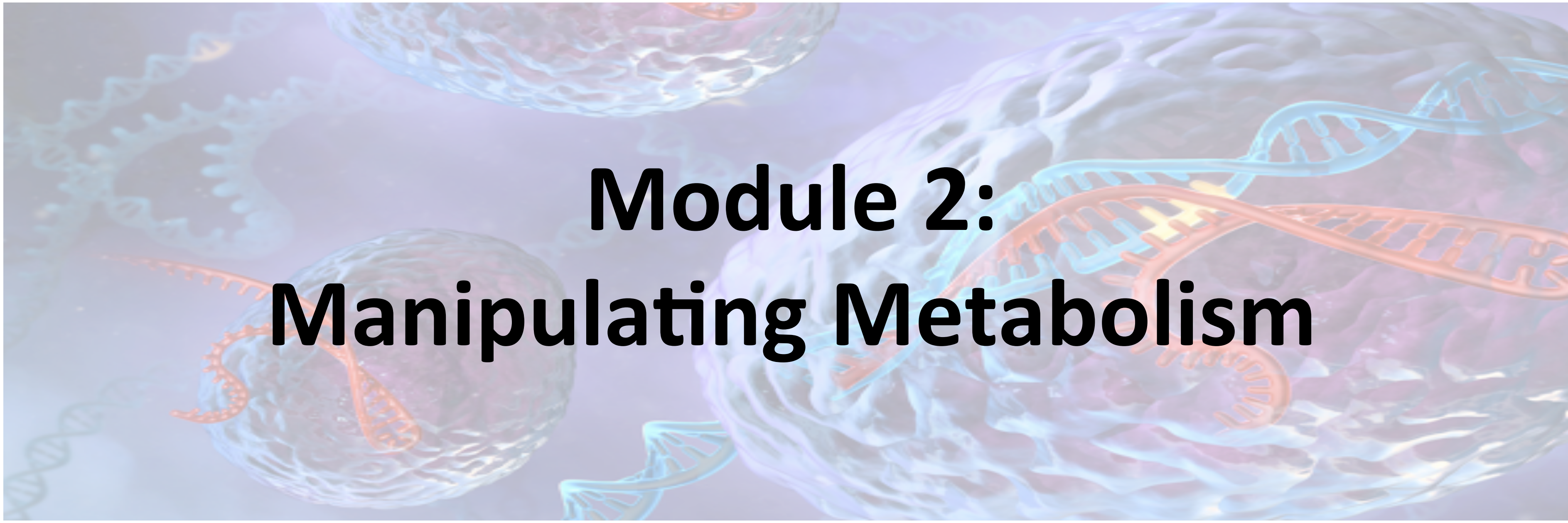

Module 2: Manipulating Metabolism

CRISPR: adaptive immunity

10/23/18

Why communicate your science?

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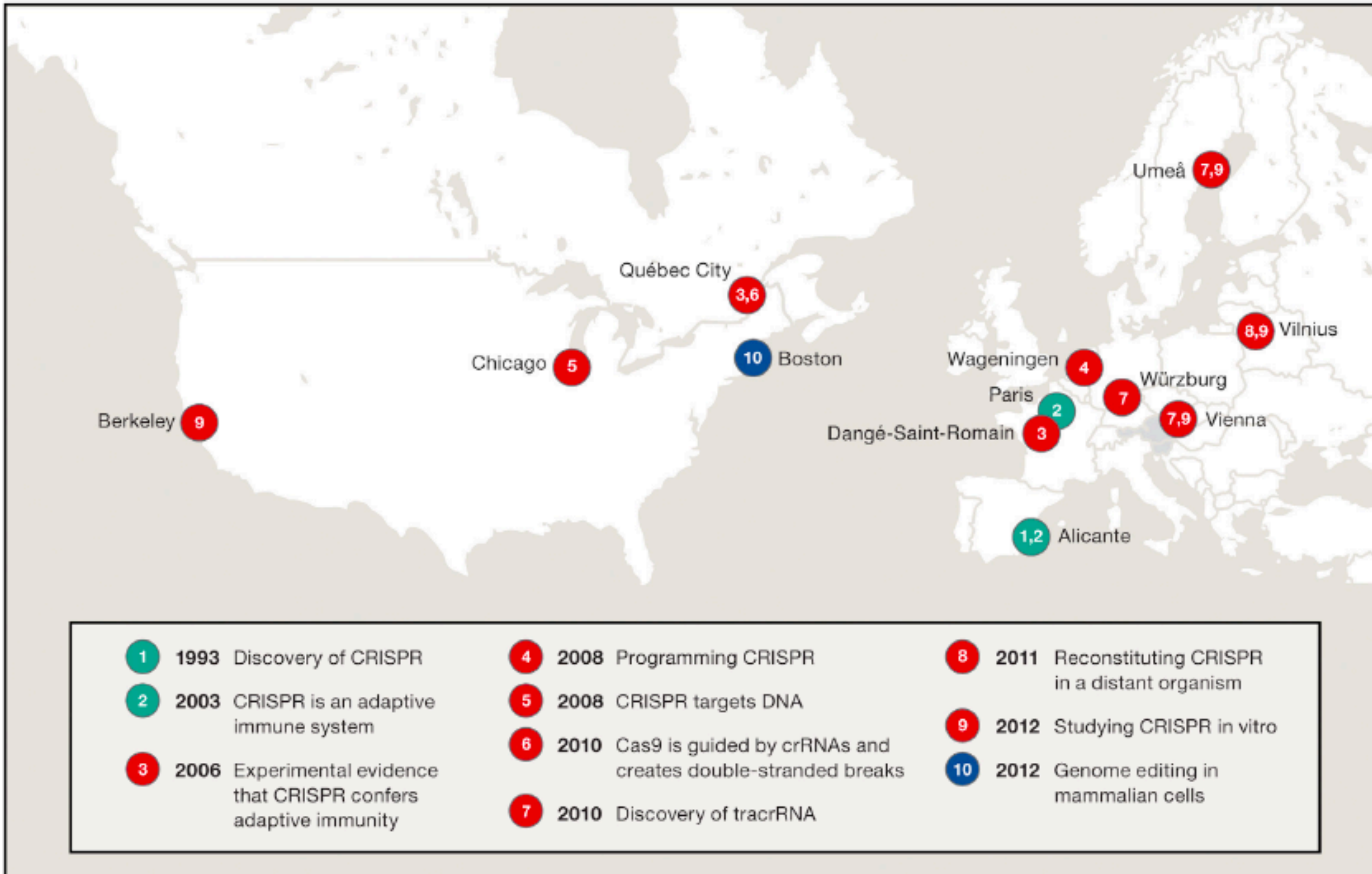


Discovery of repeat sequences in archaea

- 1993 While studying non-related anomaly in DNA fragments, identified multiple copies of 30 base repeats separated by 36 base spacers

Francis Mojica

- 1995 Found similar repeats in related organisms
  - Other work reported repeat sequences in *E. coli*
- 2000 Repeat loci identified in 20 microbes
- 2003 Spacer sequence from *E. coli* matched to P1 phage

Proposed role for repeat sequences

- 2003 88/4500 spacer sequences similar to phage
  - 2/3 matched phage known to infect host microbe
- 2005 *Y. pestis* spacer sequences similar to prophage present with genome of strains
  - New spacers present at the 'front' end of loci

MICROBIOLOGY
Publishing high-quality research since 1947
C. Pourcel,[1] G. Salvignol[1] and G. Vergnaud[1,2]

- 2005 Speculated that transcripts from spacers worked via anti-sense RNA inhibition

MICROBIOLOGY
Publishing high-quality research since 1947
Alexander Bolotin, Benoit Quinquis, Alexei Sorokin and S. Dusko Ehrlich

Evidence of adaptive immunity

- 2004 Correlation between spacers and phage resistance in *Streptococcus thermophilus*
- 2007 Genetic selections used to isolate phage-resistant *S. thermophilus*
  - Strains carried phage sequences at repeat loci
  - Insertion of multiple spacers correlated with increased resistance
- 2007 Phage with mutations in corresponding spacer sequence able to infect microbial host

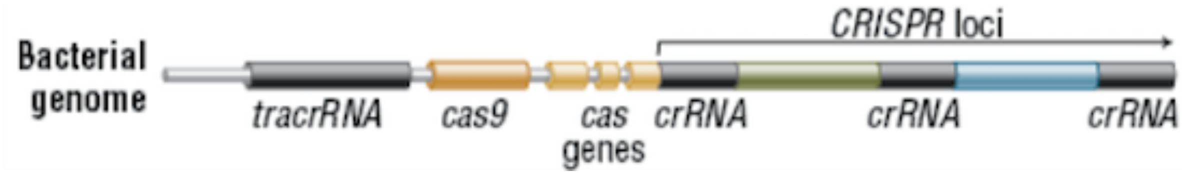
Science AAAS

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,*]

Discovery of genes associated with repeats

- 2000 Genes identified in the immediate vicinity of repeat sequences
  – Assumed to be related to spacer function
  – Hypothesized roles: gene regulation, replicon partitioning, DNA repair, etc.
- 2007 Cas7 required in acquisition of resistance, but not in resisting phage attack
- 2007 Cas9 required for resistance
  – Contains two nuclease motifs: HNH and RuvC

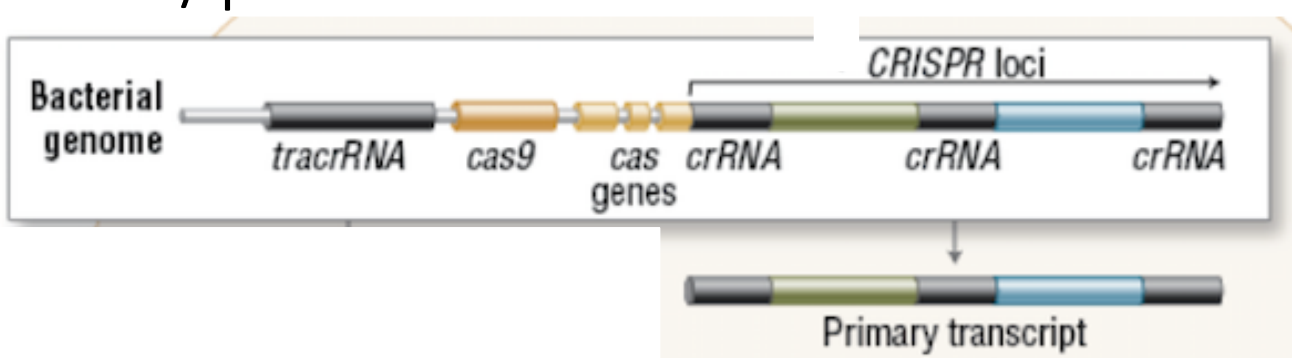
CRISPR loci components



- Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)
  - Repeats are roughly perfect, palindromic sequences
  - Spacers correspond to phage sequences
- CRISPR-associated (Cas) genes

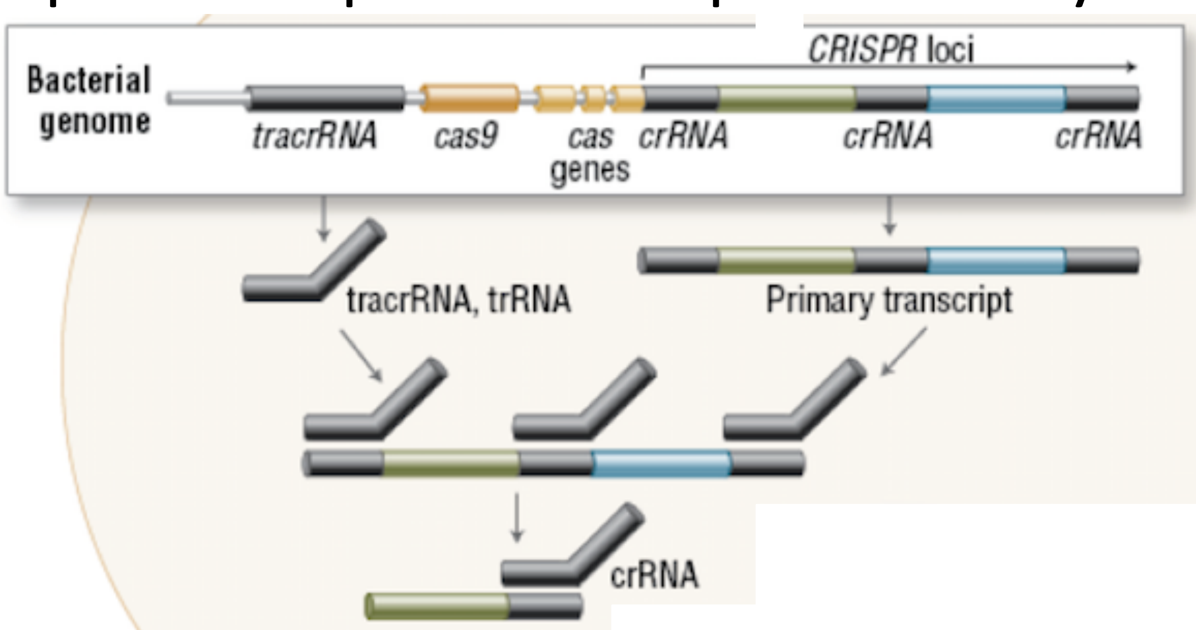
Function of CRISPR RNA (crRNA)

- Precursor RNA transcribed from CRISPR loci is cleaved into crRNAs by RNase III
  - Cleaved sequences start with last 8 bp of repeat (5’ handle), followed by complete spacer, end with first bp of repeat (3’ handle that forms hairpin)
  - Cas9 required for primary processing
    - Binds / positions molecules



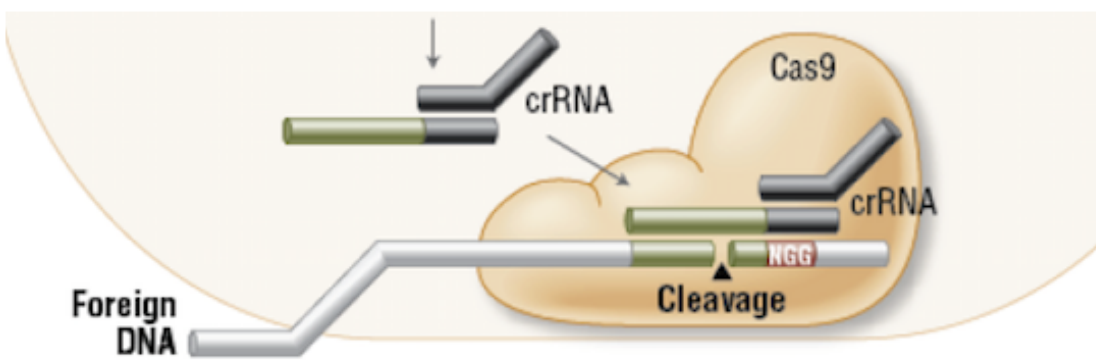
Function of *trans*-activating CRISPR RNA (tracrRNA)

- Third most abundant type of transcript
- Encoded by sequence immediately adjacent to CRISPR loci
  - 25 bp of near-perfect complementarity to repeats



DNA cleavage mediated by Cas9 with crRNA and tracrRNA

- crRNA / tracrRNA complex promotes structural change in Cas9
  - Formation of central channel that binds DNA
- Cas9 / RNA scan DNA for crRNA target (PAM)
  - Bind target sequence to enable strand displacement
- Cas9 cleaves DNA via single blunt cut



DNA is the target of Cas9 cleavage

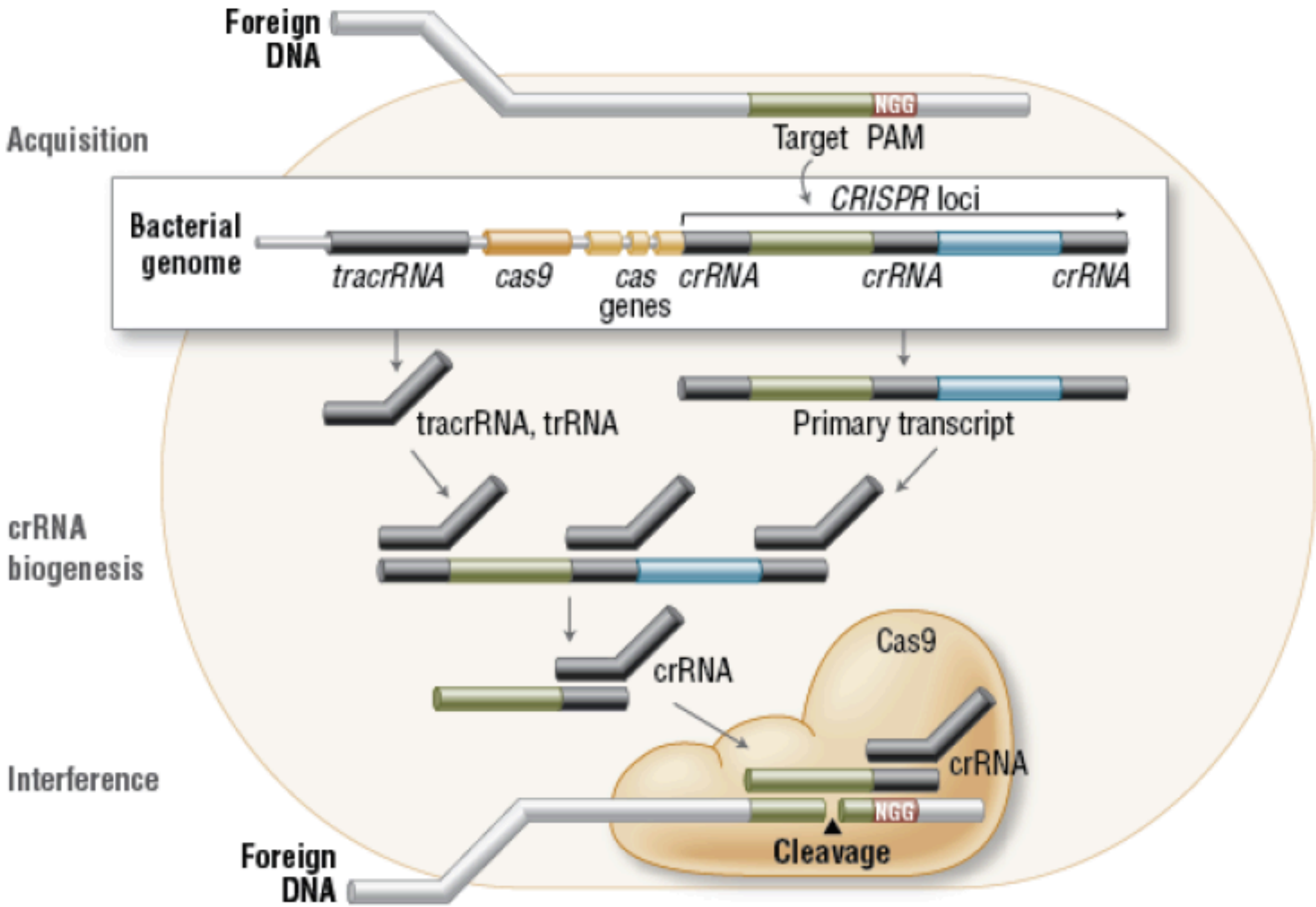
- Plasmid conjugation blocked in *S. epidermidis* strains that carried corresponding spacer
- Modified plasmid such that self-splicing intron disrupted target corresponding to spacer
  - If target is RNA, sequence will 'splice out' and CRISPR/Cas9 will recognize and cleave
  - If target is DNA, sequence will not be recognized and CRISPR/Cas9 will not recognize and cleave

https://www.youtube.com/watch?v=MbJ7Hnc2K3Q

Acquisition of immunity

- Phage DNA recognized and fragmented
  - Possible synergy with restriction enzyme system
- Suitable spacers selected by detection of protospacer adjacent motif (PAM)
- Spacer inserted into CRISPR loci by Cas1/Cas2
  - Leader end nicked for insertion
  - PAM-dependent orientation

Taken together, …



Other roles for CRISPR system

- Group behavior in *Myxococcus xanthus*
  - Disruption of *cas7*, *cas5* decreases sporulation
- Virulence in *Campylobacter jejuni*
  - Expression of cas9 in CRISPR- strain increases virulence
  - Absence of *cas9* in CRISPR+ strain increases swarming, decreases cytotoxicity
- DNA repair in *E. coli*
  - Deletion of *cas1* increases sensitivity to DNA damaging agents