Genome Editing and Engineering

Course No: BT-637

LECTURE-16

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History of CRISPR

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

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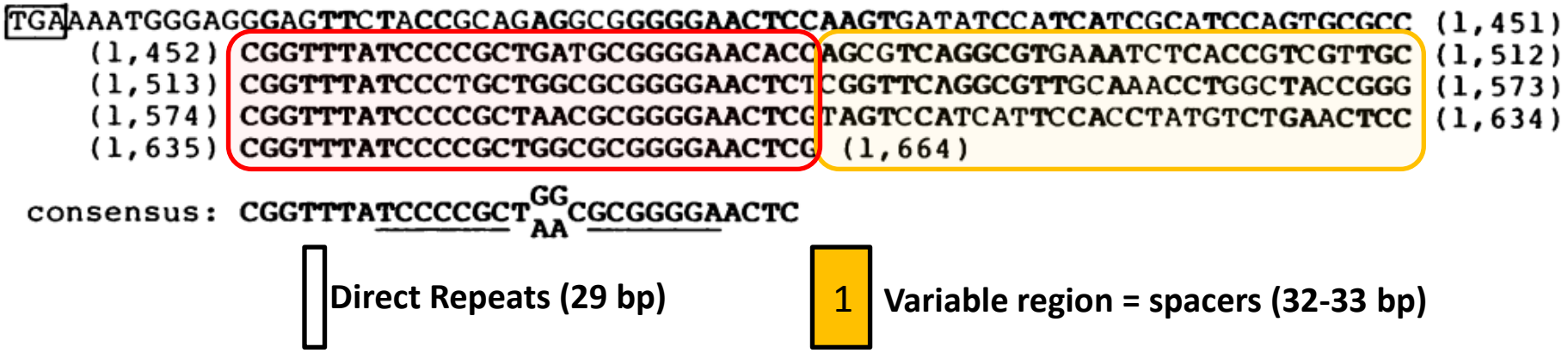
Department of Experimental Chemotherapy, The Research Institute for Microbial Diseases, Osaka University, 3-1 Yamadaoka, Suita, Osaka 565, Japan

Received 1 May 1987/Accepted 22 August 1987

IAP flanking region sequencing



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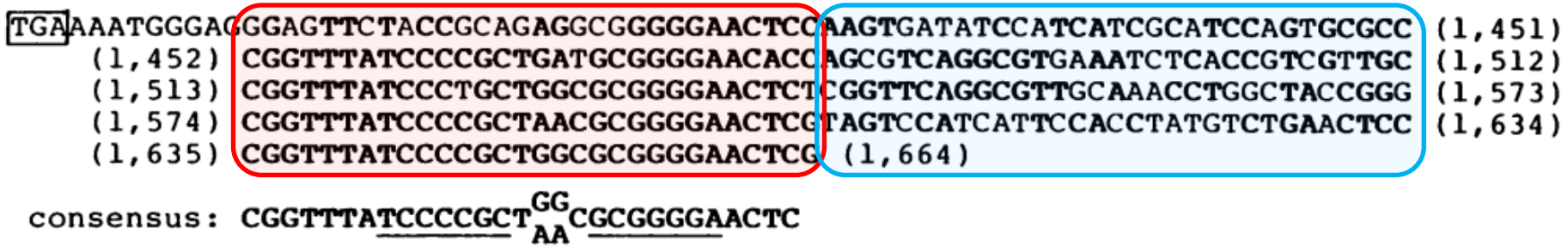
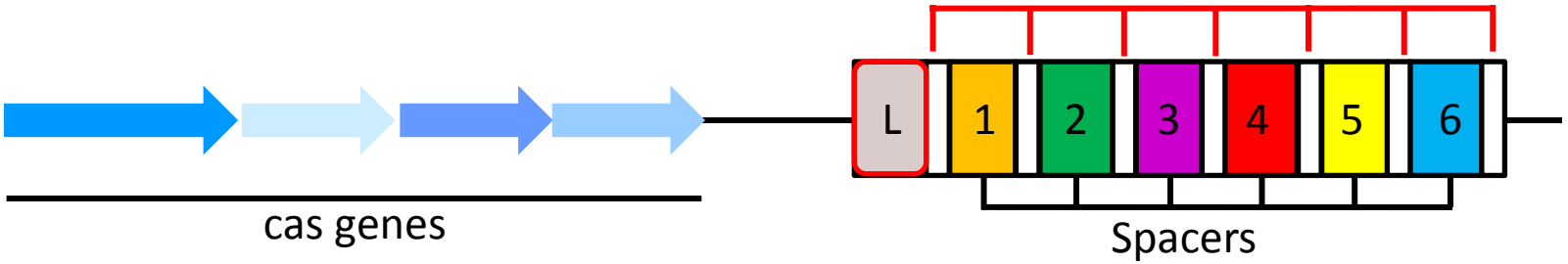


An unusual structure was found in the 3′-end flanking region of *iap* (Fig. 5). Five highly homologous sequences of 29 nucleotides were arranged as direct repeats with 32 nucleotides as spacing. The first sequence was included in the putative transcriptional termination site and had less homology than 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.

History of CRISPR (1987-2002)



DVR (Direct Variable Repeats)
TREP (Tandem Repeats)
LTRR (Long Tandemly Repeated Repetitive Sequences)
SRSR (Short Regularly Spaced Repeats)
LCTR (Large Clusters of Tandem Repeats)
SPIDR (Spacer Interspersed Direct Repeats)

CRISPR
Clustered Regularly Interspaced Short Palindromic Repeats

Jansen et al., molecular microbiology 2002

History of CRISPR (1987-2002)

Molecular Microbiology (2002) **43**(6), 1565–1575

Identification of genes that are associated with DNA repeats in prokaryotes

Ruud. Jansen,[1]* Jan. D. A. van Embden,[2]
Wim. Gaastra[1] and Leo. M. Schouls[2]
[1]*Department of Infectious Diseases and Immunology, Bacteriology Division, Veterinary Faculty, Utrecht University, Yalelaan 1, 3584 CL Utrecht, The Netherlands.*
[2]*Laboratory of Bacteriology of the Research Laboratory of Infectious Diseases, National Institute of Public Health and Environmental Protection, A. van Leeuwenhoeklaan 1, 3720 BA Bilthoven, The Netherlands.*

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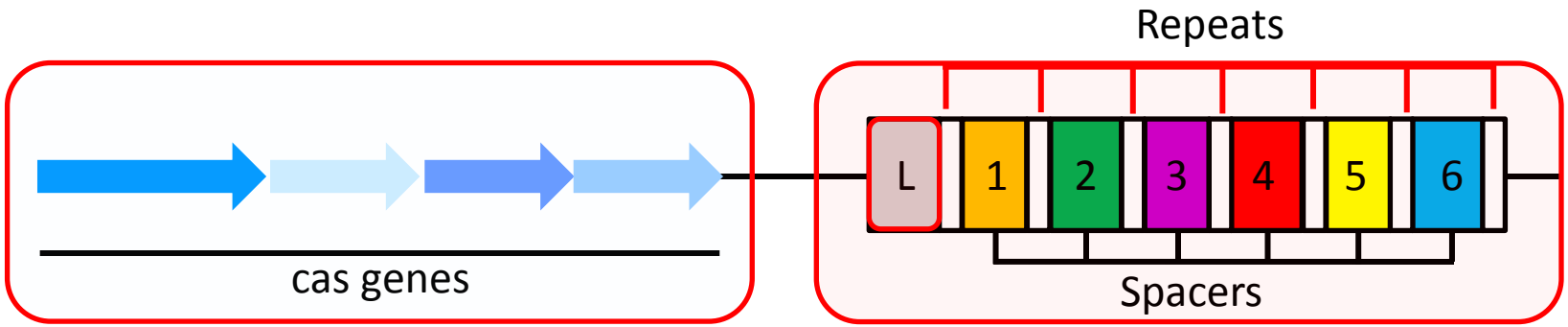

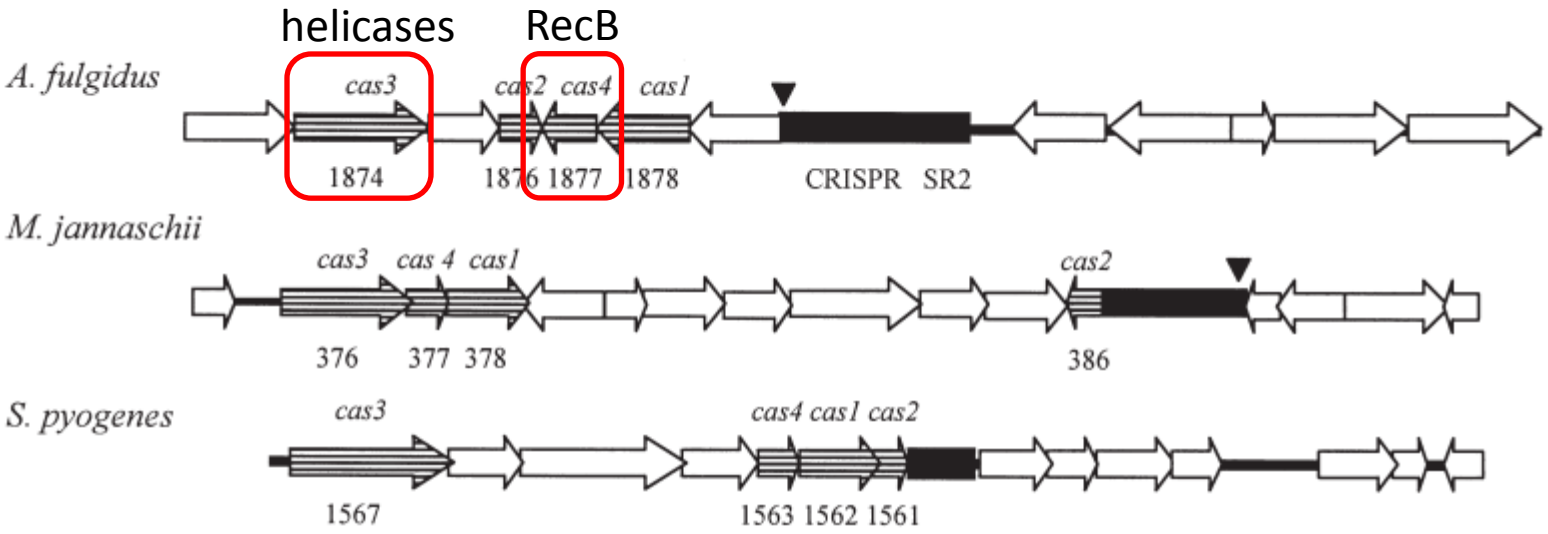

DNA metabolism or gene expression

History of CRISPR (2005)

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

JOURNAL OF MOLECULAR EVOLUTION

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

División de Microbiología, Departamento de Fisiología, Genética y Microbiología, Universidad de Alicante, Campus de San Vicente, E-03080, Spain

CRISPR elements in *Yersinia pestis* acquire new repeats by preferential uptake of bacteriophage DNA, and provide additional tools for evolutionary studies

Microbiology (2005),

C. Pourcel,[1] G. Salvignol[1] and G. Vergnaud[1,2]

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[2]Centre d'Etudes du Bouchet, 5 rue Lavoisier, 91710 Vert le Petit, France

Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extrachromosomal origin

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History of CRISPR (2005)

Table 4. Features of the sequences most similar to CRISPR spacers from *Methanothermobacter thermoautotrophicum*

Location	Phage	Activity	Alignment[a]
40 bp 3′ from ORF31	ΨM100	Not applicable	cttctagcaagagacattgacgatatacacaagtac |||||||||||||||||||||||||||||||||| cttctagcaagagacattgacgatatacacaagtac
ORF31	ΨM100	Unknown	aagcgccgggcagacagcacacatacaagacttcacaa |||||||||||||||||||||||||||||||||||| aagcgccgggcagacagcacacatacaagacttcacaa
ORF31	ΨM100	Unknown	tttcacgatgactctgttgagttcatcgtattctttcc |||||||||||||||||||||||||||||||||| || tttcacgatgactctgttgagttcatcgtattcttccc
ORF21	ΨM100	Tail protein	tgatgttgggaaggtttggccatctgaatgatttga |||||||||||||||||||||||||||||||||| tgatgttgggaaggtttggccatctgaatgatttga
peiP	ΨM2	Pseudomurein endoisopeptidase	aatattgaaacgttcaaggacatgttgaagaggtatg |||||||||||||||||||||||||||||||||| aatattgaaacgttcaaggacatgttgaagaggtatg
ORF6	ΨM2	Unknown	agtatgtgcagtatcctctctatgtccccttcattc |||||||||||||||||||||||||||||||||| agtatgtgcagtatcctctctatgtccccttcattc
ORF6	ΨM2	Unknown	aacttcacagaaaagcctccatggagcaagtgctct |||||||||||||||||||||||||||||||||| aacttcacagaaaagcctccatggagcaggtgctcc

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Table 2. Sequence of newly characterized spacers and their position outside the CRISPR loci

Chr. region	Spacer	Sequence	Position in CO92	YPO gene	Gene or product
I	z	AGATCGTGATGATAAACACACTTCCAACACAT	684 074–684 043	Between YPO0622 and YPO0623	
	y	TTGTAAGCCTTGGAACTCTGGACGCATTTTTC	684 222–684 191	YPO0623	*aspC*
II	g2	TAACGAGAAAGTCTTAATCTTTAATTCATCAG	2 357 592–2 357 561	Between YPO2075 and YPO2076	
		Between 2 363 016 and 2 409 384 putative defective lambdoid bacteriophage			
	n	TCACCAATGAGGGCGACCATGCCGAGGACTTG	2 370 263–2 370 232	Between YPO2094 and YPO2095	
	h2	TTAACGTAGCCAGGGCGTGTGGAACATGCCTAGT	2 374 566–2 374 597	YPO2101	Phage protein
	g	AAAAAGAATTTGGGATTAAAGTTACCCATCAG	2 374 731–2 374 762	YPO2102	Phage protein
	o	ACGTCATCCTGAAGGCTAGGCAGCTCGGCTTC	2 375 250–2 375 281	YPO2103	Unknown protein
	i2	TGGGACGCTTTACAGTCTGCACGTCTCTGAGT	2 375 515–2 375 546	YPO2103	
	i	GATGAGTAATGCCTTCAGCGCATTTCTCTTCA	377 2 7512 377 720	Between IS*285* and YPO2106	
	n2	ACATCTGGCCCACGACAAACATCGCGAACCGT	2 378 009–2 377 978	YPO2106	Phage protein
	d3	TTGGCAATCATGTTTGGCTGCGCTTGGTTAAAC	2 378 885–2 378 853	Between YPO2106 and YPO2108	
	s	CAGGAATGTTGGCCGCGATTGTTGCAGCTTGG	2 379 140–2 379 171	Between YPO2106 and YPO2108	
	e3	TGTCAGGCTGGGACTCTGATTTTTCAATTCGT	2 379 295–2 379 263	Between YPO2106 and YPO2108	
	k	TCAGTCCCGTTATGGTGCTGGTGTTGCCCGTAAG	2 379 357–2 379 389	YPO2108	Phage protein
	m2	TATGAGTGACAGCCGTTTTCACCACCGCCGTG	2 379 919–2 379 888	YP02108	Phage protein
	m	TTATCCGTGACCGACTCAAATACACGCTGGAACG	2 380 022–2 280 053	YPO2108	Phage protein
	a2	TCTGTACGCATACCGCCATCTTGCATCAGTCT	2 380 328–2 380 297	YPO2108	Phage protein
	u	AGCAATAAATCCCAAGGGGACAGCATGCTATT	2 380 514–2 380 545	Between YPO2108	

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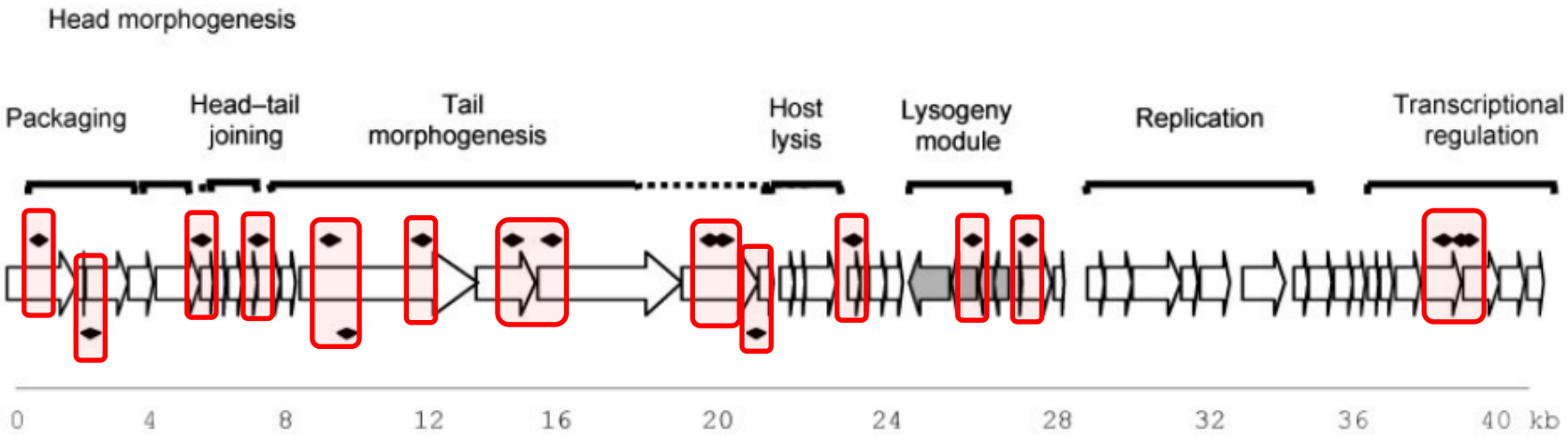


Fig. 4. Localization of spacer-matching sequences along the phage Sfi21 genome. The phage genetic map is drawn after GenBank entry NC_000872 (ORFs are shown as arrows), the regions involved in different stages of phage development, identified by comparative analysis (Desiere *et al.*, 2002), are indicated above the map, and the scale (in kb) below it. Phage regions having a BLAST E score $<0{\cdot}001$ with the CRISPR spacers are indicated by the diamonds placed above or below the map, denoting homology with the top or the bottom DNA strand, respectively.

Alexander Bolotin, Benoit Quinquis, Alexei Sorokin and S. Dusko Ehrlich

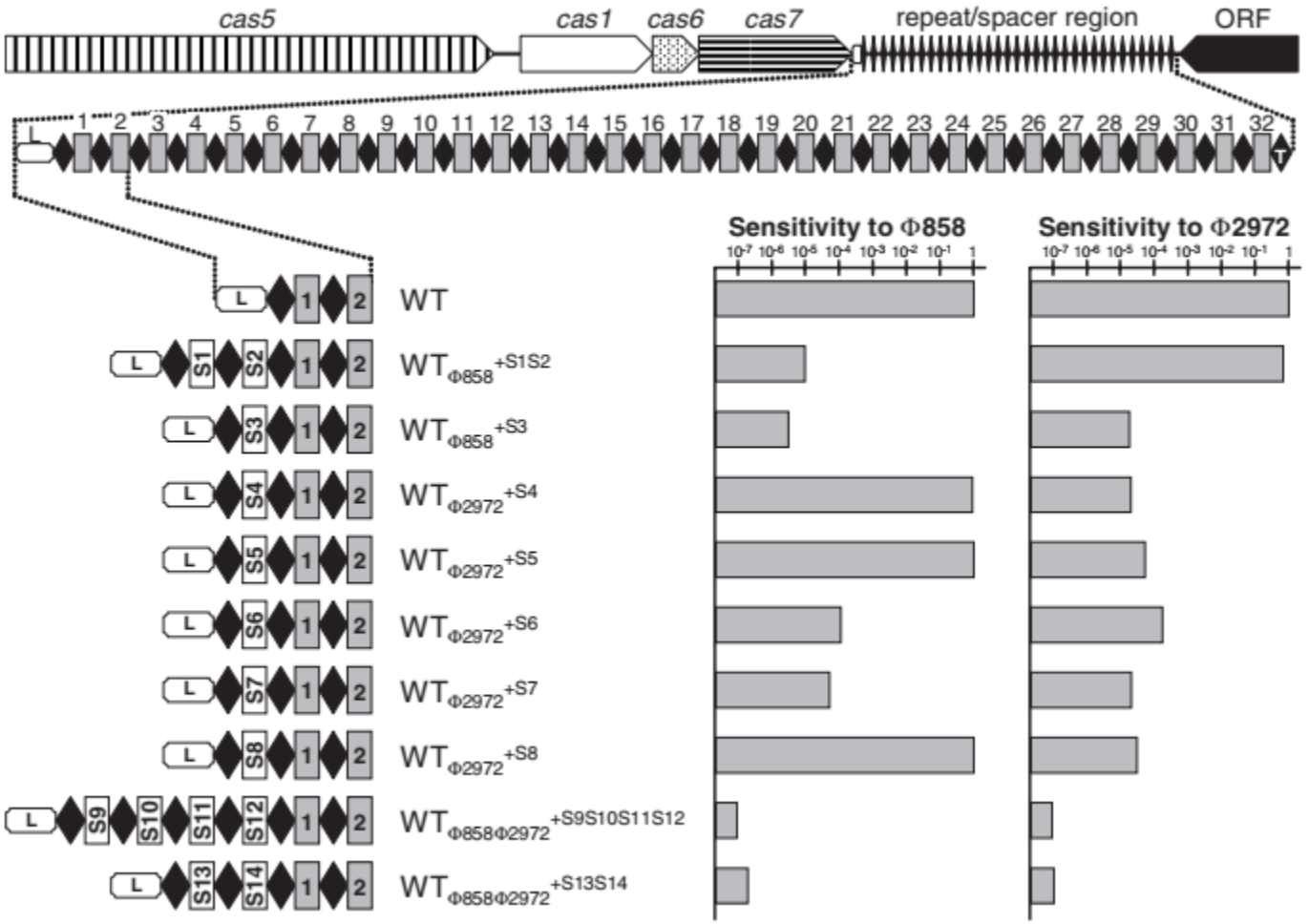
History of CRISPR- experimental evidence (2007)

CRISPR Provides Acquired Resistance Against Viruses in Prokaryotes

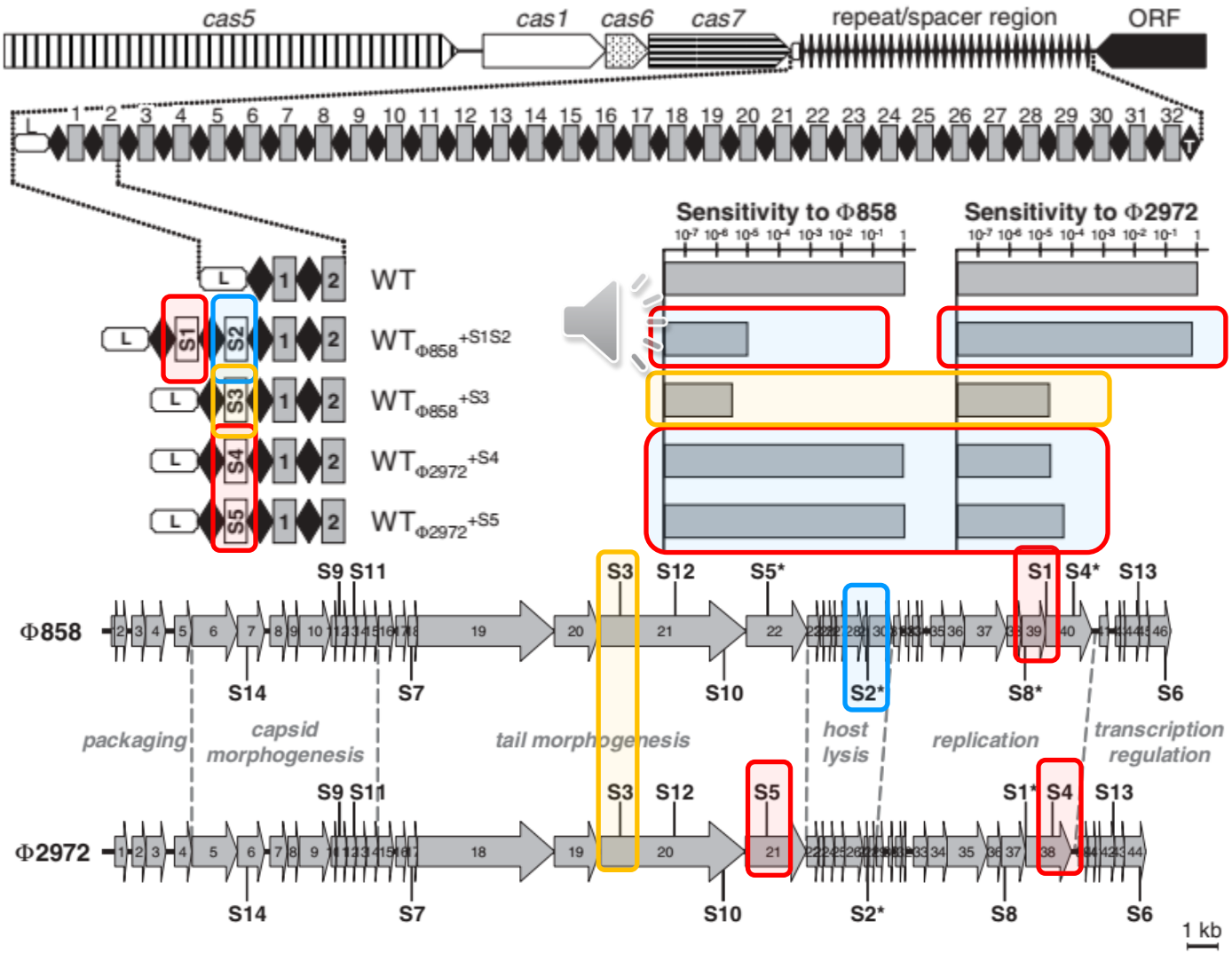
Rodolphe Barrangou,[1] Christophe Fremaux,[2] Hélène Deveau,[3] Melissa Richards,[1] Patrick Boyaval,[2] Sylvain Moineau,[3] Dennis A. Romero,[1] Philippe Horvath[2]*

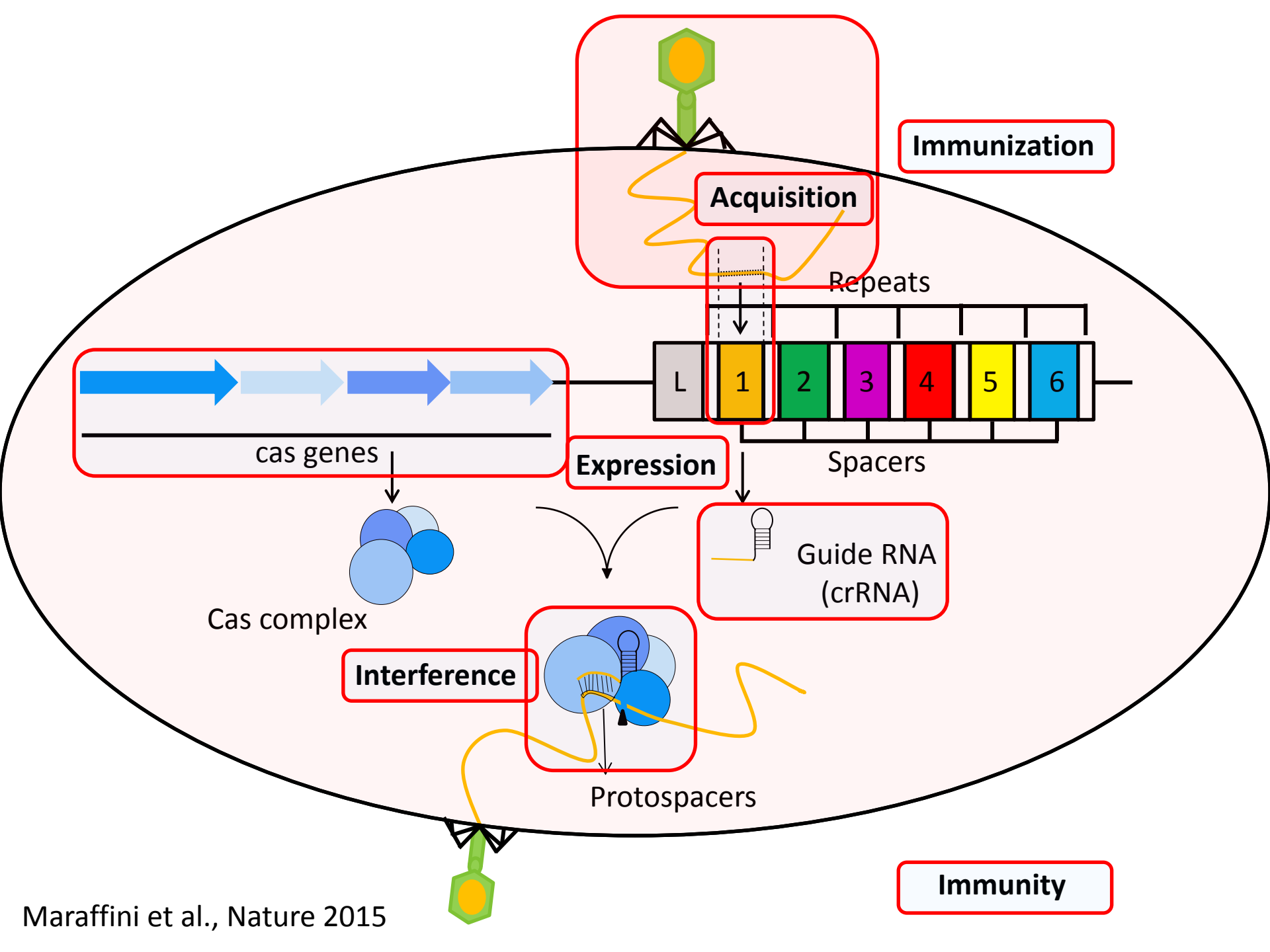
SCIENCE VOL 315 23 MARCH 2007

Acquired resistance against phage



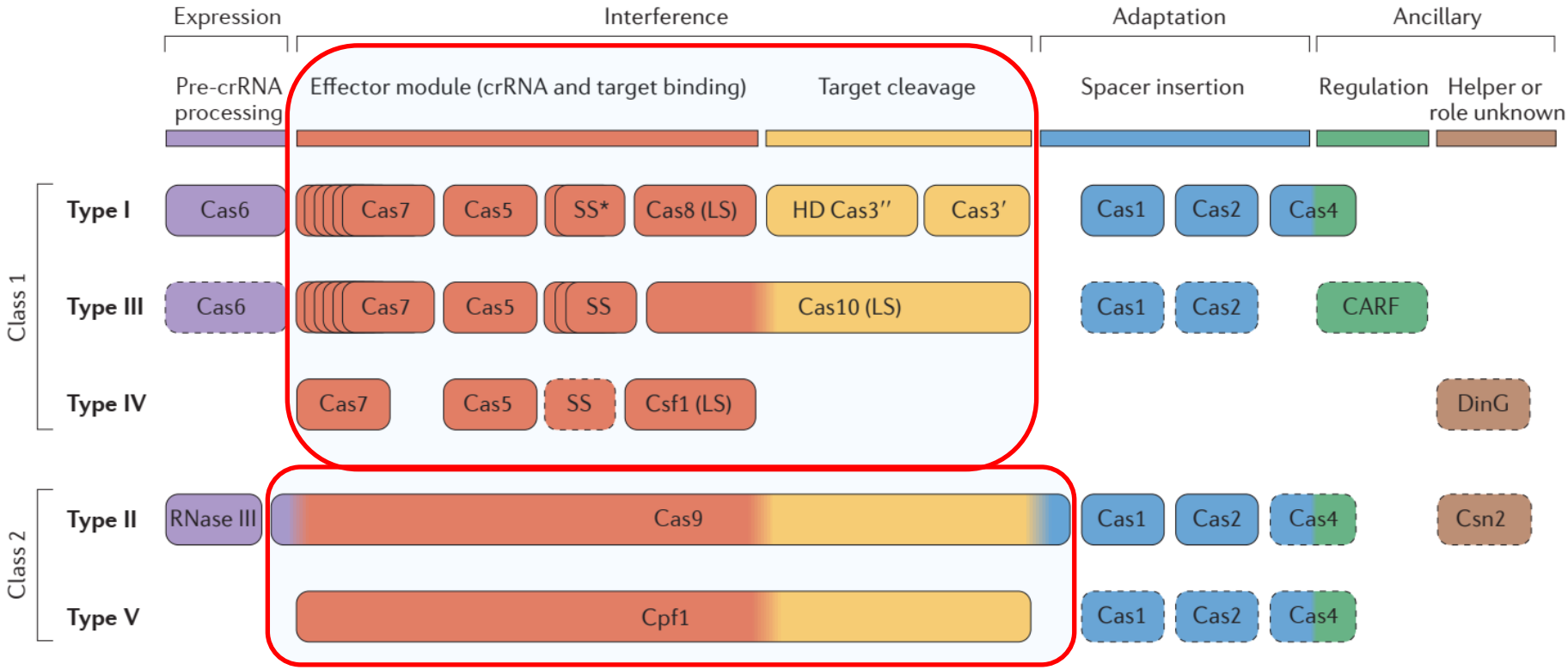
Acquired resistance against phage





Maraffini et al., Nature 2015

The diversity of CRISPR-Cas system



Makarova et al., Nature Rev Microbiol. 2015

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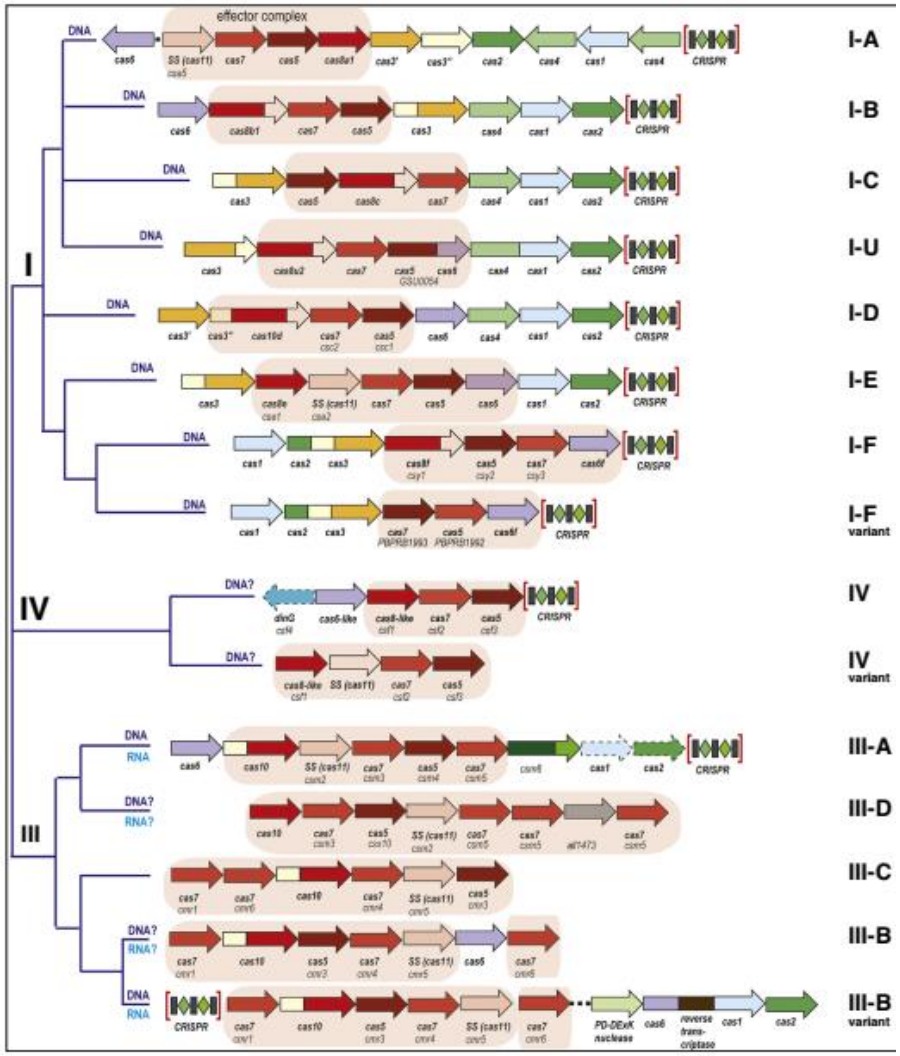

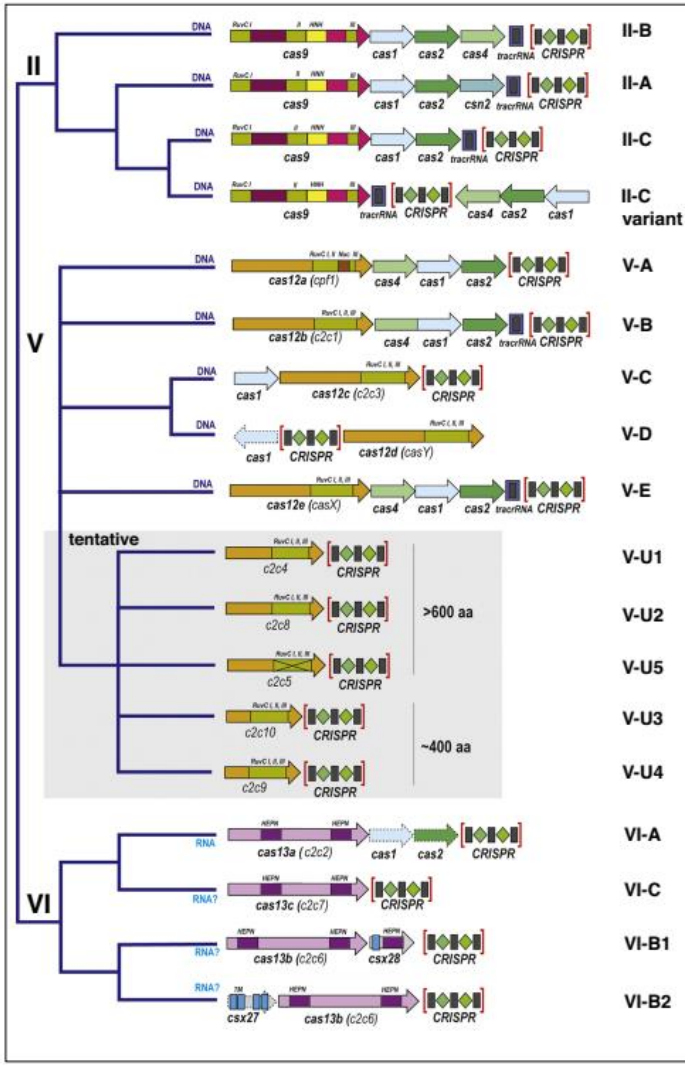

Koonin et al., Current Opinion Microbiol. 2017

Conclusions of Lecture-16

- The first article of IAP gene
- Origin of Spacers = extrachromosomal (Phage gene)
- Acquired spacers = Resistance to selective phage
- Three steps = Acquisition, Expression & Interference
- Diversity of CRISPR-Cas system
- Next = Type II-A system

Questions??