CRISPR-Cas - introduction

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CRISPR-Cas

- **CRISPR** – clustered regularly interspaced palindromic repeats
- **Cas** – CRISPR-associated genes & proteins
- present in genomes of 40% of bacteria and 85% of archaea

CRISPR-Cas – 2 classes

Class 1
- Cas3
- Cascade-like
- Cas1/Cas2

Class 2
- Cas9-like
- Cas1/Cas2

many CRISPR spacers are homologous to viruses or plasmids

experimental evidence: adaptive & heritable immunity
CRISPR-Cas – class 1

Class 1 cas operon
Cascade-like Cas1/Cas2 CRISPR
leader

First insights in molecular basis of CRISPR interference

Natural defense & Engineering
- maturation pre-crRNA > crRNA
- RNP complex (Cascade/crRNA)
- DNA nuclease (Cas3)
- RNA-guide DNA interference
- crRNA design & spec. targeting

Brouns et al. (2008) Science
CRISPR-Cas mechanism – 3 stages

Spacer acquisition

Guide expression

Target interference

Van der Oost (2014) Nat Rev Microbiol
CRISPR-Cas mechanism – *auto*-immunity?

Van der Oost (2014) Nat Rev Microbiol
CRISPR-Cas – self / non-self discrimination

Van der Oost (2014) Nat Rev Microbiol
CRISPR-Cas – *self / non-self discrimination*

Spacer acquisition

Target interference

non-self
CRISPR-Cas – self / non-self discrimination

Protospacer Adjacent Motif (PAM)

Target interference

Seed

E. coli Cascade & Cas3 – target interference

CRISPR Application – genome editing

Fusion of a Nuclease domain (FokI) with DNA-binding domain (ZF, TALE Cascade)
Fusion of a Nuclease domain (FokI) with DNA-binding domain (ZF, TALE, Cascade)
CRISPR-Cas – 2 classes

Class 1: Cascade-like Cas1/Cas2

cas operon

leader

crRNA

Class 2: Cas9-like Cas1/Cas2

cas operon

leader

crRNA

Class 2 / Type II – Cas9

Type II – unique features
- single subunit (Cas9)
- two DNase domains
- PAM at 3’ side
- crRNA & tracrRNA
- RNase-III (non-Cas)
- dsDNA break, blunt ends

Class 2 / Type II – Cas9

- **A**: Restriction nucleases
- **B**: Homing endonuclease
- **C**: CRISPR-associated nuclease

- **D**: Zinc finger nuclease
- **E**: TAL effector nuclease
- **F**: TFO nuclease

CRISPR Application – genome editing

Hsu & Zhang (2014) Cell
Class 2 – Cas9 & Cpf1

Type II
Cas9

Class 2 – Cas9 & Cpf1

Type II
Cas9

Type V
Cpf1

Class 2 – Cas9 & Cpf1

comparable efficiency of Cas9 and Cpf1 to generate knockouts in human cells

Genome editing systems

- Peptide-guided: Restriction & Homing enzymes
- Peptide-guided / engineered: TALEN & ZFN nucleases
- Oligo DNA/RNA-guided: Argonaute & CRISPR-Cas

“beat their swords into ploughshares”
Collaborators

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Berkeley
Jennifer Doudna

Bozeman
Blake Wiedenheft
CRISPR Application – genome editing

AsCpf1  LbCpf1  SpCas9

comparable efficiency of Cas9 and Cpf1 to generate knockouts

Zetsche et al. (2015) Cell
Class 1 & 2 - summary

Type I
Cascade + Cas3

Type III
Csm / Cmr

Type II
Cas9

Type V
Cpf1
CRISPR-Cas is more than Cas9
CRISPR-Cas is an anti-virus system of bacteria and archaea ...

... and there is a huge diversity of CRISPR-Cas systems
CRISPR-Cas – diversity

Class 1

E. coli Cascade & Cas3

CRISPR-Cas – diversity

Class 2

Streptococcus Cas9

Francisella Cpf1

CRISPR-Cas – diversity

CRISPR Application (3) – genome editing

guided RNP complex

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CRISPR-Cas – self / non-self discrimination

Interference

Scanning for PAM

Seed nucleation

Complete R-loop formation

CRISPR-Cas diversity – 2 classes

- **Type I**: Cas6, Cas7, Cas5, SS*, Cas8/LS, HD Cas3”, Cas3’
- **Type III**: Cas6, Cas7, Cas5, SS, Cas10/LS
- **Type IV**: ?, Cas7, Cas5, SS, LS
- **Type II**: RNase III, Cas9
- **Type V**: Cpf1
- **Type VI**: ?, C2c2

Mohanraju, Zhang, Koonin, Van der Oost (2016) Science
CRISPR-Cas diversity

Class 1 – Cascade

Class 2 – Cas9

Mohanraju, Zhang, Koonin, Van der Oost (2016) Science
CRISPR-Cas diversity

Class 2 Cas effector proteins (Cas9 / Cpf1) are multi-functional

Mohanraju, Zhang, Koonin, Van der Oost (2016) submitted
Class 2 – Cas9 & Cpf1

- *in silico* prediction of structural differences

Class 2 – Cas9 & Cpf1

- *Francisella novicida Cas9*

- *Francisella novicida Cpf1*

- *in silico* prediction of functional differences
Unlike Cas9, Cpf1 can process its own guides, which may facilitate multiplex knockouts by Cpf1 with CRISPR array. The efficiency of homologous recombination is under investigation.

CRISPR Application (1) – Flu Shot

Protect good bacteria from bad viruses

engineering of anti-virus immunity of *E. coli* BL21

requires:
- Cas3 - nuclease
- Cascade - complex
- (design) anti-virus crRNA

Brouns et al. (2008) *Science*
CRISPR Application (2) – Phage therapy 2.0

Engineer good viruses (with Cas9) to target bad bacteria

Exploiting CRISPR-Cas nucleases to produce sequence-specific antimicrobials

David Bikard, Chad W Euler, Wenyan Jiang, Philip M Nussenzweig, Gregory W Goldberg, Xavier Duportet, Vincent A Fischetti & Luciano A Marraffini

Bacteriophage-based delivery of Cas9 that specifically targets virulence genes, and as such only kills virulent bacteria

Bikard et al. (2014) Nat Biotech
CRISPR Application (3) – genome editing

Hsu & Zhang (2014) Cell
CRISPR-Cas mechanism – *adaptive immunity*

Van der Oost (2014) Nat Rev Microbiol
virus infection in prokaryotes
prokaryotic defence systems

- Restriction Enzymes
- Prokaryotic Argonautes

CRISPR-Cas systems

RNA-guided DNA interference by CRISPR-Cas

from exploration to exploitation

- CRISPR-Cas discovery & mechanism
  - Cas9-like complexes & applications
CRISPR-Cas system

- CRISPR = clustered regularly interspaced palindromic repeats
- Cas-CRISPR-associated genes & proteins
- present in genomes of 40% of bacteria and 85% of archaea

\[ \text{cas operon} \quad \text{leader} \quad \text{CRISPR} \]
RNA-guided DNA interference by CRISPR-Cas

from exploration to exploitation

- CRISPR-Cas discovery & mechanism
- Cas9-like complexes & applications
CRISPR-Cas9

Type II – unique features

• single subunit (Cas9)
• crRNA & tracrRNA
• PAM at 3’ side
• two DNase domains
• dsDNA break, blunt ends
CRISPR-Cpf1

Class 2

Type II
Cas9

Type V
Cpf1

CRISPR Application – genome editing

Hsu & Zhang (2014) Cell
Bacterial Defence systems

- Restriction Enzymes
- Prokaryotic Argonautes
- CRISPR-Cas systems

“beat their swords into ploughshares”

Pacifistic prophecy, not by John Lennon, but by Isaiah (Jesaja) Isaiah 2:4

He shall judge between the nations, and shall arbitrate for many peoples; they shall beat their swords into ploughshares, and their spears into pruning hooks; nation shall not lift up sword against nation, neither shall they learn war any more.
Collaborators

**Wageningen**
Prarthana Mohanraju
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Irene Mathijssen

**Utrecht**
Niels Geijsen

**Berkeley**
David Taylor
Jennifer Doudna
CRISPR Application – genome editing

Multiplex knockouts by Cpf1 with CRISPR array

Efficiency of homologous recombination is under investigation

CRISPR-Cas diversity

Class 2 Cas effector proteins (Cas9 / Cpf1) are multi-functional

Mohanraju, Zhang, Koonin, Van der Oost (2016) submitted
CRISPR Application (1) – Flu Shot

Protect good bacteria from bad viruses

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requires:
- Cas3 - nuclease
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Engineer good viruses (with Cas9) to target bad bacteria

Bacteriophage-based delivery of Cas9 that specifically targets virulence genes, and as such only kills virulent bacteria

Bikard et al. (2014) Nat Biotech
CRISPR-Cas diversity

Class 1 – Cascade

Class 2 – Cas9

Mohanraju, Zhang, Koonin, Van der Oost (2016) submitted
RNA-guided DNA interference by CRISPR-Cas

from exploration to exploitation

- CRISPR-Cas discovery & mechanism
- class 1 – Cascade-like complexes
- class 2 – Cas9-like complexes
- applications
CRISPR Application (3) – genome editing

Hsu & Zhang (2014) Cell
Class 2 / Type V – Cpf1

**Type II – unique features**
- single subunit (Cas9)
- two DNase domains
- PAM at 3’ side
- crRNA & tracrRNA
- RNase-III (non-Cas)
- dsDNA break, blunt ends

**Type V – unique features**
- single subunit (Cpf1)

Class 2 / Type V – Cpf1

Type II – unique features
- single subunit (Cas9)
- two DNase domains
- PAM at 3’ side
- crRNA & tracrRNA
- RNase-III (non-Cas)
- dsDNA break, blunt ends

Type V – unique features
- single subunit (Cpf1)
- single DNase domain (RuvC)

Class 2 / Type V – Cpf1

Type II – unique features
- single subunit (Cas9)
- two DNase domains
- PAM at 3’ side
- crRNA & tracrRNA
- RNase-III (non-Cas)
- dsDNA break, blunt ends

Type V – unique features
- single subunit (Cpf1)
- single DNase domain (RuvC)
- **PAM at 5’ side**

Francisella novicida Cas9
5’ side                              3’ side

Francisella novicida Cpf1
5’ side                              3’ side

• *in silico* prediction

• experimental confirmation

Zetsche et al. (2015) Cell
Class 2 / Type V – Cpf1

Functional expression of *cpf1* locus (variants) in *E. coli*

RNA-guided DNA interference

**Type II – unique features**
- single subunit (*Cas9*)
- two DNase domains
- PAM at 3’ side
- crRNA & tracrRNA
- RNase-III (non-Cas)
- dsDNA break, blunt ends

**Type V – unique features**
- single subunit (*Cpf1*)
- **single** DNase domain (*RuvC*)
- PAM at 5’ side
- crRNA, **no** tracrRNA
Class 2 / Type V – Cpf1

**Type II – unique features**
- single subunit (**Cas9**)
- two DNase domains
- PAM at 3’ side
- crRNA & tracrRNA
- RNase-III (non-Cas)
- dsDNA break, blunt ends

**Type V – unique features**
- single subunit (**Cpf1**)
- single DNase domain (**RuvC**)
- PAM at 5’ side
- crRNA, no tracrRNA
- no RNase, cleavage by Cpf1
Class 2 / Type V – Cpf1

Type II – unique features
• single subunit (Cas9)
• two DNase domains
• PAM at 3’ side
• crRNA & tracrRNA
• RNase-III (non-Cas)
• dsDNA break, blunt ends

Type V – unique features
• single subunit (Cpf1)
• single DNase domain (RuvC)
• PAM at 5’ side
• crRNA, no tracrRNA
• no RNase, cleavage by Cpf1
• dsDNA break, sticky ends

Zetsche et al. (2015) Cell
CRISPR-Cas system - discovery

- CRISPR – clustered regularly interspaced palindromic repeats

Oshino (1987), She (2001)
CRISPR-Cas system - discovery

- CRISPR – clustered regularly interspaced palindromic repeats

Oshino (1987), She (2001)
CRISPR-Cas system - discovery

- CRISPR – clustered regularly interspaced palindromic repeats
- Cas – CRISPR-associated genes & proteins

many CRISPR spacers are homologous to viruses or plasmids
CRISPR-Cas – self / non-self discrimination

Spacer acquisition
- fragmentation
- PAM screen
- integration

Class 1 / Type I – target interference

- Cascade and Cas3 required for crRNA-guided DNA interference
- crRNA guide processing by Cas6 ribonuclease subunit of Cascade
- Functional design CRISPR allows for directed targeting
<table>
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<tr>
<th>Expression</th>
<th>Interference</th>
<th>Adaptation</th>
<th>Ancillary</th>
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<tr>
<td>Pre-crRNA processing</td>
<td>Effector module (crRNA and target binding)</td>
<td>Target cleavage</td>
<td>Spacer insertion</td>
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<td>Regulation, dormancy</td>
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<td>Helper, role unknown</td>
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</table>

**Class 1**

- **Type I**
  - Cas8
  - Cas7
  - Cas5
  - SS*
  - Cas8/LS
  - HD Cas3″
  - Cas3′
  - Cas1
  - Cas2
  - Cas4

- **Type III**
  - Cas8
  - Cas7
  - Cas5
  - SS
  - Cas10/LS
  - Cas1
  - Cas2
  - CARF

- **Type IV**
  - Cas7
  - Cas5
  - SS
  - LS

**Class 2**

- **Type II**
  - RNase III
  - Cas9
  - Cas1
  - Cas2
  - Cas4
  - Csn2

- **Type V**
  - Cpf1
  - Cas1
  - Cas2
  - Cas4

- **Type VI**
  - C2c2
  - Cas1
  - Cas2

*Mohanraju, Zhang, Koonin, Van der Oost (2016) submitted*
Class 2 / Type V – Cpf1

Francisella novicida Cas9

5’ side                              3’ side

Francisella novicida Cpf1

5’ side                              3’ side

Type II – unique features
• single subunit (Cas9)
• two DNase domains
• PAM at 3’ side
• crRNA & tracrRNA
• RNase-III (non-Cas)
• dsDNA break, blunt ends

Type V – unique features
• single subunit (Cpf1)
• single DNase domain (RuvC)

BLAST search with CRISPR spacers as query
hits of prophages in related strains

Zetsche et al. (2015) Cell
CRISPR Application (3) – genome editing

guided complex (Cas9, Cascade, Ago) with nuclease domains (FokI)
Class 1 / Type I – target interference

- Cascade and Cas3 required for crRNA-guided DNA interference
- crRNA guide processing by Cas6 ribonuclease subunit of Cascade
- Functional design CRISPR allows for directed targeting

Class 1 / Type I – target interference

Class 1

Cas3  Cascade  Cas1/Cas2  crRNA

Class 2 Cas effector proteins (Cas9 / Cpf1) are multi-functional
anti-virus systems in prokaryotes

**established mechanisms**
- inhibition of adsorption (Omp)
- inhibition of DNA injection (Sie)
- degradation of DNA (R/M)
- abortive infection systems (T/AT)

**guided interference systems**
- CRISPR-Cas
- Argonaute (pAgo)

CRISPR Application (3) – genome editing

Cpf1 as alternative for Cas9: genome editing 2.0

RNAi and DNAi systems - applications

guided complex of inactive Cas9 (dCas9) with nuclease domains (FokI)

Guilinger (2014)
Figure 6. Applications of Cas9 as a Genome Engineering Platform

(A) The Cas9 nuclease cleaves DNA via its RuvC and HNH nuclease domains, each of which nicks a DNA strand to generate blunt-end DSBs. Either catalytic domain can be inactivated to generate nickase mutants that cause single-strand DNA breaks.

(B) Two Cas9 nickase complexes with appropriately spaced target sites can mimic targeted DSBs via cooperative nicks, doubling the length of target recognition without sacrificing cleavage efficiency.

(C) Expression plasmids encoding the Cas9 gene and a short sgRNA cassette driven by the U6 RNA polymerase III promoter can be directly transfected into cell lines of interest.

(D) Purified Cas9 protein and in vitro transcribed sgRNA can be microinjected into fertilized zygotes for rapid generation of transgenic animal models.

(E) For somatic genetic modification, high-titer viral vectors encoding CRISPR reagents can be transduced into tissues or cells of interest.

(F) Genome-scale functional screening can be facilitated by mass synthesis and delivery of guide RNA libraries.

(G) Catalytically dead Cas9 (dCas9) can be converted into a general DNA-binding domain and fused to functional effectors such as transcriptional activators or epigenetic enzymes. The modularity of targeting and flexible choice of functional domains enable rapid expansion of the Cas9 toolbox.

(H) Cas9 coupled to fluorescent reporters facilitates live imaging of DNA loci for illuminating the dynamics of genome architecture.

(I) Reconstituting split fragments of Cas9 via chemical or optical induction of heterodimer domains, such as the cib1/cry2 system from Arabidopsis, confers temporal control of dynamic cellular processes.
CRISPR-Cas / Type I – Cascade & Cas3

Hochstrasser & Doudna (2014) PNAS

Gong et al. (2014) PNAS
RNAi and DNAi systems - applications

Class 1 / Type I – spacer acquisition

**cas operon**
- Cas3
- Cascade
- Cas1/Cas2

**CRISPR**
- leader
- crRNA

Class 1 / Type I – target interference

- cas operon
  - Cas3
  - Cascade
  - Cas1/Cas2

- CRISPR
  - leader
  - crRNA