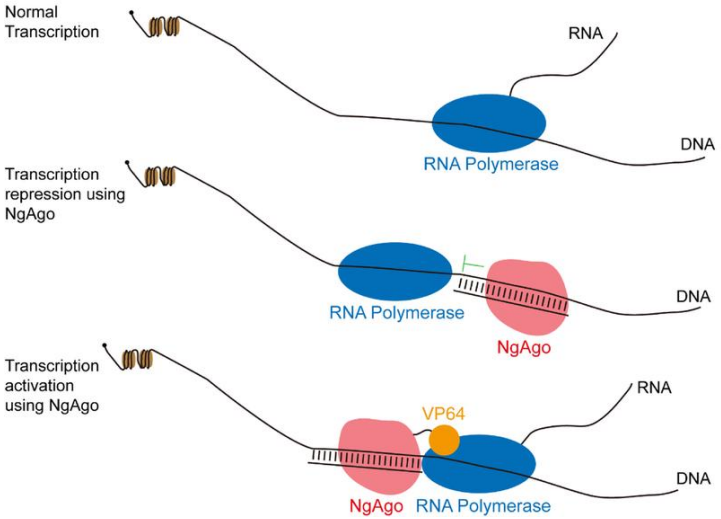
西北农林科技大学 https://www.nature.com/articles/nbt.3547

“东方不亮西方亮，黑了南方有北方”

When the east is dark, the west is still bright; when it's dark in the south but bright in the north

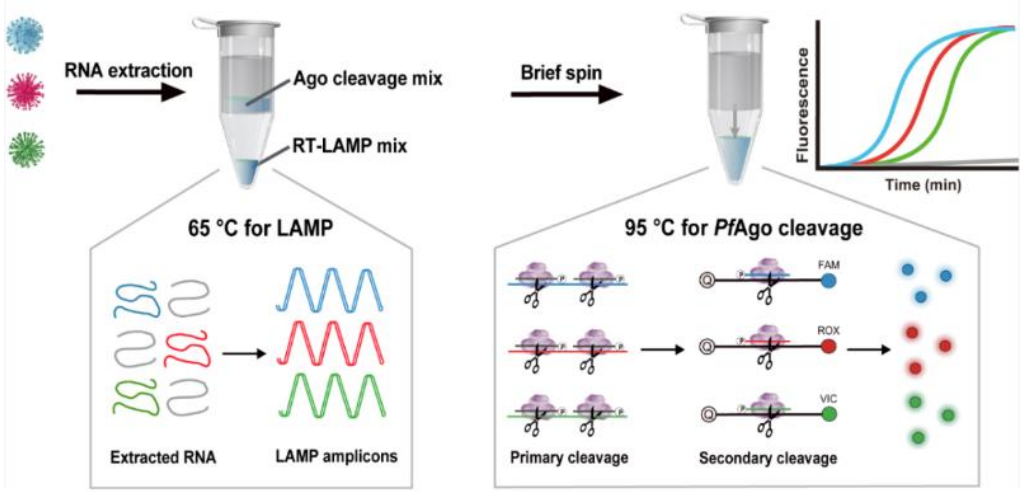
AGOi & AGOa

Transcription Manipulation using NgAgo



AGO Detection

MULAN (Multiplex Argonaute-based Nucleic Acid Detection)



https://pubmed.ncbi.nlm.nih.gov/35334329/

Argonaute for DNA(transcription) and RNA (translation) interferences

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

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Argonaute

9 languages

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From Wikipedia, the free encyclopedia

For the French ships, see French ship Argonaute.
Not to be confused with Argonaut (disambiguation).

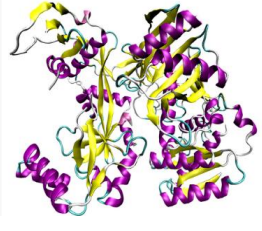
The **Argonaute** protein family, first discovered for its evolutionarily conserved stem cell function,[1] plays a central role in RNA silencing processes as essential components of the RNA-induced silencing complex (RISC). RISC is responsible for the gene silencing phenomenon known as RNA interference (RNAi).[2] Argonaute proteins bind different classes of small non-coding RNAs, including microRNAs (miRNAs), small interfering RNAs (siRNAs) and Piwi-interacting RNAs (piRNAs). Small RNAs guide Argonaute proteins to their specific targets through sequence complementarity (base pairing), which then leads to mRNA cleavage, translation inhibition, and/or the initiation of mRNA decay.[3]

The name of this protein family is derived from a mutant phenotype resulting from mutation of AGO1 in *Arabidopsis thaliana*, which was likened by Bohmert et al. to the appearance of the pelagic octopus *Argonauta argo*.[4]

RNA interference [edit]

RNA interference (RNAi) is a biological process in which RNA molecules inhibit gene expression, via either destruction of specific mRNA molecules or suppressing translation.[5] RNAi has a significant role in defending cells against parasitic nucleotide sequences [*citation needed*]. In eukaryotes, including animals, RNAi is initiated by the enzyme Dicer. Dicer cleaves long double-stranded RNA (dsRNA, often found in viruses and small interfering RNA) molecules into short double stranded fragments of around 20 nucleotide siRNAs. The dsRNA is then separated

Argonaute Piwi domain



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Eukaryotic Argonaute:
RNAi

Prokaryotic Argonaute:
AGOi
AGOa
AGO Detection

But still not gene edting

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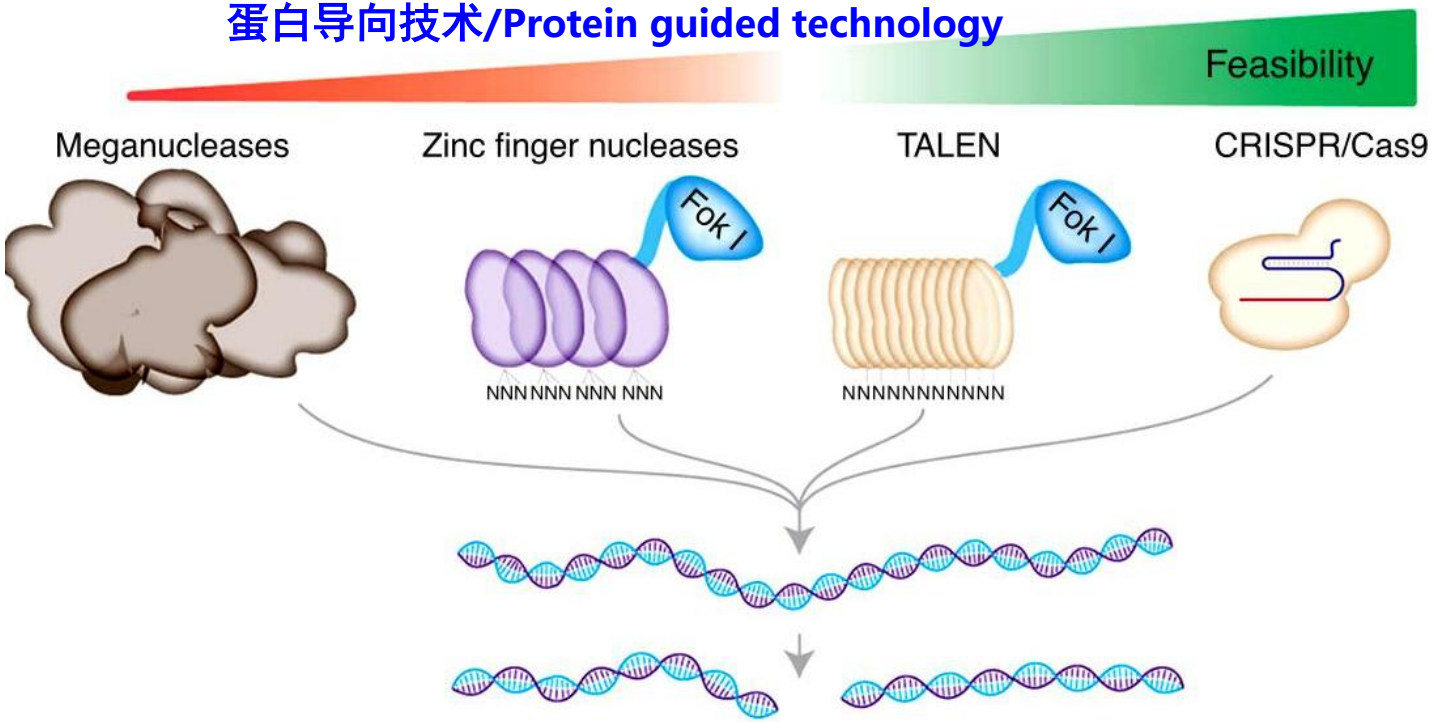
A lot of similar tools emerged

西北农林科技大学

基因剪刀手/Gene Scissors—

人工特异性核酸酶技术/Programmable specific endonuclease technology

蛋白导向技术/Protein guided technology



RNA导向技术/RNA guided:

CRISPR/Cas9 Series

CRISPR/Cas12 Series

OMEGA/IscB Series

OMEGA/TnpB Series

OMEGA/Fanzor Series

TIGA/Tas Series

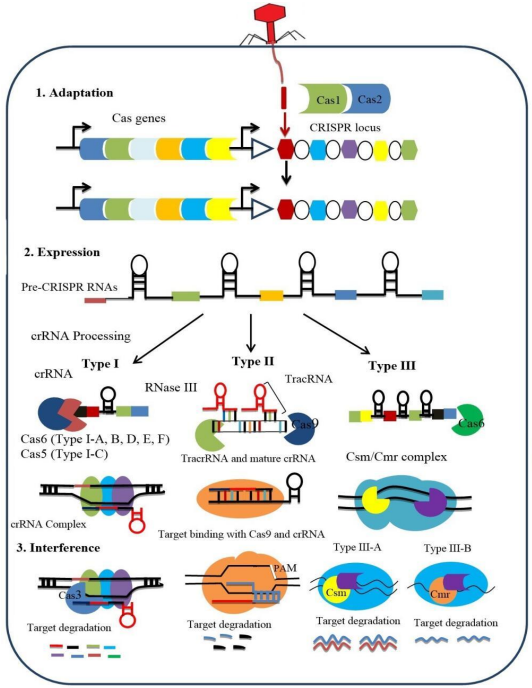
Guided and Cut by a single RNA

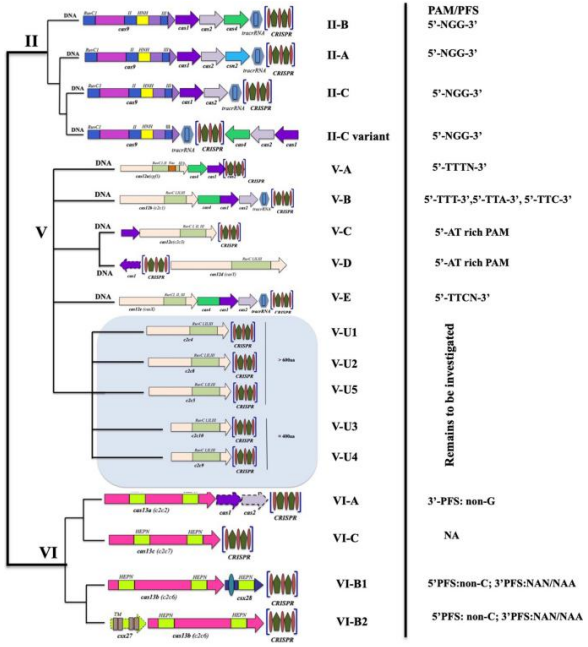
HYER: Hydrolytic endonucleolytic ribozyme

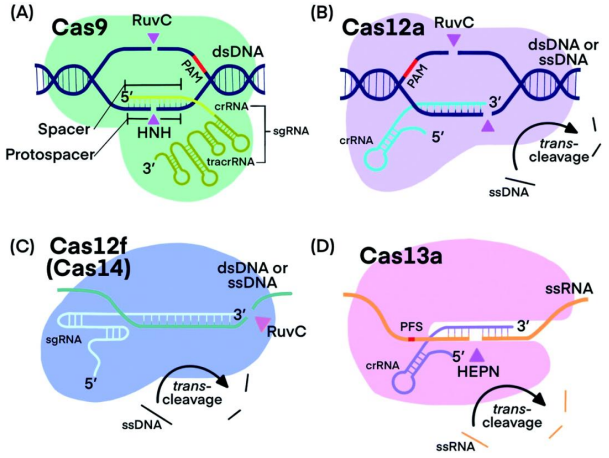
DNA导向技术/DNA guided?

gDNA/NgAGO, failed

丰富多样的CRISPR基因剪刀手/Variety of CRISPR gene scissors







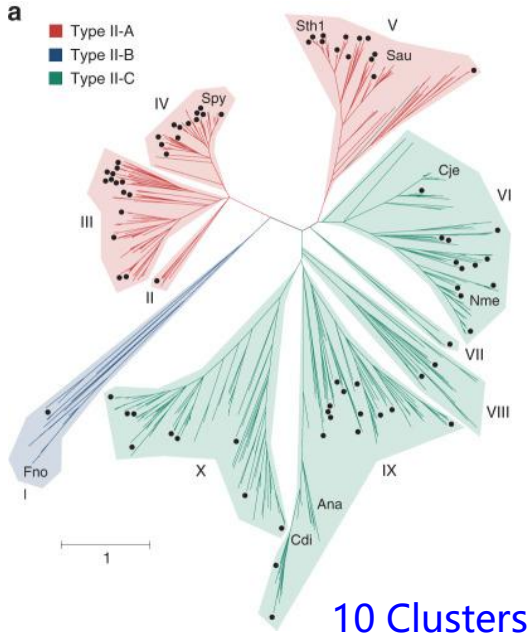
ZHANG Feng (张锋)

Jennifer A. Doudna

西北农林科技大学

各式各样的CRISPR/Cas9系统 / Diverse CRISPR/Cas9 systems

CRISPR-Cas工具酶介绍 (qq.com)



10 Clusters

表2. 本实验室筛选的Cas9工具酶

名字	Type II	大小	PAM	活性	特异性	时间
SauriCas9	A	1061	NNGG	高	中	2020.3
SlugCas9	A	1054	NNGG	高	低	2021.4
SlugCas9-HF	A	1054	NNGG	高	高	2021.4
ShaCas9	A	1055	NNGGV	中	中	2021.4
SlutrCas9	A	1054	NNGRR	中	中	2021.4
SchCas9	A	1054	NNGR	中	中	2022.2
Sha3Cas9	A	1055	NNGRC	高	中	2022.2
Nsp2Cas9	C	1067	NNNNCC	中	高	2022.8
NarCas9	C	1070	NNNNC	低	-	2022.8
Sha2Cas9	A	1058	NNGG	高	中	2022.11
Sha2Cas9-HF	A	1058	NNGG	高	高	2022.11
SpeCas9	A	1058	NNGG	高	中	2022.11
SpeCas9-HF	A	1058	NNGG	高	高	2022.11
SmiCas9	A	1063	NNGG	中	高	2022.11
Hsp1Cas9	C	1057	NNNNRAA	中	低	2023.4
Hsp2Cas9	C	1067	NNNNCC	中	低	2023.4
CcuCas9	C	1032	NNNNCNA	中	低	2023.4

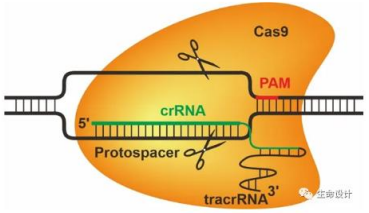
有的Cas9识别独特的PAM，但是活性不高。将识别PAM的结构域替换到活性/特异性高的同系物中，得到的嵌合体Cas9性能更好（表3）

表3. Cas9嵌合体工具酶

名字	Type II	大小	PAM	活性	特异性	时间
Sa-SlugCas9	A	1055	NNGG	高	高	2021.4
Sa-SchCas9	A	1055	NNGR	中	中	2022.2
Nsp2-SmuCas9	C	1072	NNNNC	中	高	2022.8
Hsp1-Hsp2Cas9	C	1048	NNNNCY	中	低	2023.4
Hsp1-Hsp2Cas9-Y	C	1048	NNNNCY	中	高	2023.4
Hsp1-Hsp2Cas9-KY	C	1048	NNNNCY	中	高	2023.4

表1. Cas9工具酶

名字	Type II-	大小	PAM	活性	特异性	国家	时间
SpCas9	A	1368	NGG	高	低	美国	2013.2
St1Cas9	A	1121	NNAGAAW	低	中	美国	2013.2
NmCas9	C	1082	NNNNGATT	低	高	美国	2013.8
St3Cas9	A	1409	NGGNG	中	高	中国	2015.1
SaCas9	A	1053	NNGRRT	高	高	美国	2015.4
FnCas9	B	1629	NGG	低	中	美国	2016.2
CjCas9	C	984	NNNNRYAC	低	高	韩国	2017.2
GeoCas9	C	1087	NNNNCRAA	低	低	美国	2017.11
ScCas9	A	1380	NNG	低	低	美国	2018.10
Nme2Cas9	C	1082	NNNNCC	中	高	美国	2019.2



https://pubmed.ncbi.nlm.nih.gov/33139742/

复旦大学王永明

Wang Yongming from Fudan University, China

西北农林科技大学

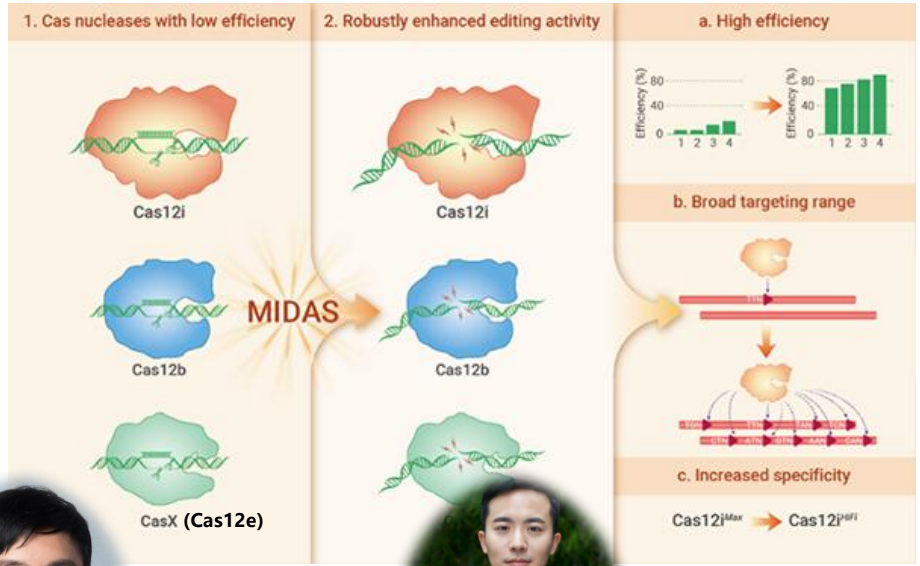
各式各样的CRISPR/Cas12系统/Diverse CRISPR/Cas12 systems

表4. Cas12工具酶

名字	大小	PAM	活性	特异性	时间
AsCas12a	1307	TTTV	高	高	2015.9
LbCas12a	1228	TTTV	中	高	2015.9
FnCas12a	1300	TTN	低	高	2015.9
Mb3Cas12a	1261	TTN	中	-	2020.9
TsCas12a	1298	TTN	中	-	2020.9
Mb2Cas12a	1251	TTN	中	-	2020.9
BsCas12a	1206	TTN	高	-	2020.9
PiCas12a	1323	KKYV	低	-	2020.6
HkCas12a	1310	YYV	低	-	2020.6
CeCas12a	1287	TTTV	中	高	2020.3
BfCas12a	1231	TTTV	中	-	2020.3
Cas12a-M29-1	1280	YYN	高	-	2020.12
Lb2Cas12a	1206	TTN	中	-	2021.12
PrCas12a	1213	TTTV	高	-	2021.12
PxCas12a	1215	TTN	中	-	2021.12
PdCas12a	1323	TTTV	低	-	2021.12
MbCas12a	1373	TTV	低	-	2021.12
EeCas12a	1282	TTTN	中	-	2021.12
ArCas12a	1266	TTN	低	-	2021.12
ErCas12a	1263	YTTN	中	-	2021.12
AaCas12b	1129	TTN	中	高	2018.11
BhCas12b v4	1108	RTTN	高	高	2019.1
BvCas12b	1112	ATTN	低	-	2019.1
DpbCas12e	986	TTCN	中	-	2019.2
PlmCas12e	978	TTCN	中	-	2022.2
Cas12i^{Max}	1054	HHN	高	低	2022.5
Cas12j-8	718	TTN	高	极高	2023
AsCas12f1	422	TTR	低	高	2021
Un1Cas12f1	529	TTTN	中	极高	202
enOsCas12f1	433	TTH	高	高	202
enRhCas12f1	415	CCD	高	高	2023

中国农大赖锦盛

中科院神经所杨辉



中科院动物所李伟

上科大季泉江

清华大学刘俊杰

From different affiliations in China

更多的 “剪刀手”/What's more

virginijus siksnys

CRISPR:

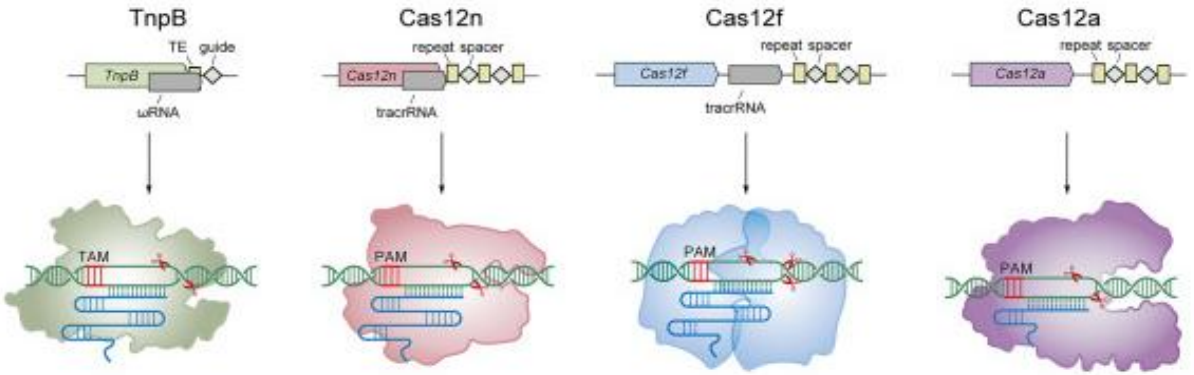
Clustered **R**egularly
Interspaced **S**hort
Palindromic **R**epeats

规律间隔成簇短回文重复序列

OMEGA：

Obligate **M**obile **E**lement
Guided **A**ctivity

具有导向活性的指定移动元件

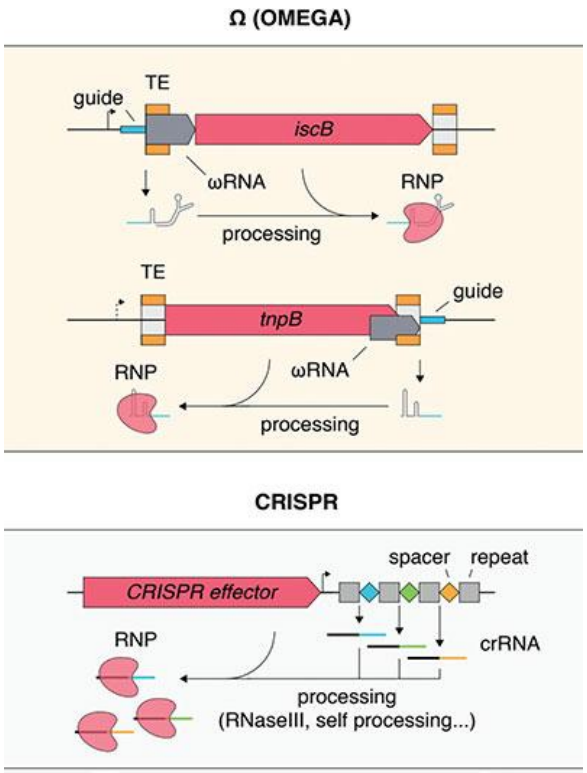


System	IS200/IS605 and IS607	CRISPR-Cas	CRISPR-Cas	CRISPR-Cas
Protein	~400 aa (monomer)	400-700 aa (likely monomer)	400-700 aa (dimer)	1000-1500 aa (monomer)
Guide RNA	ωRNA	crRNA and tracrRNA	crRNA and tracrRNA	crRNA
gRNA region	Located in protein ORF	Located in protein ORF	Exist alone	Exist alone
dsDNA target	5'TAM and target	5'PAM and target	5'PAM and target	5'PAM and target
M/PAM	5'-TTGAT / 5'-TCAN	5'-NAAN	5' T-rich PAM	5' T-rich PAM

ZHANG Feng
(张锋)

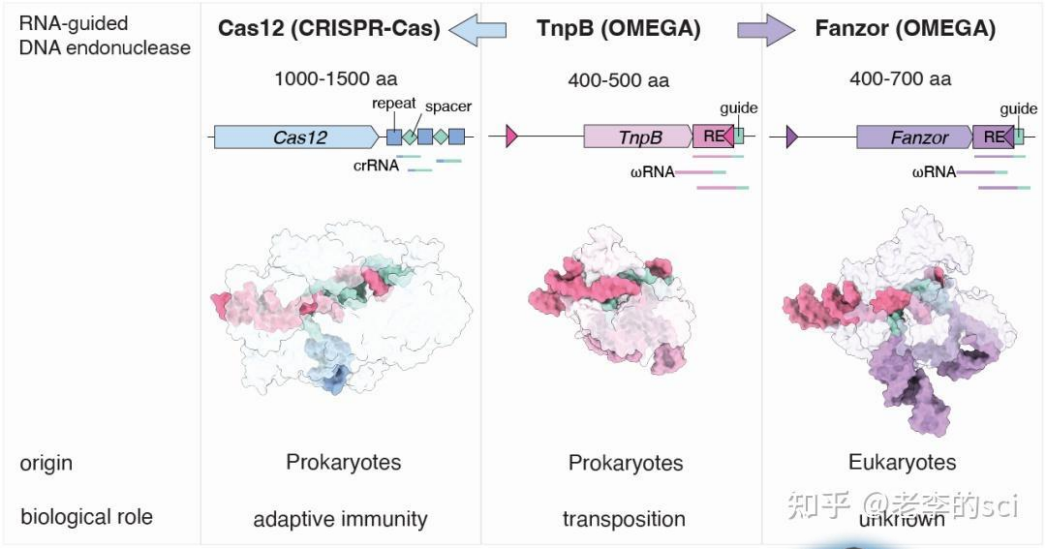
西北农林科技大学

“剪刀手”的进化/The evolution of Scissors



IscB→Cas9

TnpB→Cas12，Fanzor

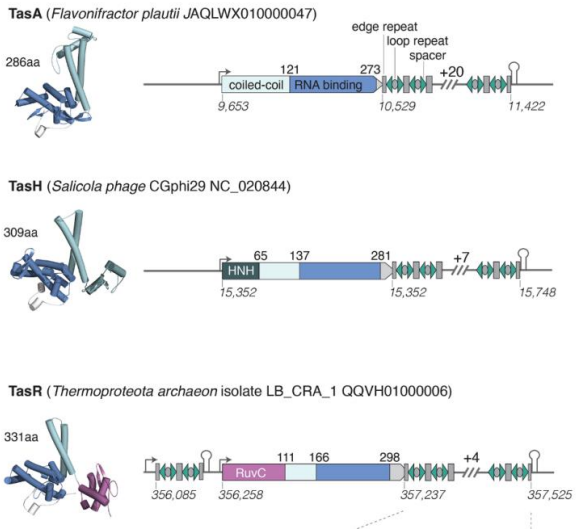


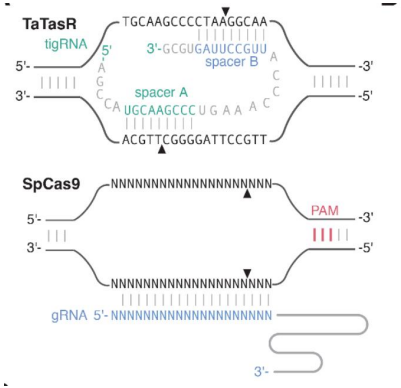
ZHANG Feng
(张锋)

西北农林科技大学

最新的基因剪刀手/The Latest PAMless Scissors

Tandem Interspaced Guide RNA (**TIGR**) array and a TIGR-associated (**Tas**),
Similar but different with CRISPR/Cas and OMEGA systems





- **PAMless**
- **Targeting both sense and antisense strands**

ZHANG Feng (张锋)

Cas9、Cas12、Cas13 IscB、TnpB、Fanzor、Tas、 CRISPR screening、CRISPR detection,,,,,

https://pubmed.ncbi.nlm.nih.gov/40014690/

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目录/Contents

Fantastic and wonderful expectations

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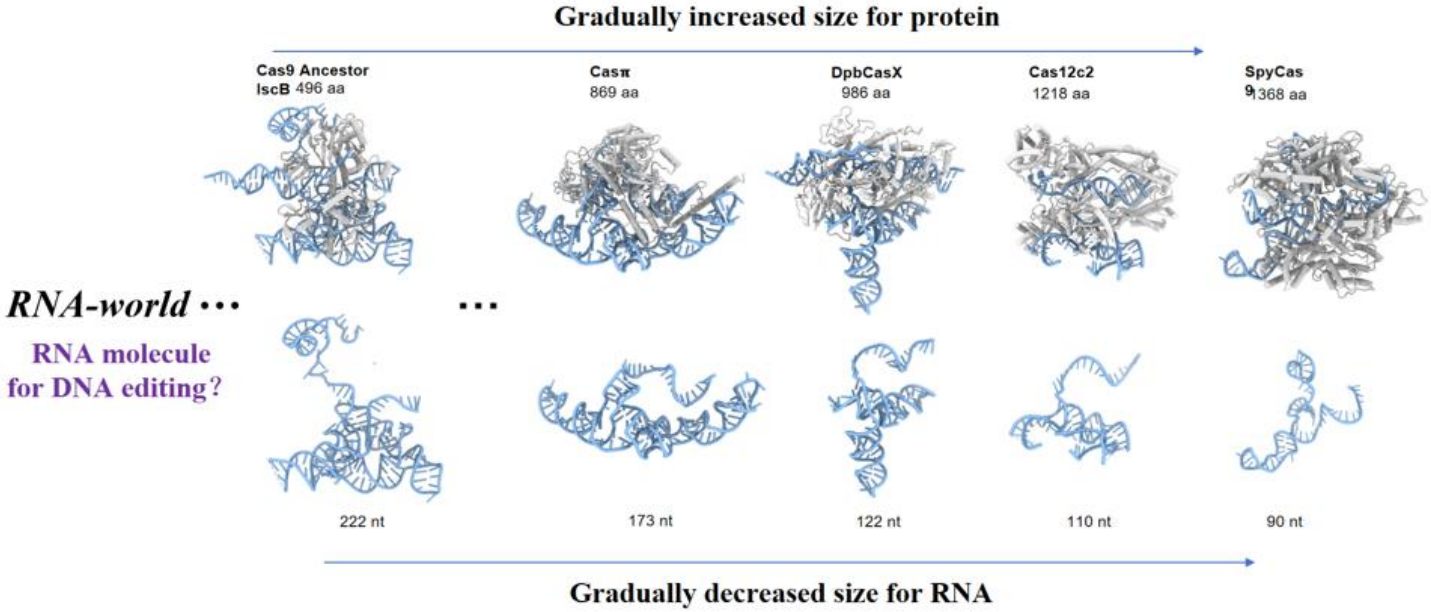
“RNA-World”

1. Background

RNA-Protein co-evolution starting from the RNA world

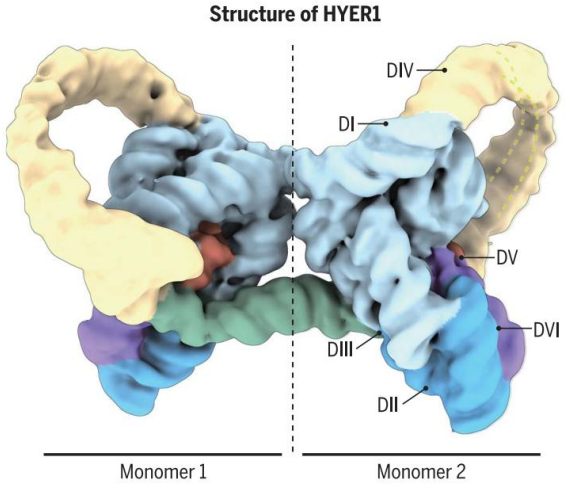
How is RNA gradually replaced by protein?

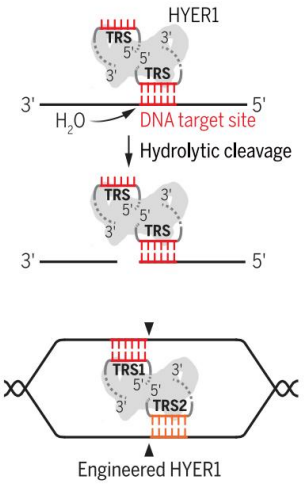


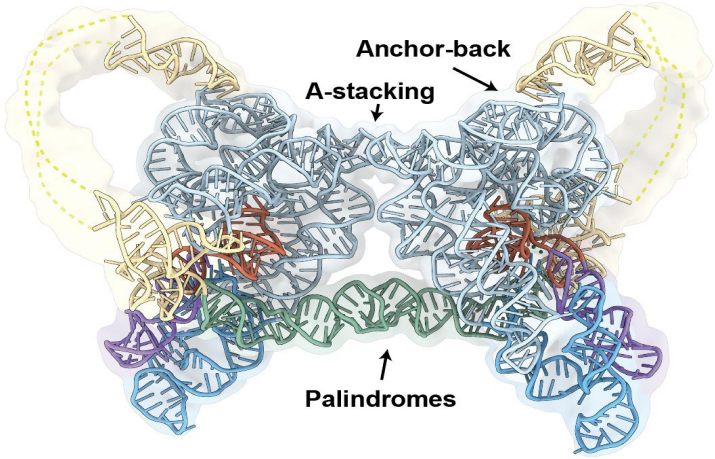
Sun A. et. al., Cell Res, 2023
Connor T.A. et al., Mol. Cell, 2022
Liu J.J. et al., Nature, 2019

清华大学刘俊杰
LIU Junjie from
Tsinghua University
China

Scissors by only RNA: Hydrolytic endonucleolytic ribozyme (HYER)







https://pubmed.ncbi.nlm.nih.gov/38301022/

Liu Z, et al. **Science**. 2024.02

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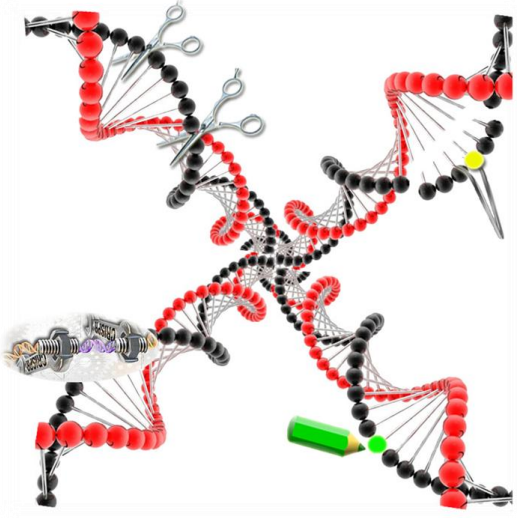
Assignments and Discussions:

1. 画图+列表，对比总结归纳上述基因剪刀手; Draw diagrams and make tables, to compare and summarize the above-mentioned gene scissors

2. 思考如何利用上述工具实现基因编辑? Think about how to utilize the above-mentioned tools to achieve gene editing?



https://www.bilibili.com/video/BV15x411Z7kf?spm_id_from=333.788.videopod.episodes&vd_source=dac2eca651ed36d05d1ed333d1c7177d

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