

Literature Report

Reporter: Chunyu Yan

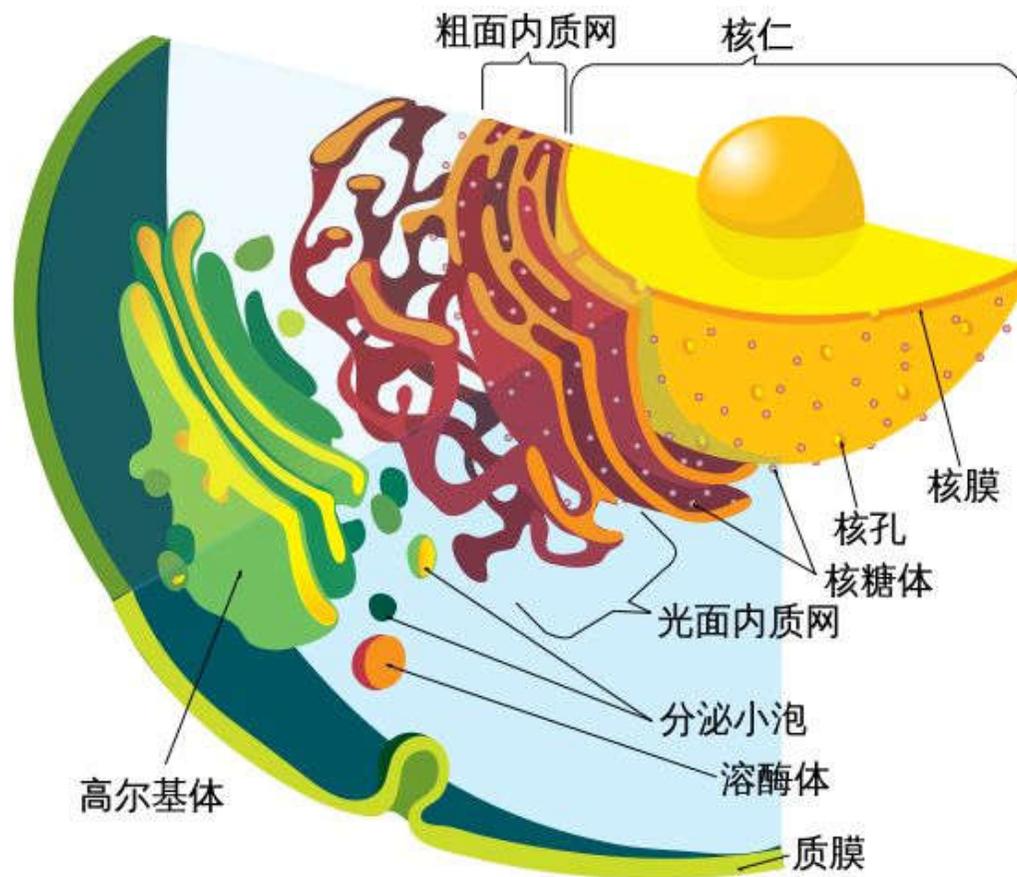
Date: 2020-12-03

Background



基因好好的，干嘛要被人编辑？

基因是潜藏在细长DNA链中的一段段序列。DNA被细胞层层叠叠的膜结构保护着，还有非常严谨的DNA修复机制应变。想对它做什么手脚，并不简单。



将生物的基因序列做某种改造，看看生物会因此出现什么变化，从而了解那个基因可能的功能。

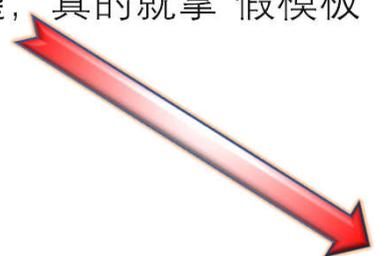
把基因拆了，改了之类的操作，就可以叫做“基因编辑”。

Background

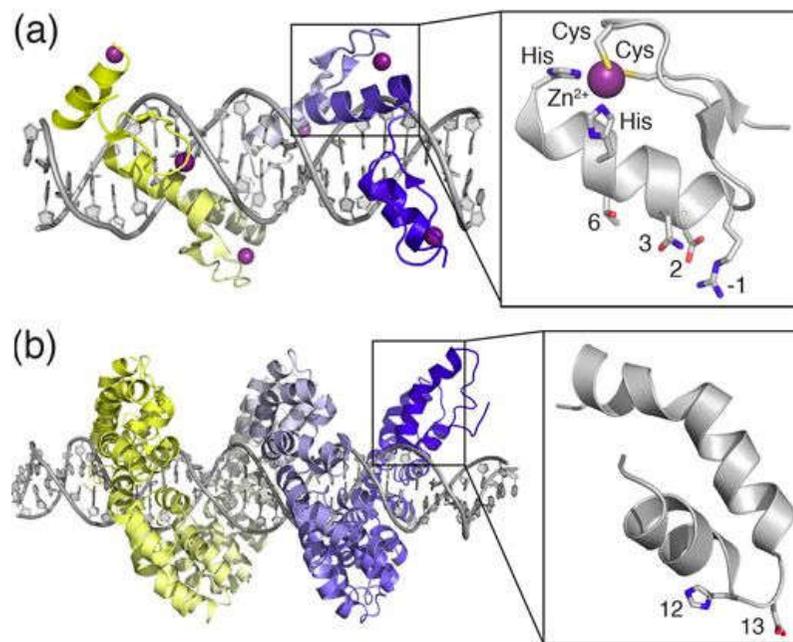


奥利弗·史密斯 (Oliver Smithies)
遗传工程的奠基人之一。
因此分享了2007年的诺贝尔生理
或医学奖。

上世纪八十年代，奥利弗·史密斯 (Oliver Smithies) 等科学家：故意塞给细胞一堆“假模板”。根据需要人工合成一些DNA，并且故意把这些DNA序列设计得和某个基因的序列有点像，然后将它们塞到细胞里面去。细胞果然没让人失望，真的就拿“假模板”修复了真的基因。



到了21世纪初，Sangamo公司主导研发的“锌指核酸酶” (ZFNs)，和另一个由全球众多科学家一点一点捣鼓出来的“转录激活因子样效应物核酸酶” (TALENs)。

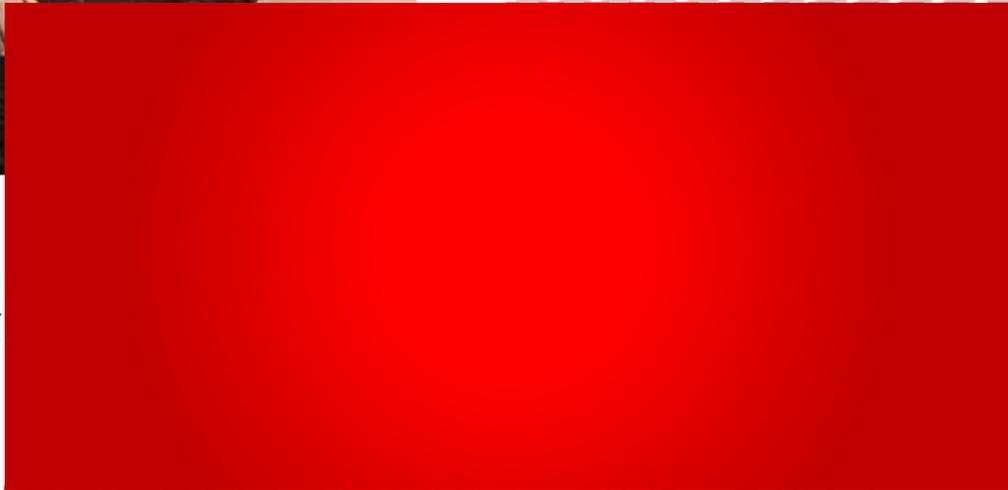


Background



2003年，西班牙微生物学家**弗朗西斯科·莫伊卡 (Francisco Mojica)** 给《自然》杂志投了个论文，惨遭拒稿。后来这篇论文又相继投递给了《美国科学院院刊》《分子微生物学》以及《核酸研究》等一系列期刊，但也都遭到了冷落。原因无它，因为这个研究的结论实在是太奇怪了。2005年，莫伊卡的研究成果才被个比较一般的学术期刊《分子演化杂志》所接收。

弗朗西斯科·莫伊卡
CRISPR系统第



因编辑工具的实力。

的最
埃
她
基

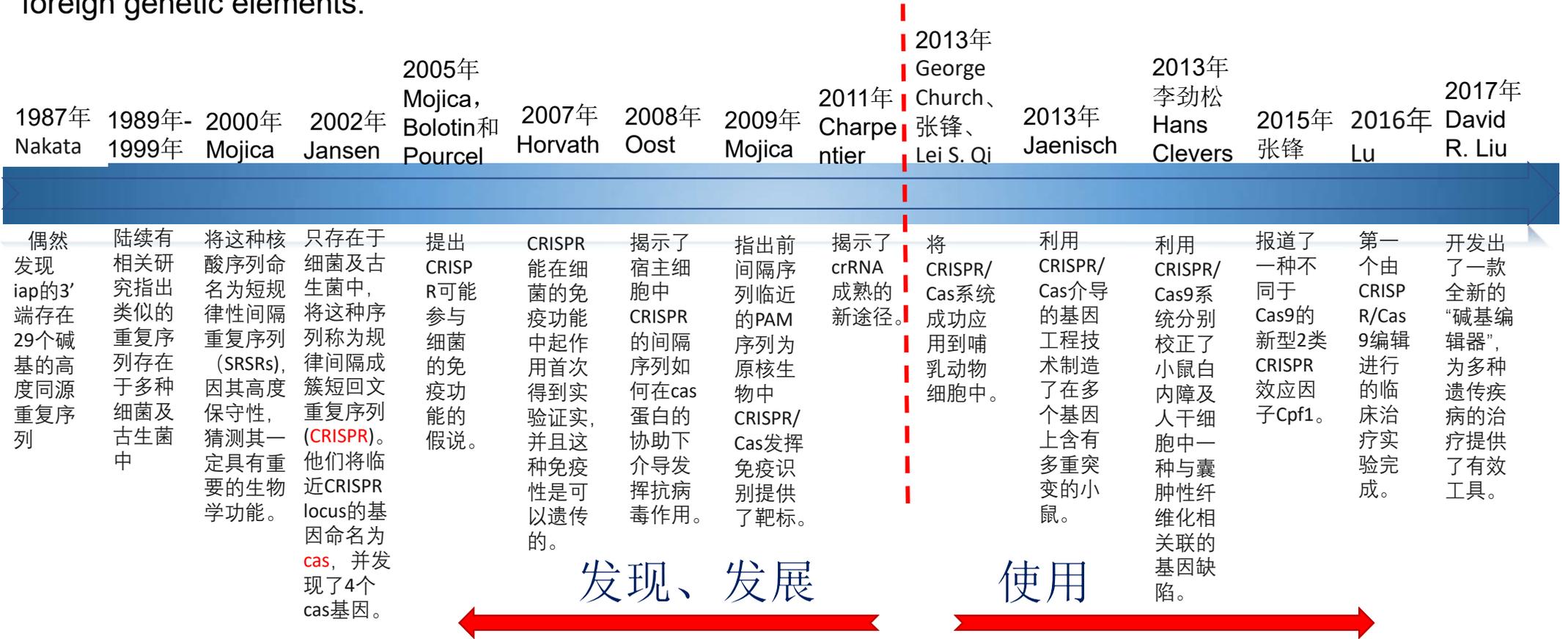


埃玛纽埃尔·卡彭蒂耶
(Emmanulle Charpentier)

Introduction



CRISPR/Cas: In **bacteria and archaea**, clustered regularly interspaced short palindromic repeats (**CRISPR**) and CRISPR-associated (**Cas**) proteins constitute an **adaptive immune system** against phages and other foreign genetic elements.

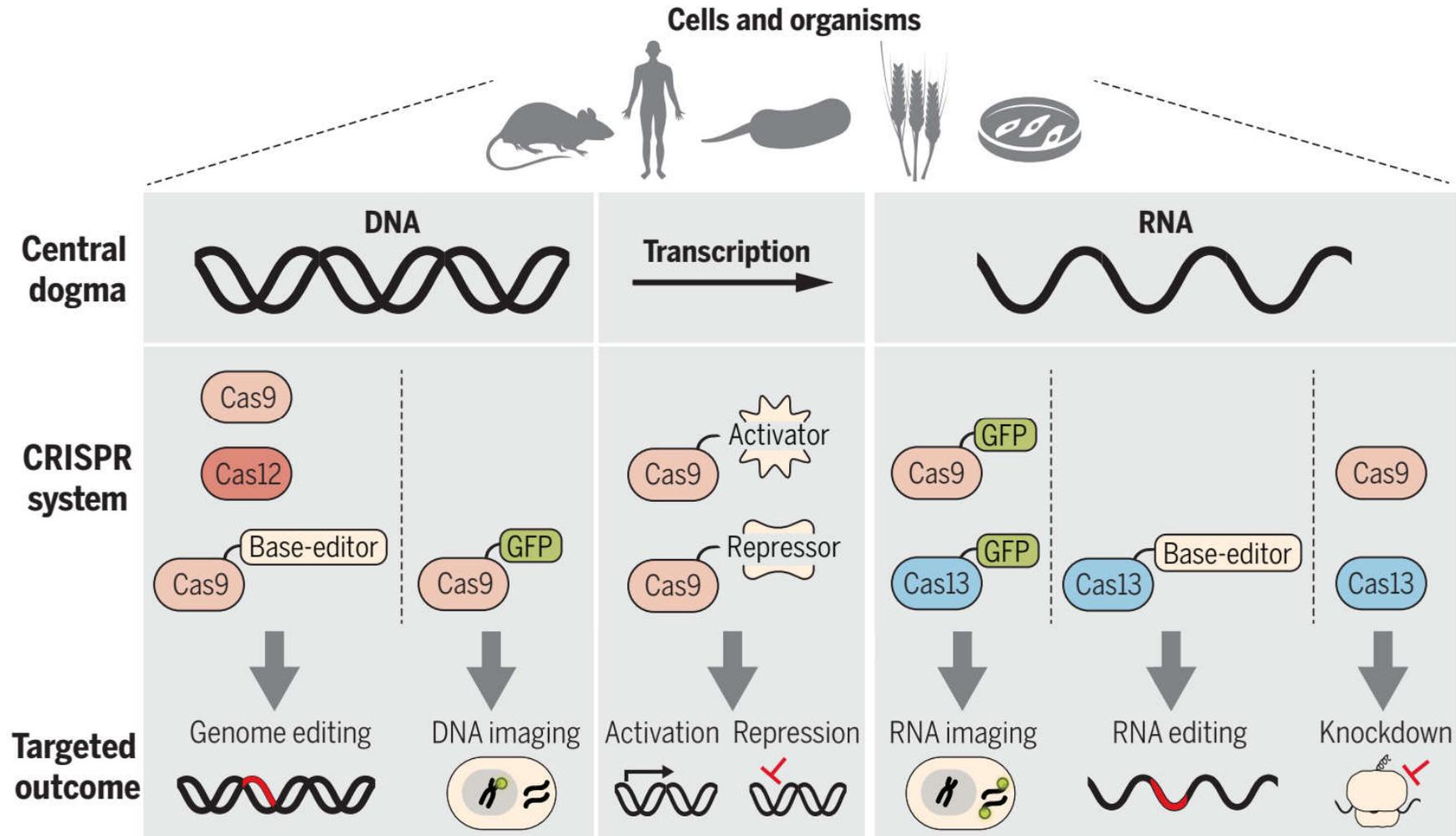


Introduction



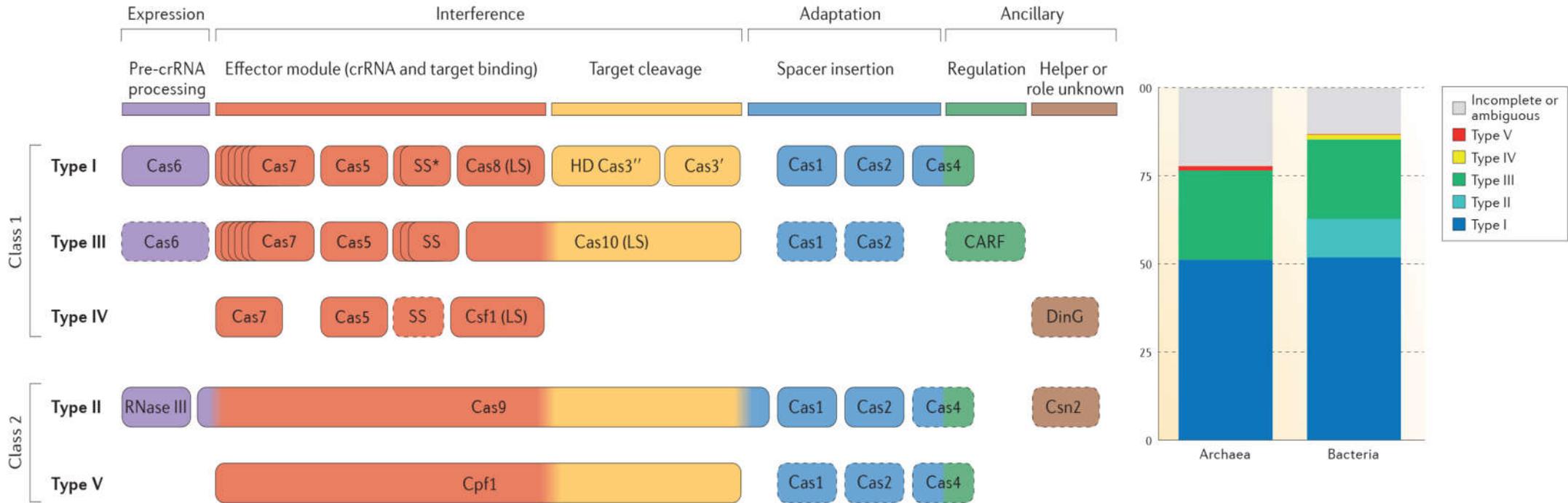
CRISPR 之争，左至右： Jennifer Doudna, Emmanuelle Charpentier, 张锋, George Church

Introduction



Knott et al., Science 361, 866–869 (2018)

Introduction



Nature Reviews Microbiology, 28,9 (2015):3569

Introduction

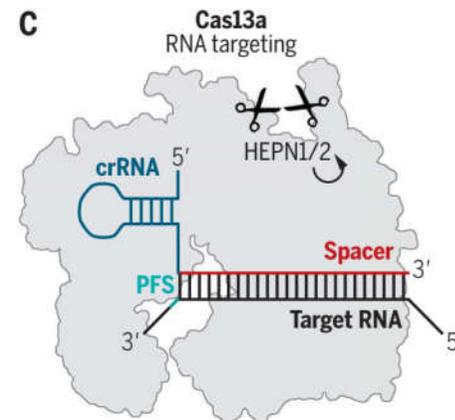
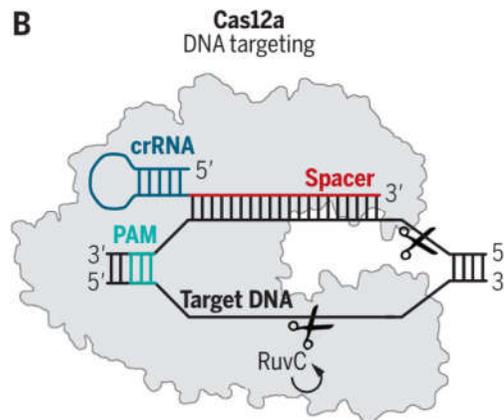
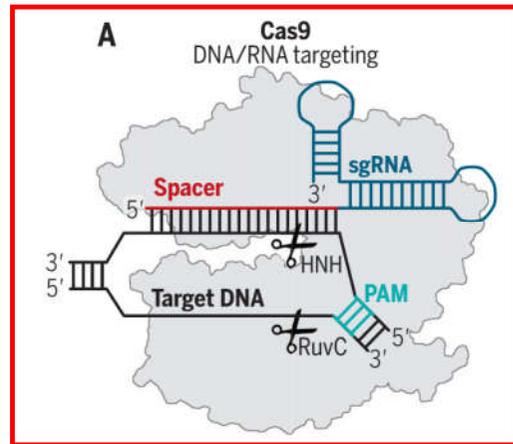
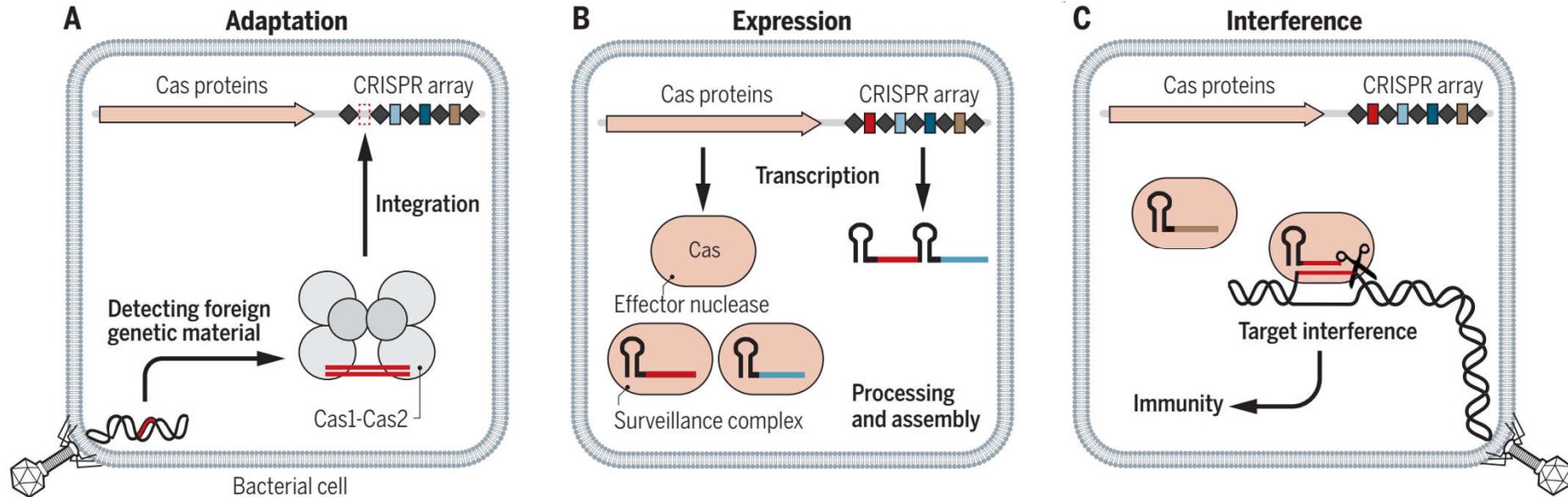


Fig. 1. CRISPR-Cas adaptive immunity.

Knott et al., Science 361, 866–869 (2018)

Problem and Solution



CASFISH: CRISPR/Cas9-mediated in situ labeling of genomic loci in fixed cells

Wulan Deng^{a,1}, Xinghua Shi^a, Robert Tjian^{a,b}, Timothée Lionnet^a, and Robert H. Singer^{a,c,d,1}

^aTranscription Imaging Consortium, Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, VA 20147; ^bDepartment of Molecular and Cell Biology, University of California, Berkeley, CA 94707; ^cDepartment of Anatomy and Structural Biology, Albert Einstein College of Medicine, Bronx, NY 10461; and ^dGruss-Lipper Biophotonics Center, Albert Einstein College of Medicine, Bronx, NY 10461

Contributed by Robert H. Singer, August 11, 2015 (sent for review July 15, 2015; reviewed by Joseph G. Gall, Arjun Raj, and Xiaowei Zhuang)

2015年, 首次提出CASFISH的概念

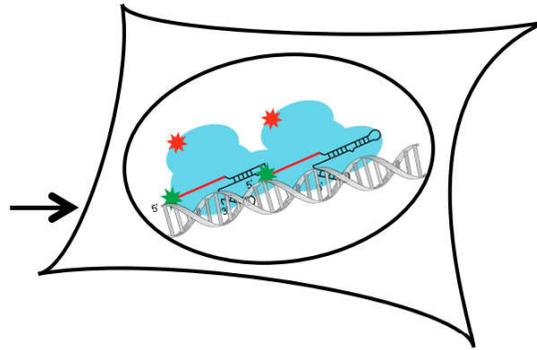
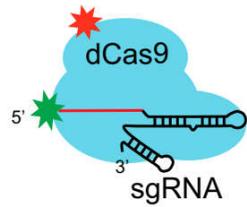
PNAS, 2015 112 (38) 11870-11875

	DNA FISH	Live Cas9 imaging	CASFISH
Probe	Nucleic acid probe	Genetically coded dCas9/sgRNA	In vitro assembled dCas9/sgRNA
Difficulty of probe generation	Reliable synthesis	Variable and time-consuming	Reliable synthesis
Experiment duration	Hours to days	Immediately	Minutes to 1 h
Color	Multicolor	One color per CRISPR system	Multicolor per CRISPR system
High-throughput multiplexing	Yes	Challenging	Yes
Global DNA denaturation	Yes	No	No
Fixed cell imaging	Yes	No	Yes
Live cell imaging	No	Yes	Possible
Tissue imaging	Yes	Possible	Yes

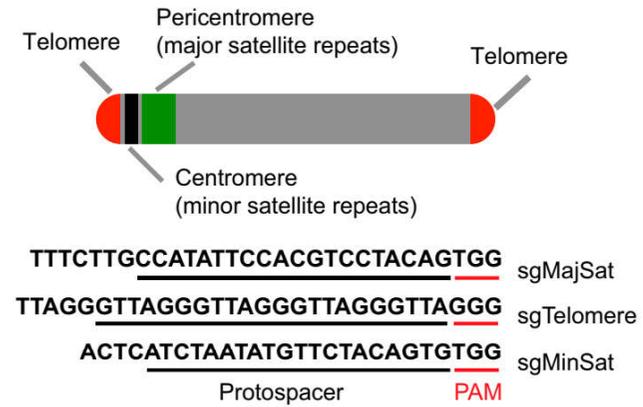
Result and Discussion



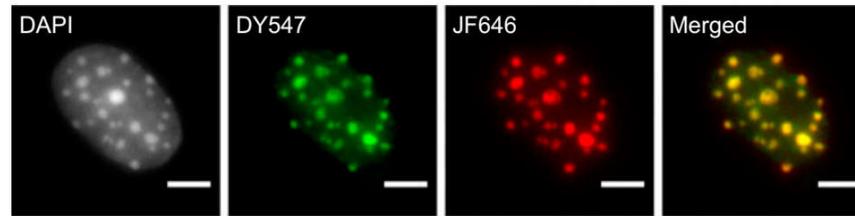
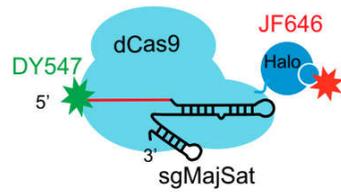
A In vitro assembled complex



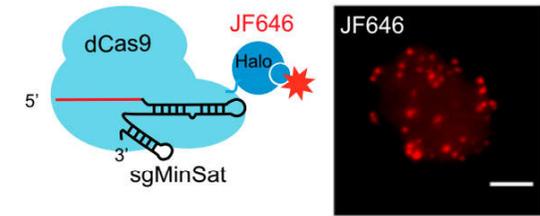
B



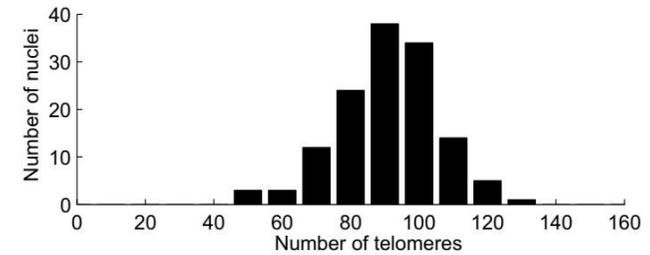
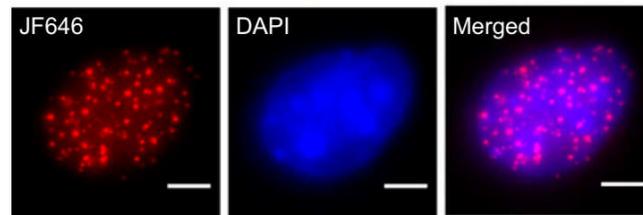
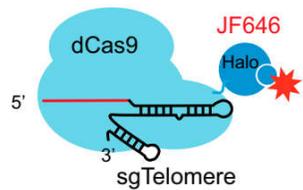
C CASFISH of pericentromeres

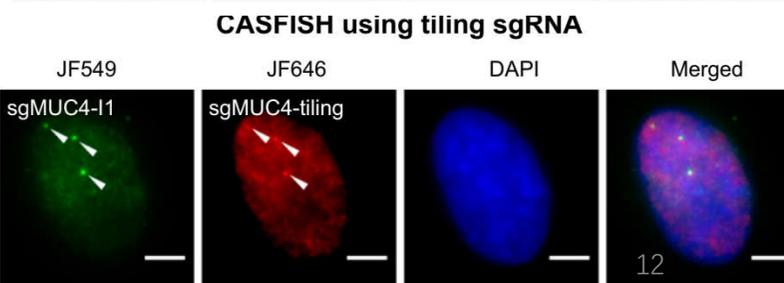
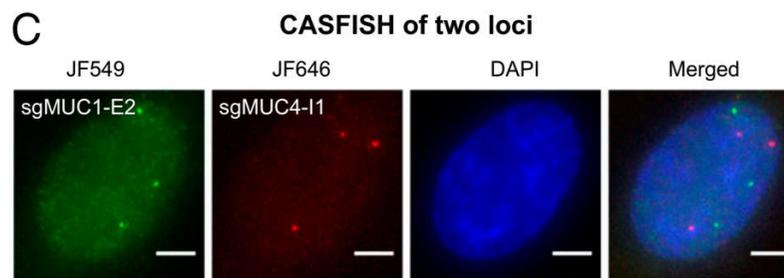
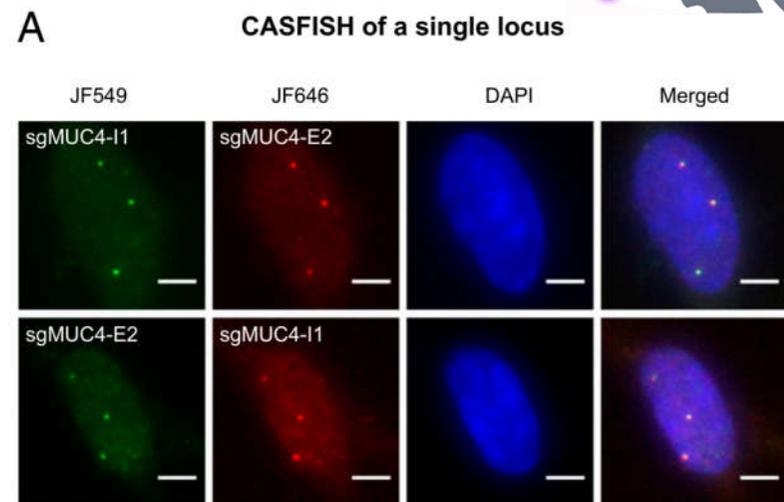
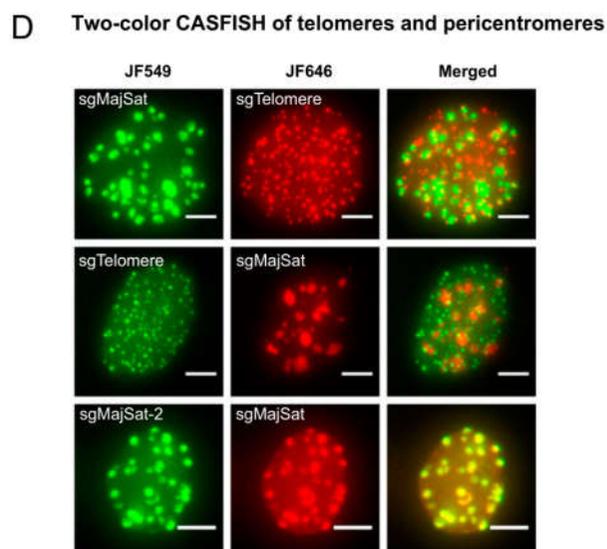
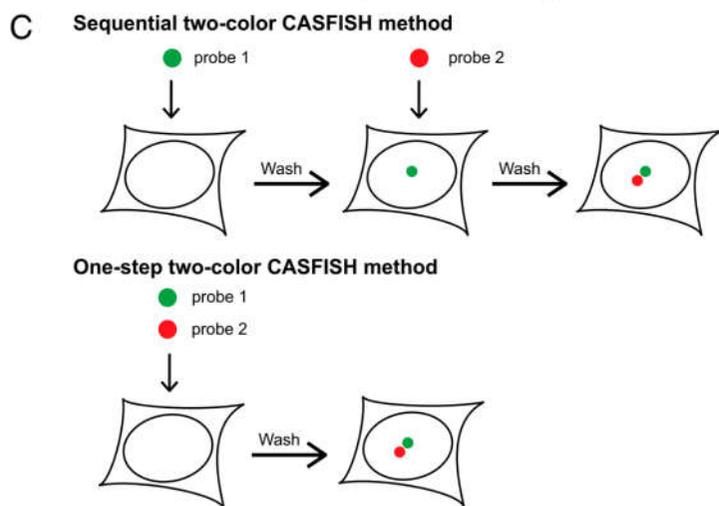
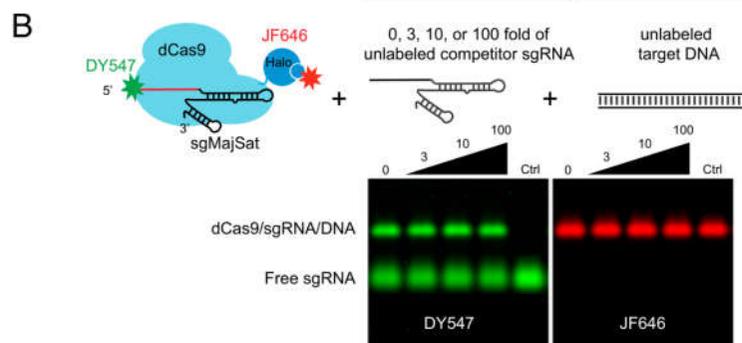
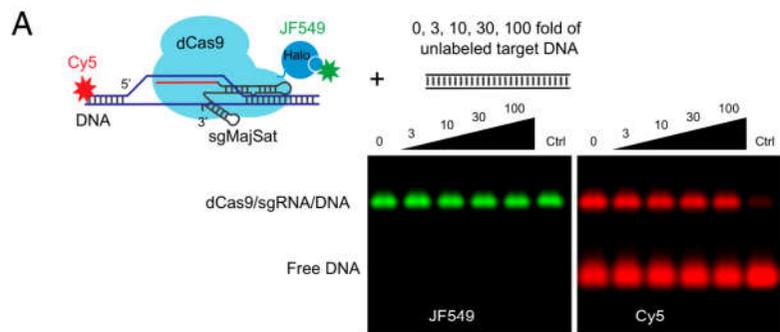


E CASFISH of centromeres



D CASFISH of telomeres

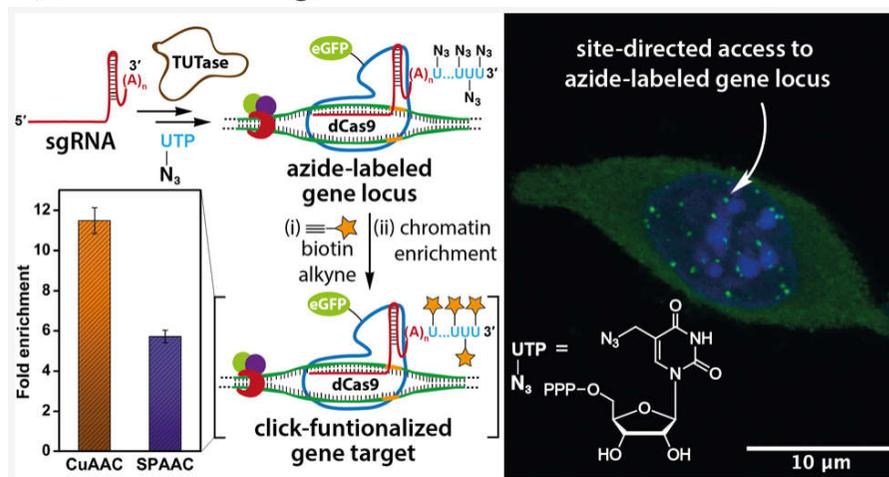






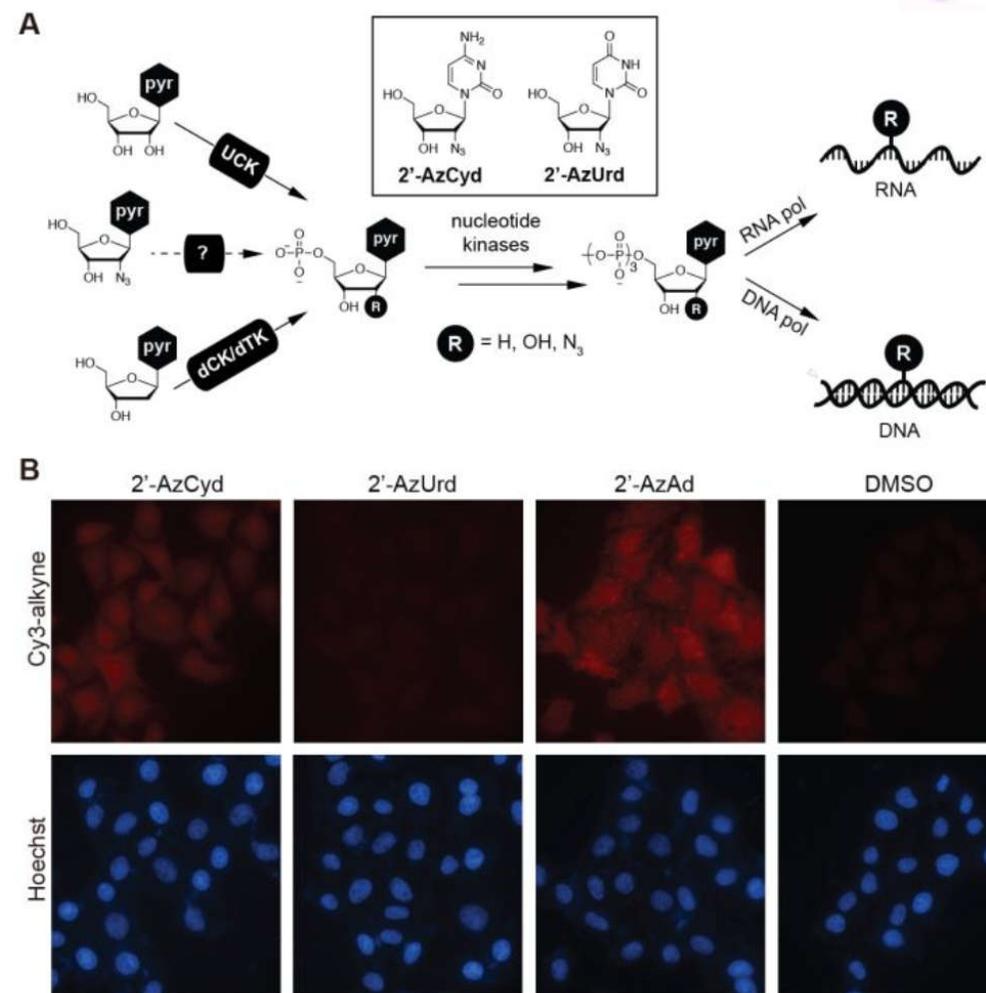
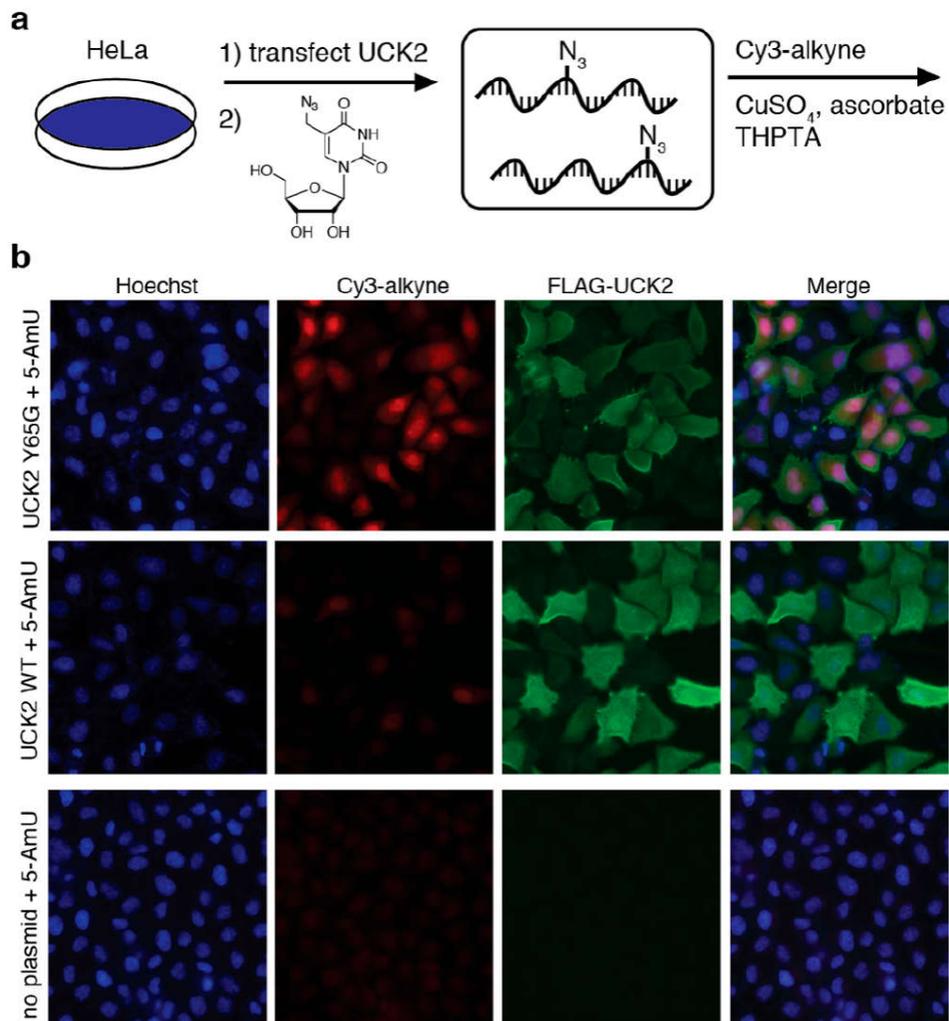
Terminal Uridylyl Transferase Mediated Site-Directed Access to Clickable Chromatin Employing CRISPR-dCas9

Jerrin Thomas George, Mohd. Azhar, Meghali Aich, Dipanjali Sinha, Uddhav B. Ambi, Souvik Maiti,* Debojyoti Chakraborty,* and Seergazhi G. Srivatsan*

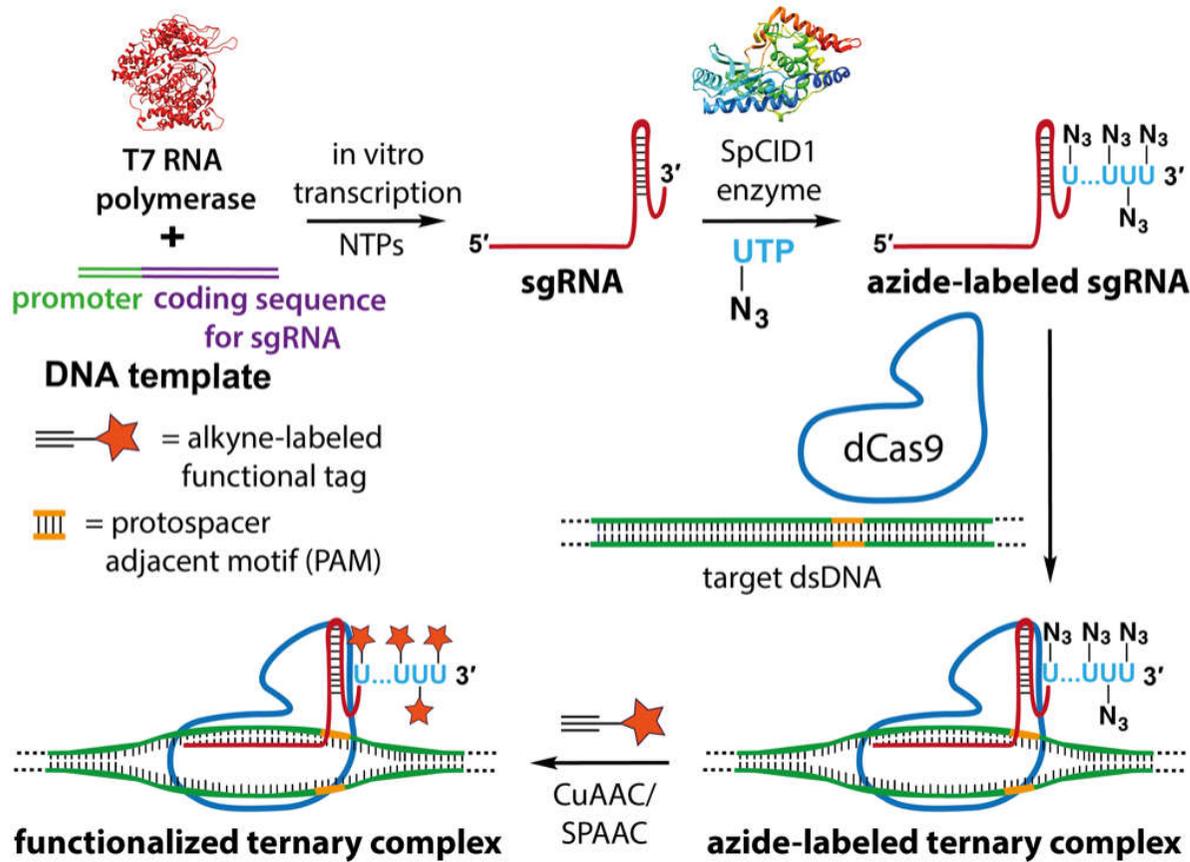


J. Am. Chem. Soc. 2020, 142, 13954–13965

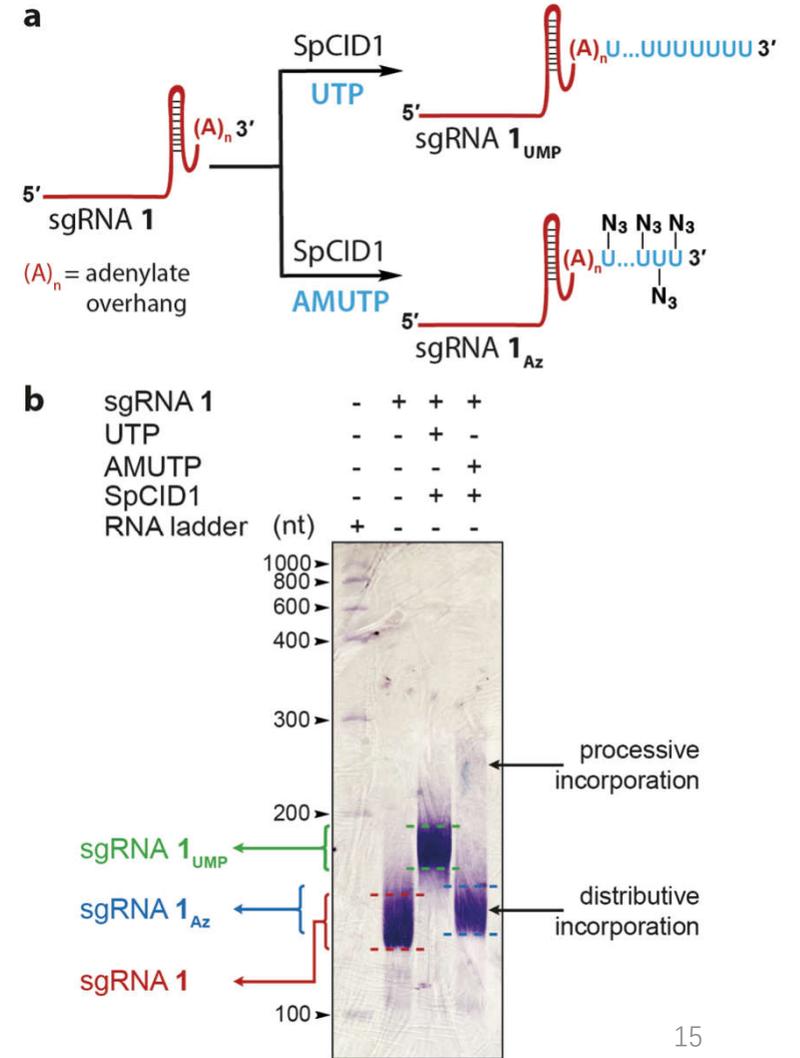
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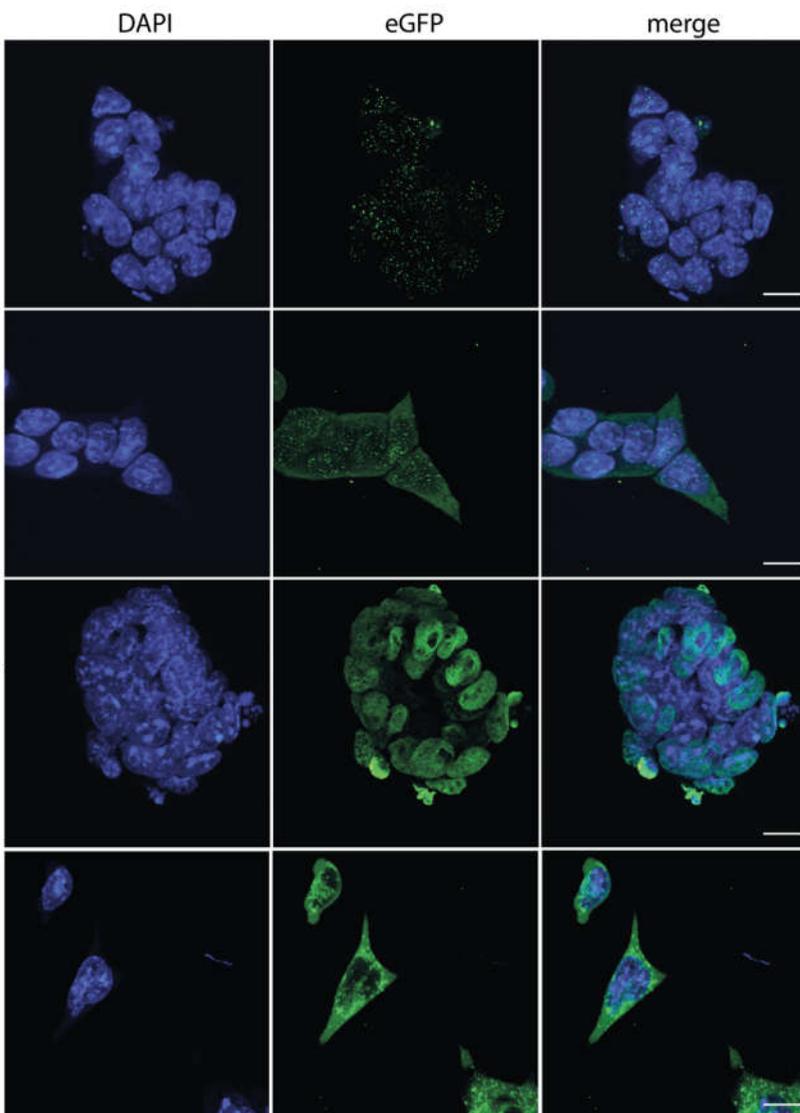
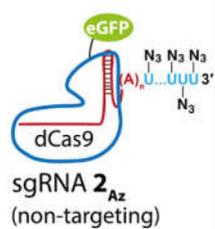
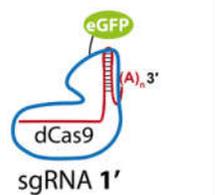
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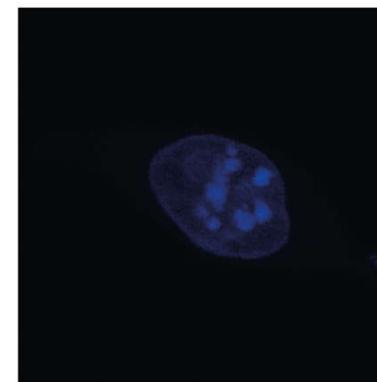


a



b

DAPI



eGFP

merge

