CRISPR-Cas systems - an insight into Next generation antimicrobials

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Situation with current antibiotics

Used and abused

- Improper clinical use
- Extensive veterinary use
- **Result** – selection and emergence of multidrug resistant organisms
- **Consequence**- Fewer treatment options
- Antibiotic pipeline running dry

Broad spectrum nature

- Targets are mostly conserved throughout bacteria
- Indiscriminatory towards harmless commensals
- **Result:** Selects for resistance amongst non disease causing species
- **Consequence:** Exchange of resistance amongst bacteria

- Understanding of the human microbiome is improving- thought to have many associated benefits
- **Result:** Disrupts the balance within microbiota
- **Consequence:** thought to impact human health; opportunistic infection (*C.difficile*)

Need for narrow spectrum antibiotics- Selectively target ‘bad’ bacteria

Table 1 Antibacterial candidates with novel mechanisms and/or narrow spectra in the pipeline

Name	Mechanism	Spectrum and/or target organisms	Development phase
Debio 1450/1452	FabI inhibitor	Narrow/Staphylococci	1/2
CG-400549	FabI inhibitor	Narrow/Staphylococci	2
POI7080	LptD inhibitor	Narrow/*Pseudomonas aeruginosa*	2
AZD0914	GyrB/ParE inhibitor	*Neisseria gonorrhoeae* and some Gram-positive bacteria	1
CRS3123	MetRS inhibitor	*C. difficile* and other Gram-positive bacteria	1
Brilacidin	Defensin mimetic	Broad/Gram-positive and Gram-negative bacteria)	2
Lefamulin	Protein synthesis inhibitor	Gram positive	2
GSK-2140944	Topoisomerase inhibitor	Gram positive	2

Silver, L. (2014). Antibacterials for any target. *Nat Biotechnol*, *32*(11), 1102-1104. doi:10.1038/nbt.3060

Problems associated with narrow spectrum antibiotics

- Target usually a single enzyme- prone to rapid development of resistance
- These are small molecules and cells are generally impermeable to them
- Need to be accompanied with rapid, accurate diagnostics in order for optimal and efficient use
- Polymicrobial infections?

Need to revise our approach

Silver, L. (2014). Antibacterials for any target. *Nat Biotechnol*, *32*(11), 1102-1104. doi:10.1038/nbt.3060

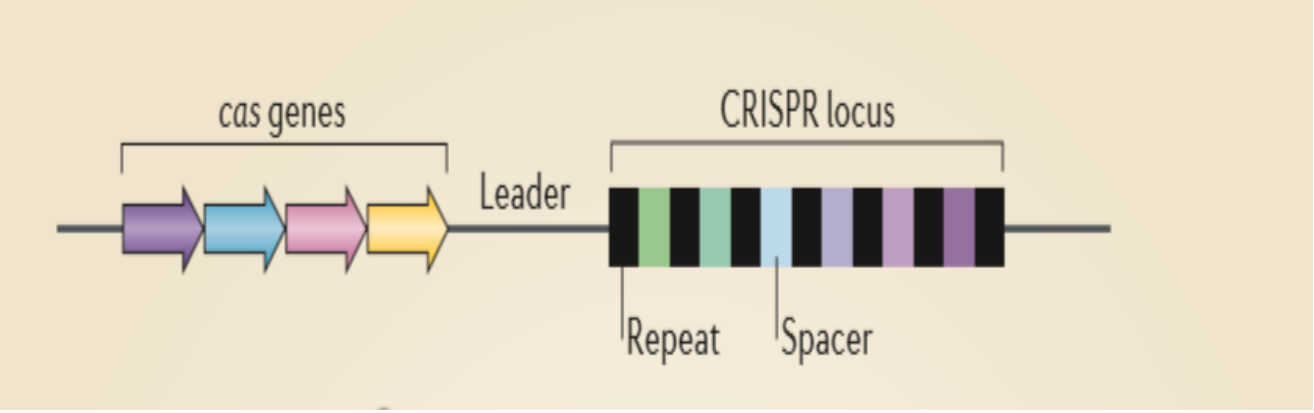
CRISPR-Cas systems as antimicrobials- the way forward?

CRISPR-Cas systems as antimicrobials

- Involves part of a bacterial and archeal adaptive immune system that uses an **RNA guided nuclease** to target complementary DNA
- These nucleases can theoretically be programmed to target a large number of DNA sequences-Potential for **sequence specific targeting**
- Sequence specific targeting can exploited to eliminate only strains harbouring certain genes – e.g. Antibiotic resistance genes or virulence genes
- Two key studies have already revealed the potential of the CRISPR-Cas system as a sequence specific antimicrobial.

What are CRISPR-Cas systems?

- CRISPR- **C**lustered **R**egularly **I**nterspaced **P**alindromic **R**epeats
- Cas – '**C**RISPR **as**sociated' proteins
- Present in almost all archea and about 50% of bacteria
- Characteristic pattern of alternating repeats and 'Spacers'
- Spacers – fragments of invading mobile genetic elements (MGE). Used for sequence specific adaptive immunity.
- **Three main types : Type I, II and III**



Modified from: van der Oost, J., Westra, E., Jackson, R., & Wiedenheft, B. (2014). Unravelling the structural and mechanistic basis of CRISPR – Cas systems. *Nat Rev Micro*. doi:10.1038/nrmicro3279

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Mechanism

1) **Adaptation** – Recognition of spacers from MGE and incorporation into CRISPR locus – Involves Cas1/Cas2 and repair and recombination enzymes?
2) **Expression** – CRISPR locus with spacers transcribed to produce pre CRISPR RNA (pre crRNA)

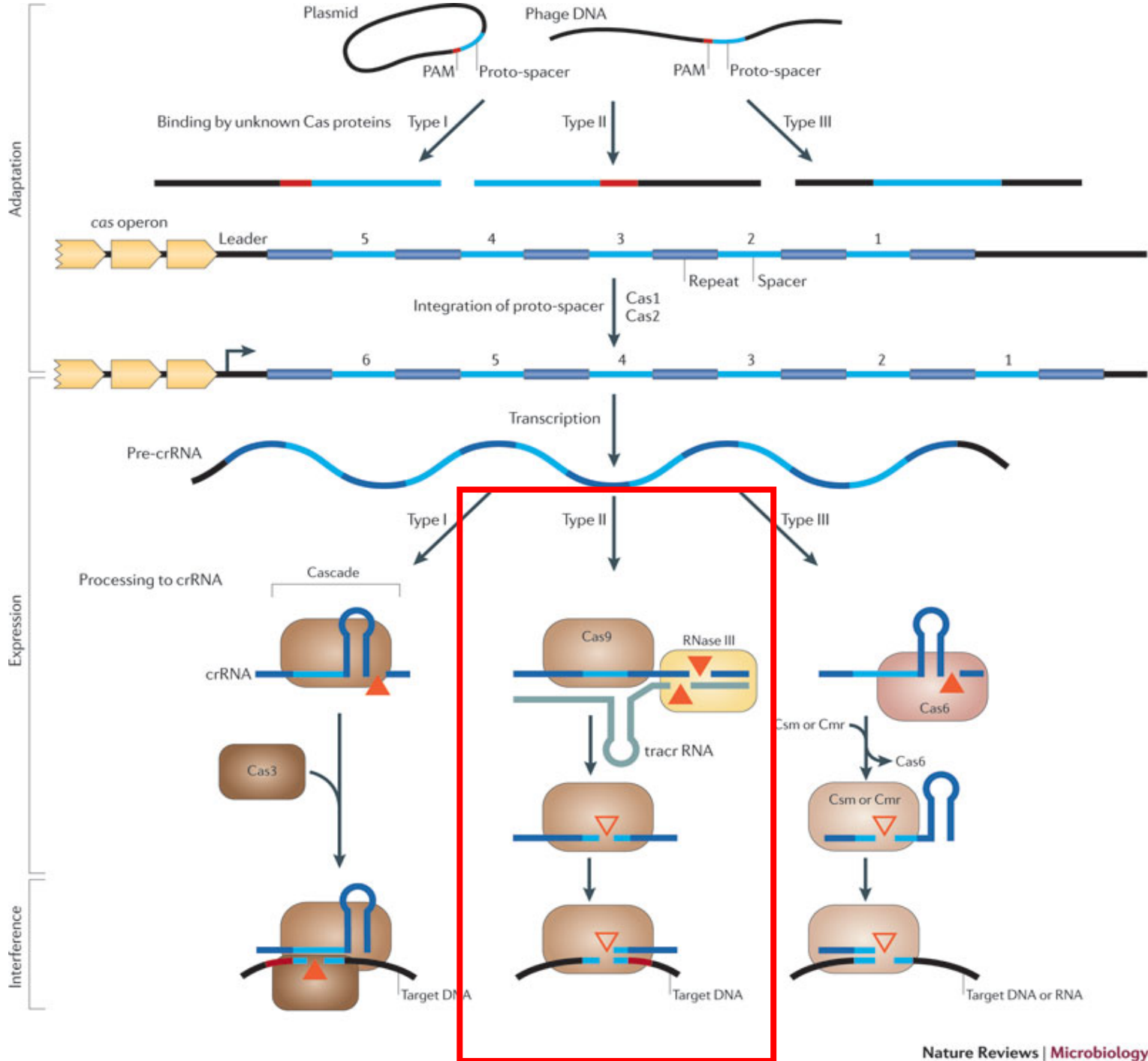


Diagram: Makarova, K., et al. (2011). Evolution and classification of the CRISPR – Cas systems. *Nat Rev Micro*, *9*(6), 467-477. doi:10.1038/nrmicro2577

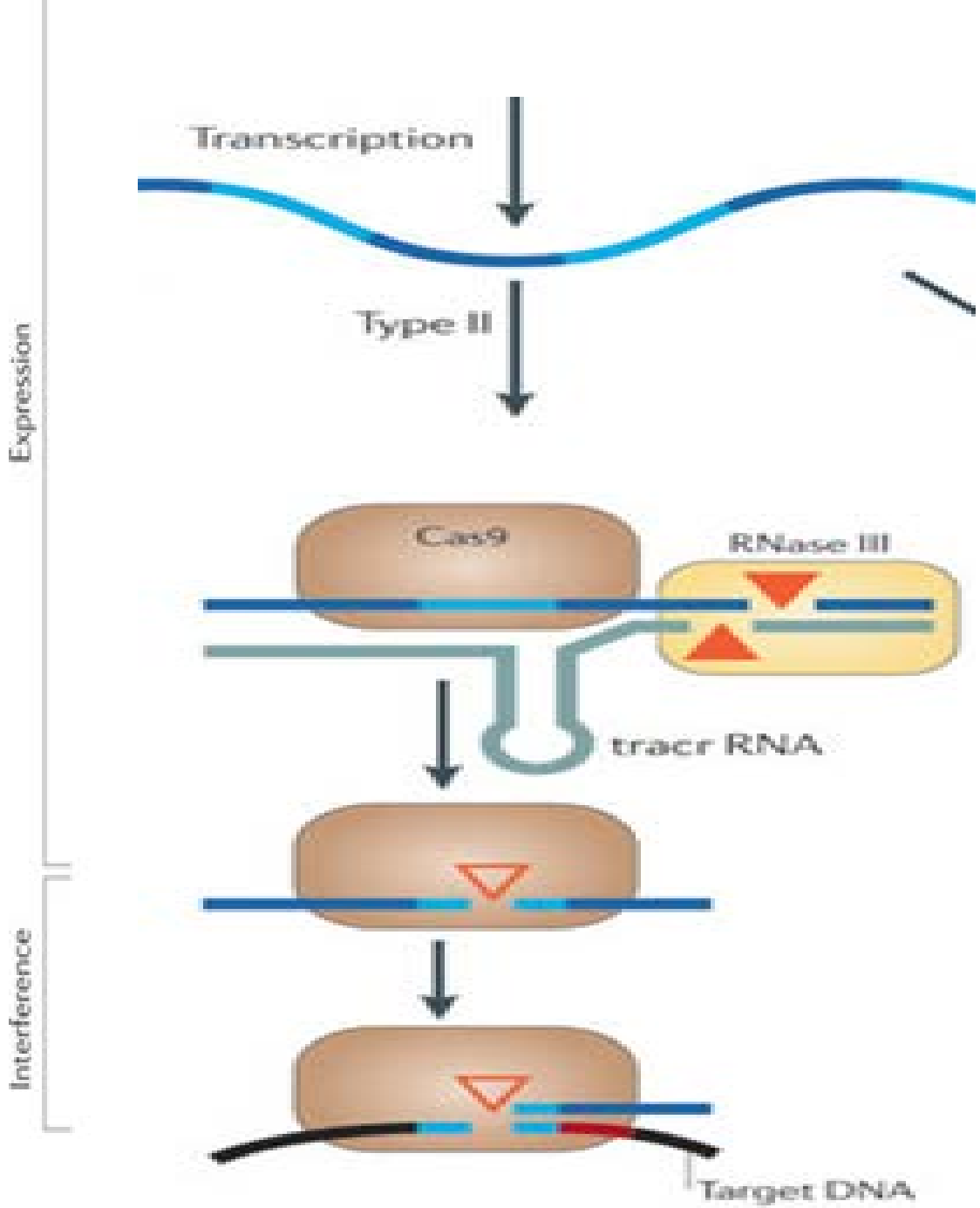
van der Oost, et al (2014). Unravelling the structural and mechanistic basis of CRISPR – Cas systems. *Nat Rev Micro*. doi:10.1038/nrmicro3279

Mechanism (continued)

Type II CRISPR-Cas systems

2) **Expression** – *trans*-encoded small RNA (tracrRNA) which binds with the ‘repeat’ part of the pre-crRNA – processing to crRNA by housekeeping Rnase III.

3) **Interference:** crRNA associates with **Cas9** (an RNA guided double stranded endonuclease). Scans invading DNA. If there is complementarity, the DNA is destroyed by the Cas9 nuclease



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Turning the bacterial immune system against them

- Specificity of the Cas9 endonuclease is based on the sequence of the spacers (transcribed into crRNAs)
- Modification of the spacers can result in the targeting of virtually any sequence.
- Many studies have shown that reprogramming the RNA guided Cas9 against sequences in bacterial genomes is cytotoxic – genomic lesions?
- Potential of the RNA guided Cas9 to be used as a programmable sequence specific antimicrobial was demonstrated [Gomaa, A. (2013).]
- Only thing lacking so far was an appropriate delivery mechanism
- Solved soon after…

Gomaa, A., Klumpe, H., Luo, M., Selle, K., Barrangou, R., & Beisel, C. (2013). Programmable Removal of Bacterial Strains by Use of Genome-Targeting CRISPR-Cas Systems. *Mbio*, *5*(1), e00928-13-e00928-13. doi:10.1128/mbio.00928-13

Phagemids as delivery vehicles

Phagemids- plasmids with a packaging sequence

LETTERS

nature biotechnology

Exploiting CRISPR-Cas nucleases to produce sequence-specific antimicrobials

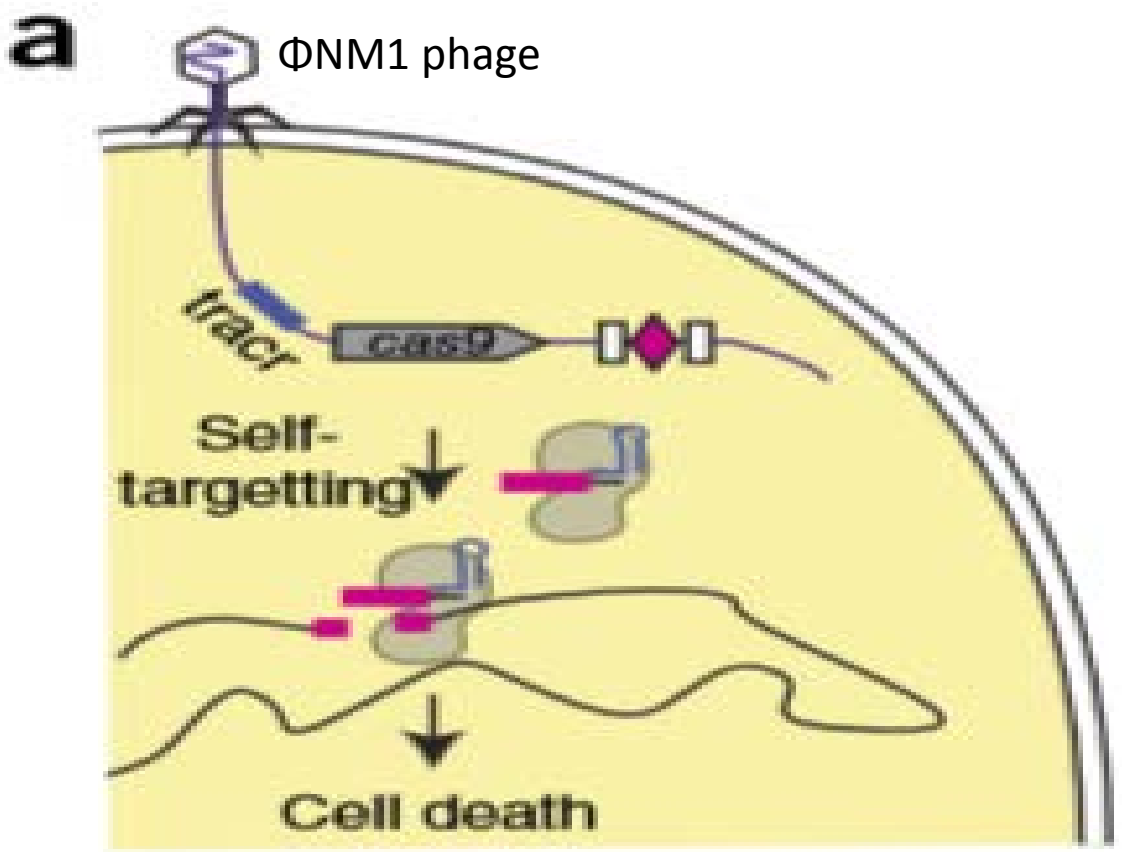
David Bikard[1,5], Chad W Euler[2,6], Wenyan Jiang[1,6], Philip M Nussenzweig[1], Gregory W Goldberg[1], Xavier Duportet[3,4], Vincent A Fischetti[2] & Luciano A Marraffini[1]

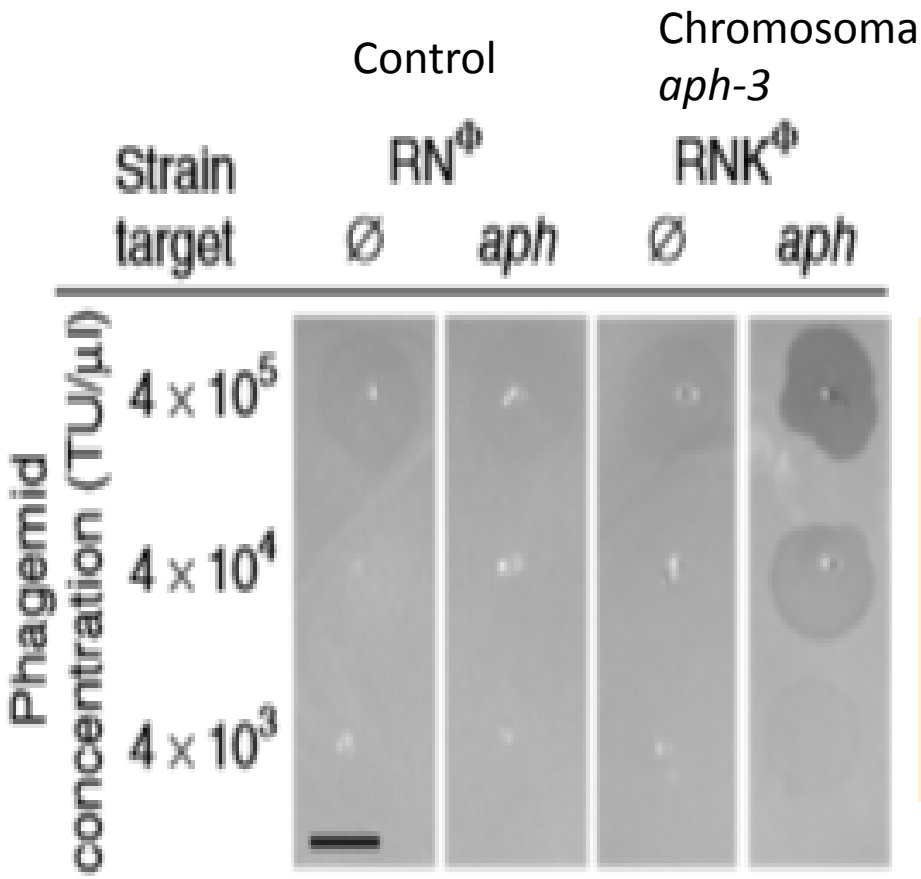
Used phagemids encoding a *Streptococcus pyrogenes* Cas9, CRISPR RNAs and tracrRNA to target chromosomal *aph-3* gene

pDB121øaph

Targeting resistance genes of *Stapylococcus aureus*

pDB121øaph elicits strong growth inhibition



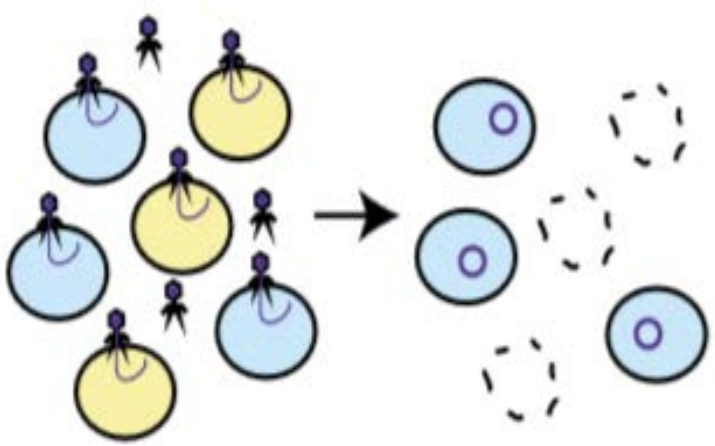


Top agar lawns

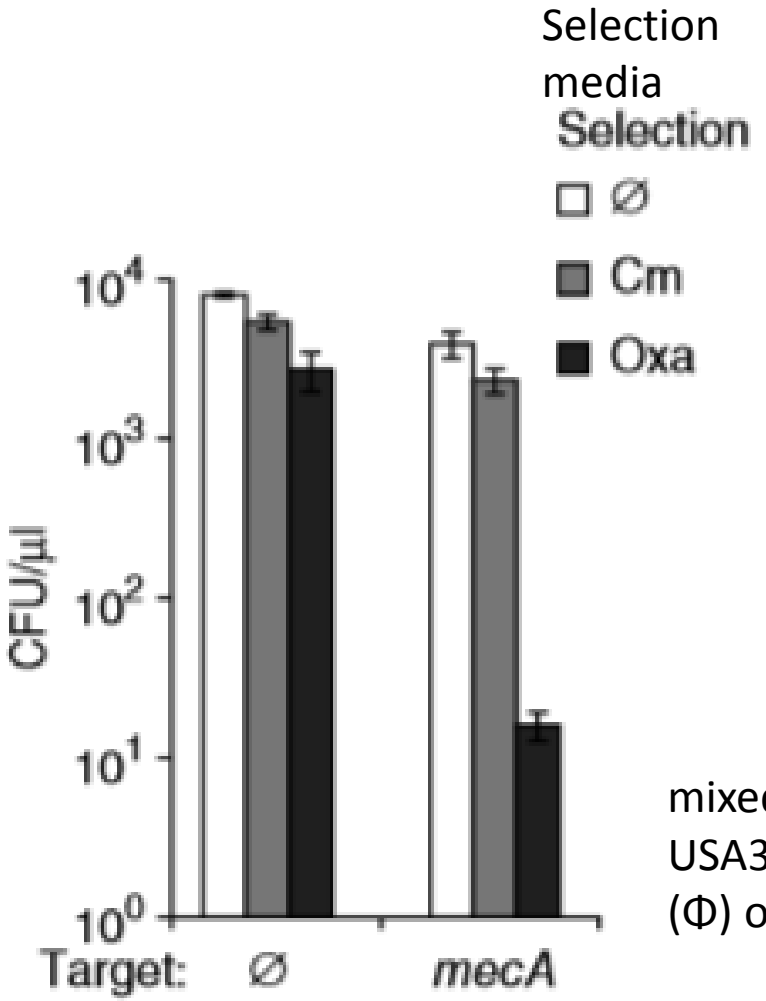
Lysates of targeting (*aph*) and non targeting (Φ) phagemid

Bikard, D., Euler, C., Jiang, W., Nussenzweig, P., Goldberg, G., & Duportet, X. et al. (2014). Exploiting CRISPR-Cas nucleases to produce sequence-specific antimicrobials. *Nat Biotechnol*, *32*(11), 1146-1150. doi:10.1038/nbt.3043

Targeting resistance genes - *mecA*



Treating a mixed population of RNΦ and USA300Φ MRSA leads to selective killing of the MRSA and immunizes survivors



mixed population of RNΦ and USA300Φ treated with non targeting (Φ) or targeting (*mecA*) phagemids.

Used phagemids encoding a *Streptococcus pyrogenes* Cas9, CRISPR RNAs and tracrRNA to target *MecA* gene

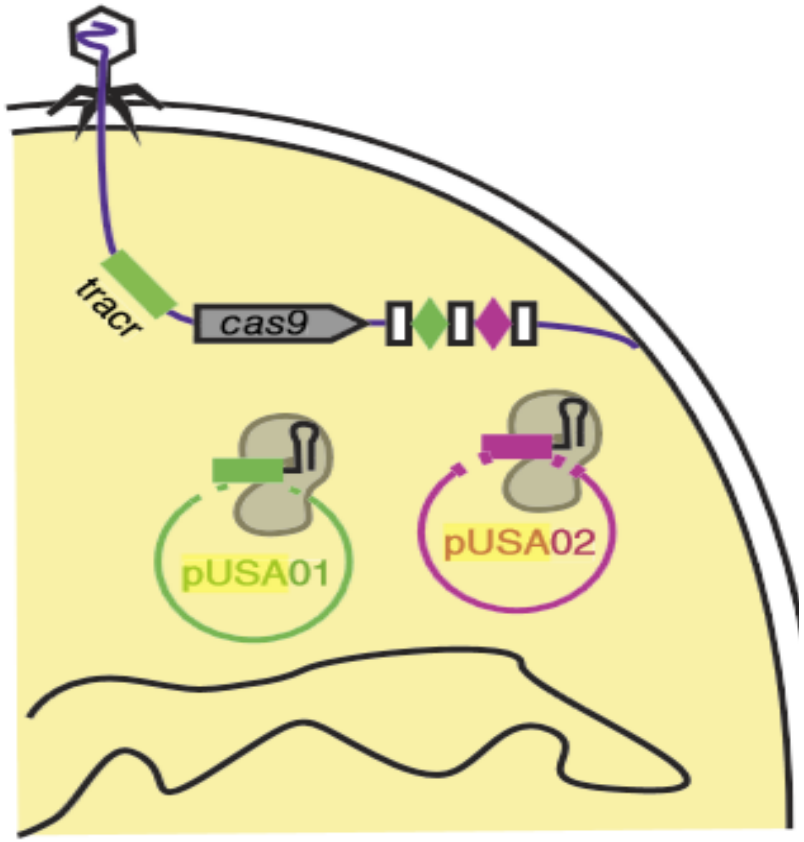
USA300– Clinical isolate (MRSA)

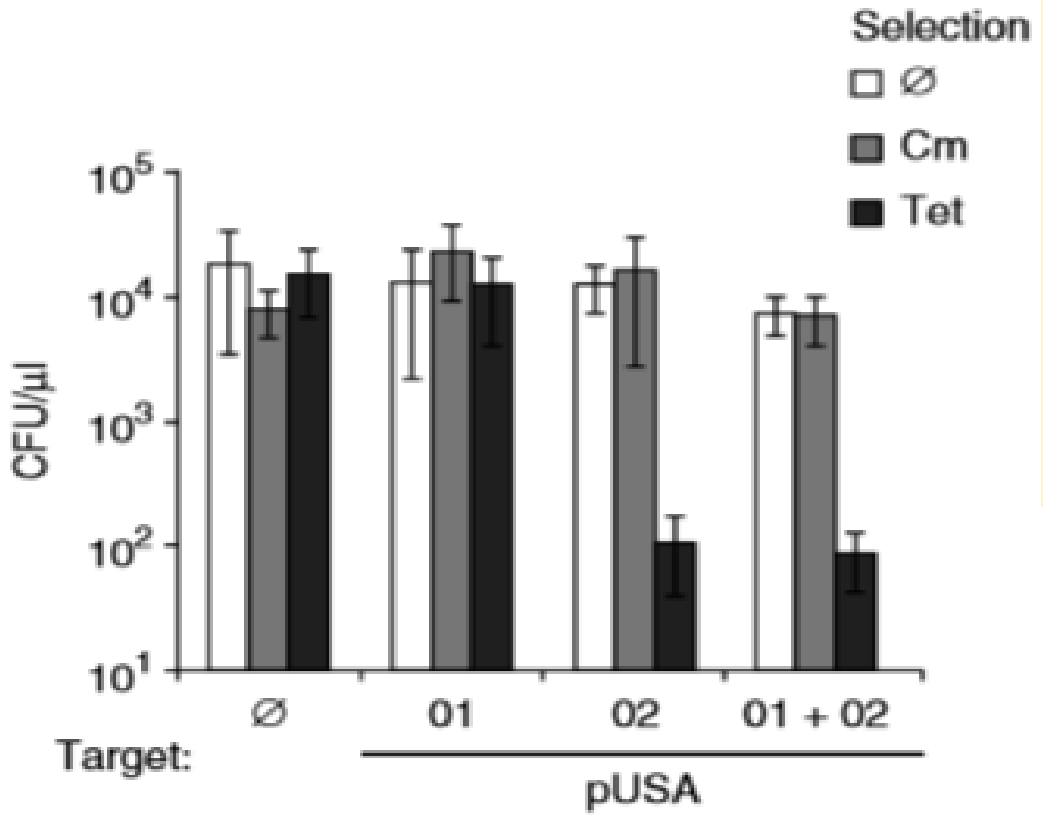
pDB121ømecA

Bikard, D., Euler, C., Jiang, W., Nussenzweig, P., Goldberg, G., & Duportet, X. et al. (2014). Exploiting CRISPR-Cas nucleases to produce sequence-specific antimicrobials. *Nat Biotechnol*, *32*(11), 1146-1150. doi:10.1038/nbt.3043

Simultaneous targeting of plasmids carrying resistance genes – pUSA01 and pUSA02

ΦNM1 phage





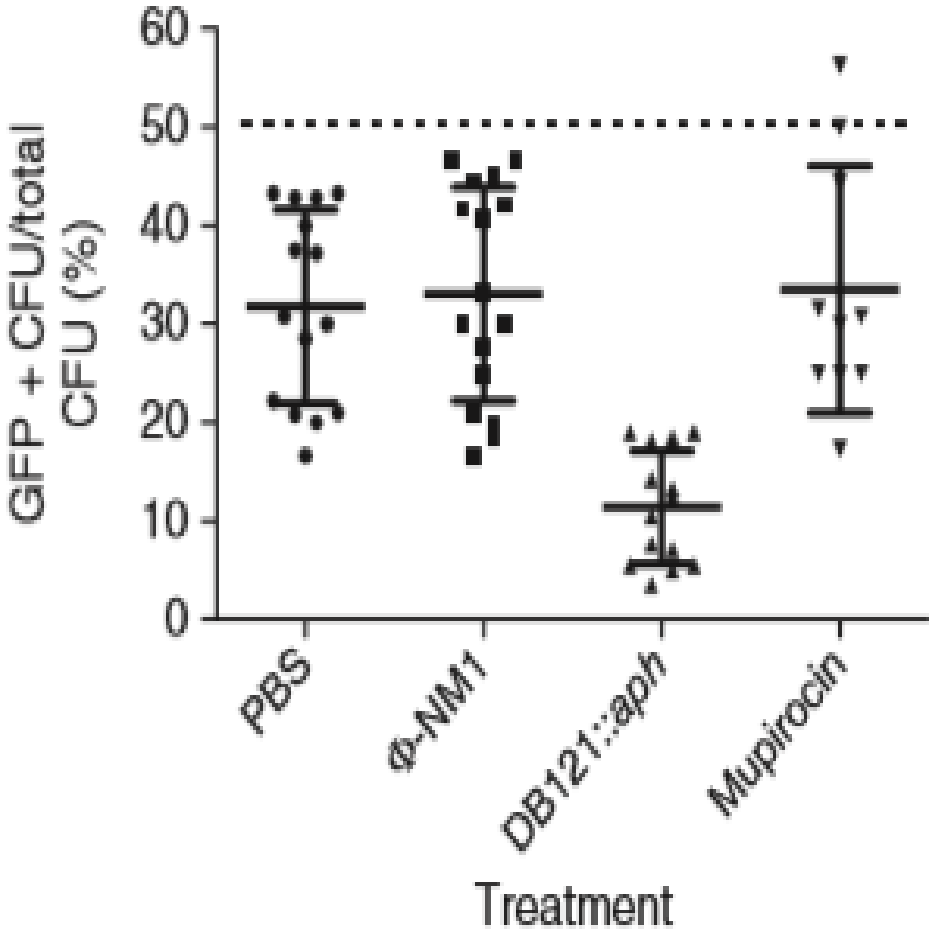
Used phagemids encoding a *Streptococcus pyrogenes* Cas9, CRISPR RNAs and tracrRNA to target each plasmid individually or in combination

pUSA02 – tetracycline resistance

Bikard, D., Euler, C., Jiang, W., Nussenzweig, P., Goldberg, G., & Duportet, X. et al. (2014). Exploiting CRISPR-Cas nucleases to produce sequence-specific antimicrobials. *Nat Biotechnol*, *32*(11), 1146-1150. doi:10.1038/nbt.3043

In vivo study

- Mouse skin colonization model
- ”An area on the back was colonized with a 1:1 mixture of RNΦ and RNKΦ” (the latter was fluorescently labelled for detection)
- Topically treated with either the CRISPR-Cas9 antimicrobial pDB121øaph and various controls



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Sequence-specific antimicrobials using efficiently delivered RNA-guided nucleases

Robert J Citorik[1,2,7], Mark Mimee[1,2,7] & Timothy K Lu[1–6]

- Cas9 programed to target β-lactamase genes (the *bla*$_{\text{NDM-1}}$ or *bla*$_{\text{SHV-18}}$) genes encoding β-lactam resistance in *E. coli*.

Plenty of challenges lie ahead…..

- Phage resistance?
- Mouse skin infection model is very simple but what about more complex environment such as gut (trillions of bacteria)?
- Variation in the expression of phage receptors?
- Once successfully delivered it must bypass host defence mechanisms such as restriction modification as well as native CRISPR-Cas

A next generation delivery approach for the next generation antimicrobial:
Polymeric nanoparticles

Beisel, C., Gomaa, A., & Barrangou, R. (2014). A CRISPR design for next-generation antimicrobials. *Genome Biol*, *15*(11). doi:10.1186/s13059-014-0516-x

Summary

- Bacterial tolerance towards our current arsenal of antimicrobials is rising at an alarming rate
- Our current strategies are very broad spectrum
- Cas9 nuclease of the type II CRISPR Cas systems can be programmed to serve as sequence specific antimicrobial
- Studies have demonstrated the promise it holds
- Significant hurdles to cross before it can be introduced as a mainstream antimicrobial
- With our current antimicrobial pipeline running dry and resistance increasing amongst bacteria, investigating into this approach may well be worth out time and effort.

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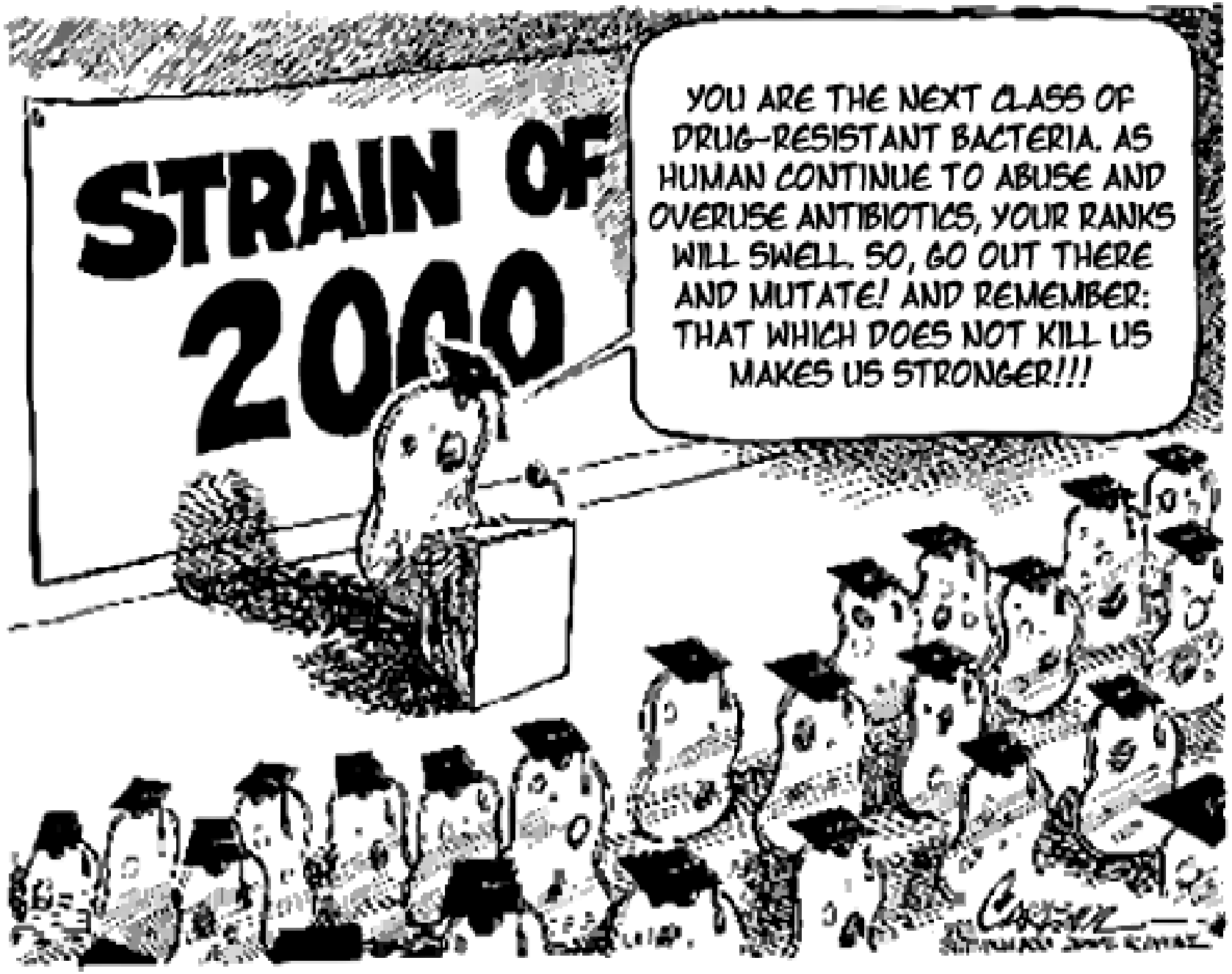

Thank you

http://deltabiology.com/2011/bacteria-cartoon-comic/cartoon10/