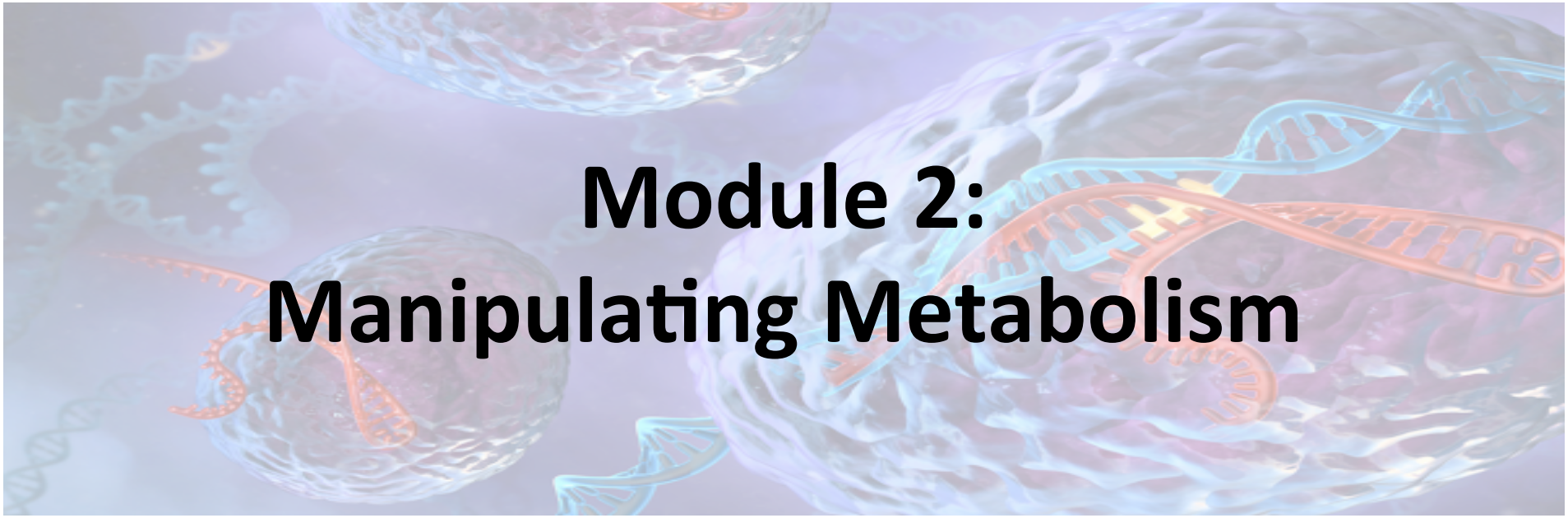


Module 2: Manipulating Metabolism

CRISPR: adaptive immunity

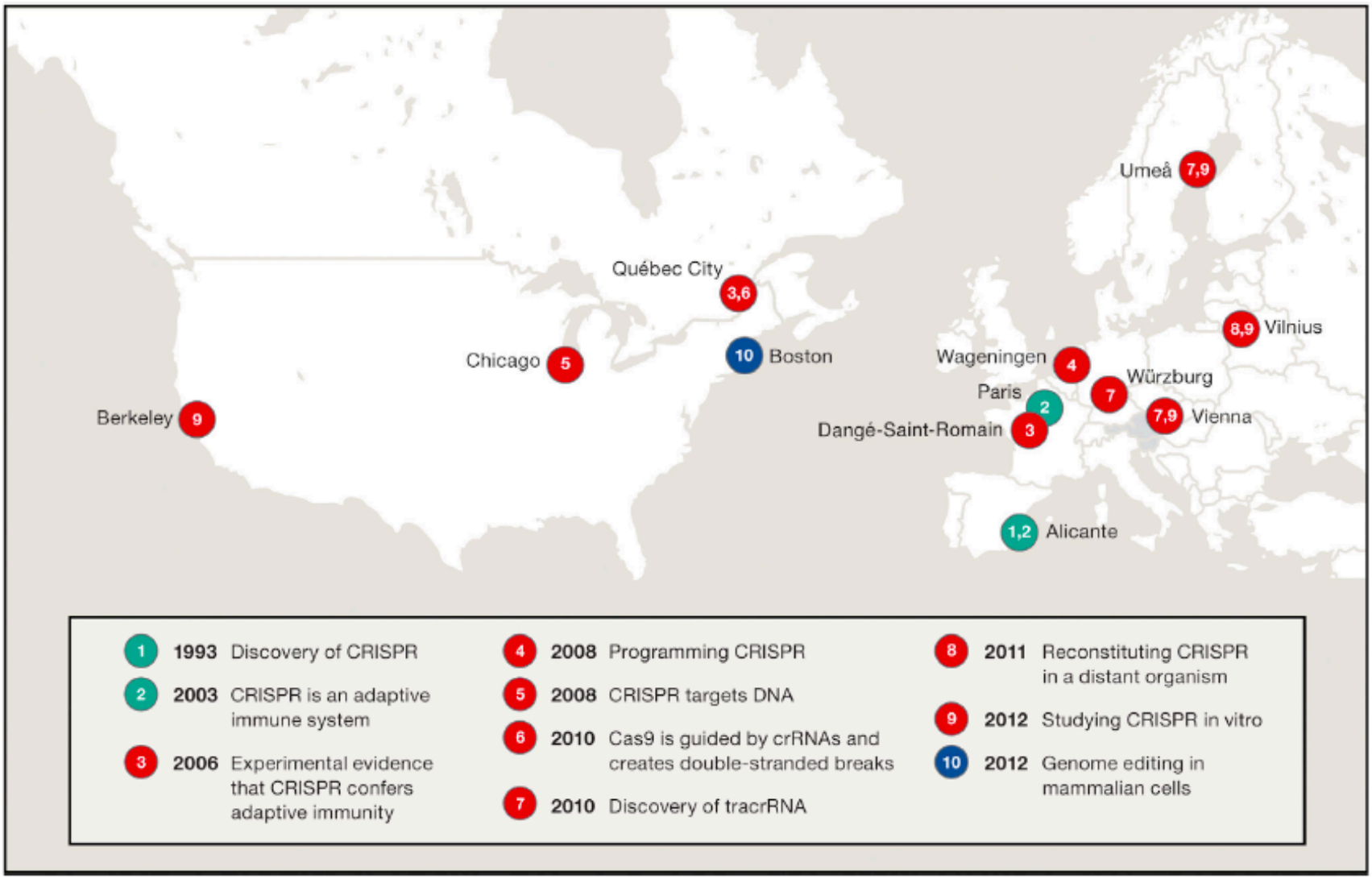
10/20/15

Thoughts on mini-presentations

- Be sure to define your jargon
  - What is a comet tail? What does it represent?
  - Explain how the data / damage is visualized
- Avoid over-reaching your data
  - How will the CometChip assay assist with personalized cancer treatment?
  - Only extend your results by a ‘single’ step

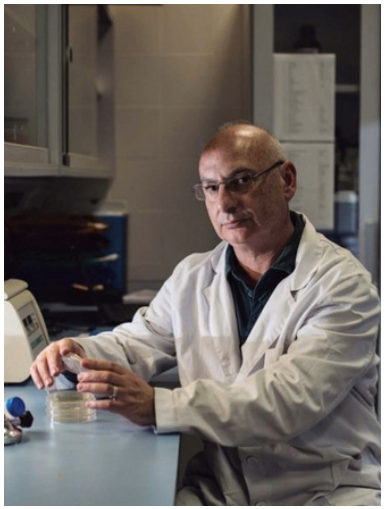
Why communicate your science?

Why communicate your science?



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*

• Genetic selections used to isolate phage-

2007 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

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.

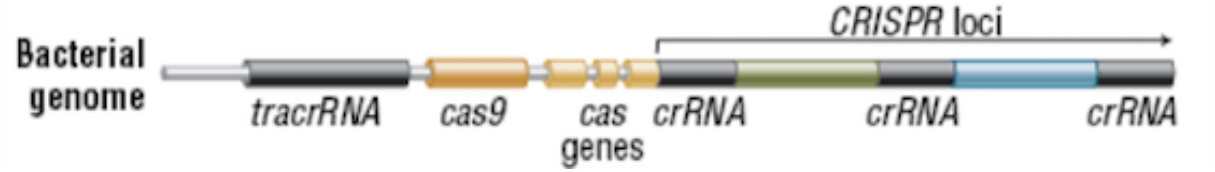
2007 • Cas7 required in acquisition of resistance, but not in resisting phage attack

2007 • Cas9 required for resistance
  - Contains two nuclease motifs

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,*]

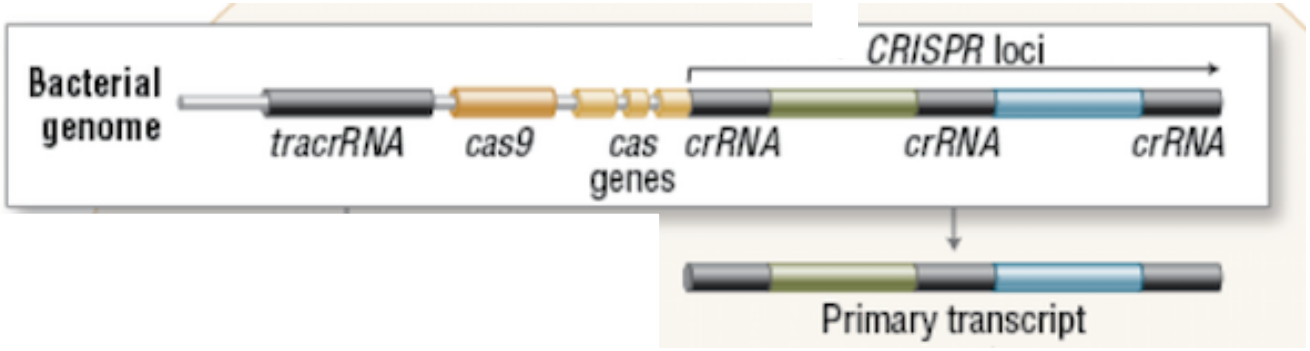
CRISPR loci components



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

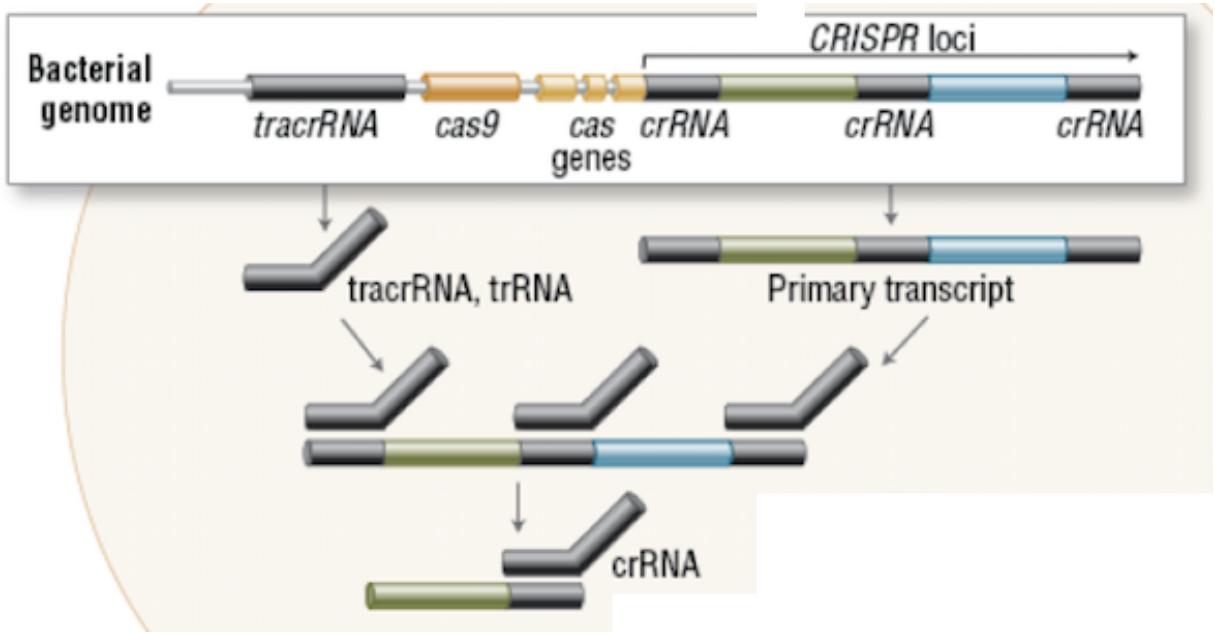
Function of CRISPR RNA (crRNA)

- 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



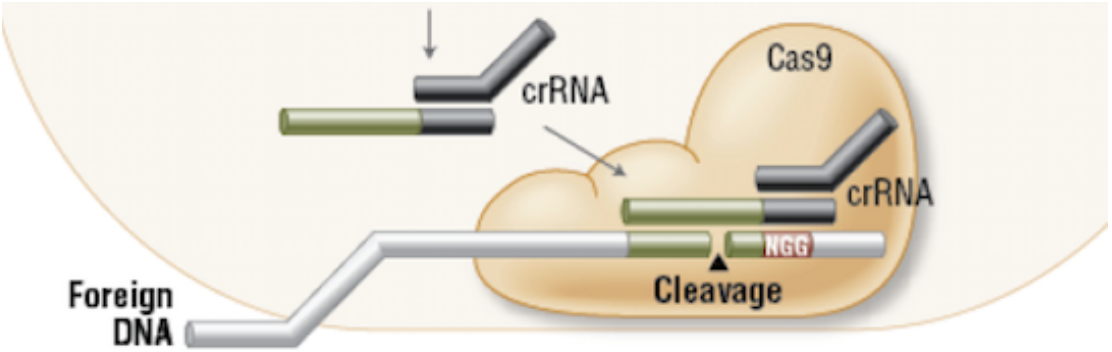
Function of *trans*-activating CRISPR RNA (tracrRNA)

- 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

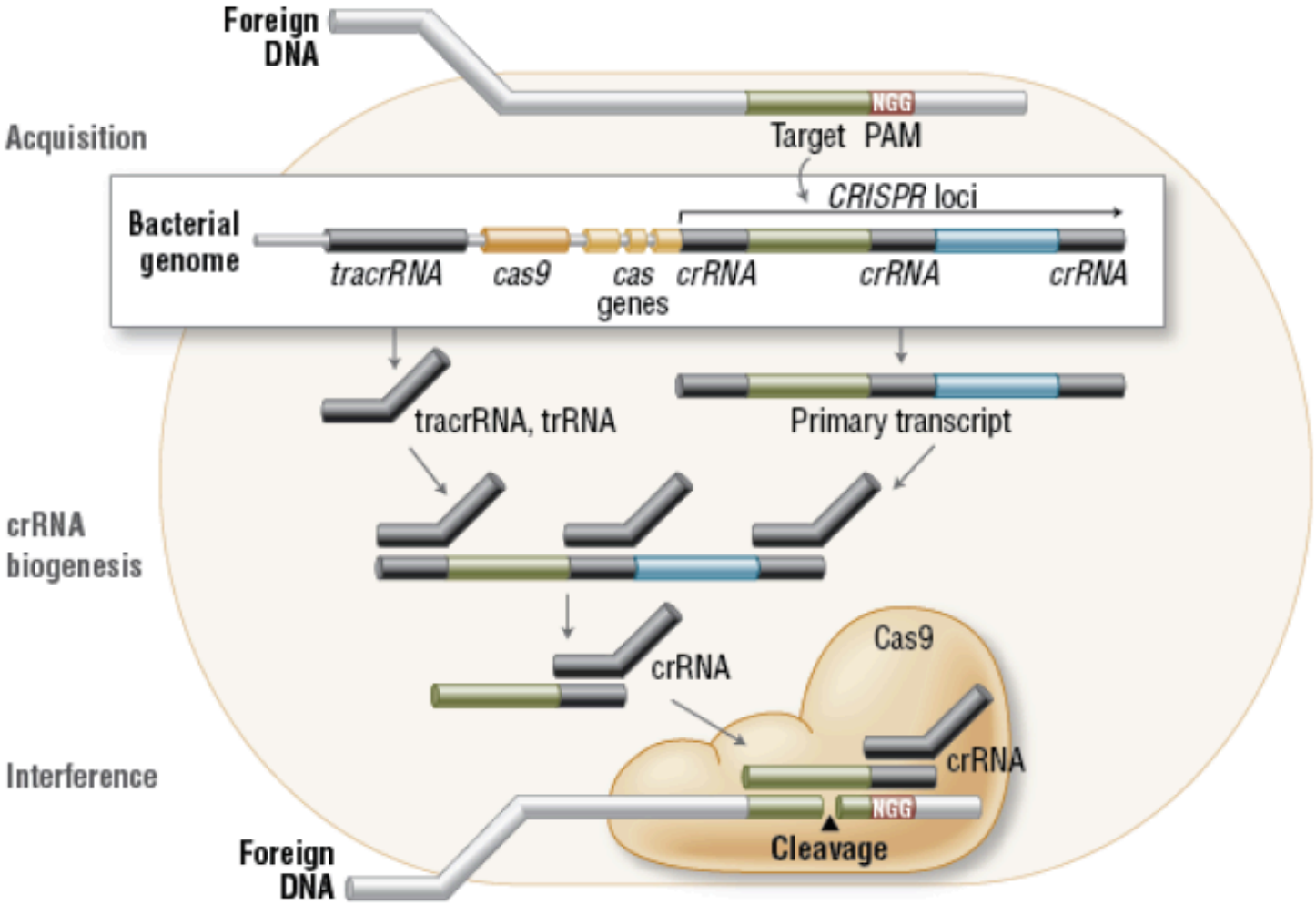
- crRNA / tracrRNA complex promotes structural change in Cas9
  - Formation of central channel that binds DNA
- Cas9 / RNA scan DNA for crRNA target (PAM)
  - Bind target sequence to enable strand displacement
- Cas9 cleaves DNA via single blunt cut



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, …

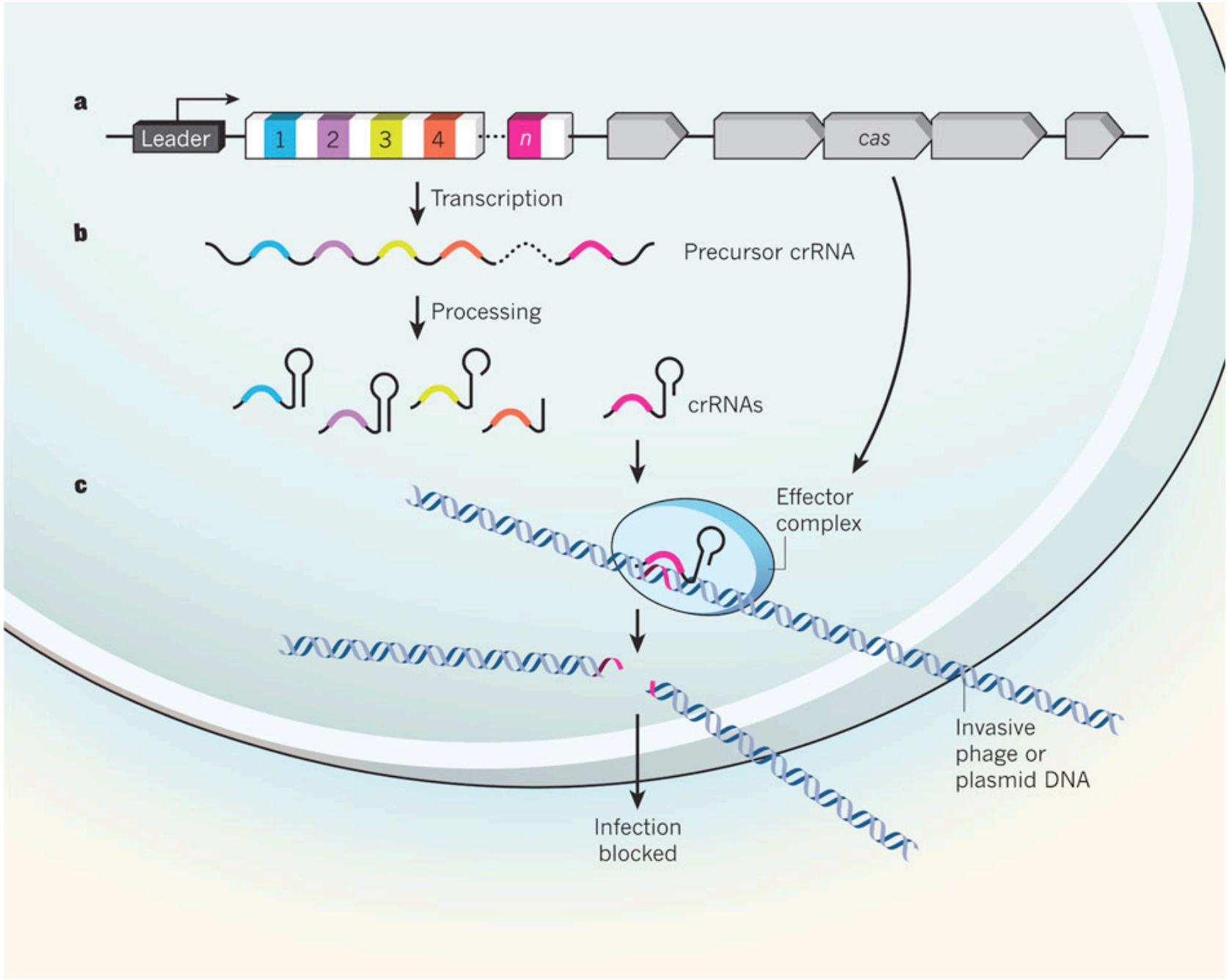


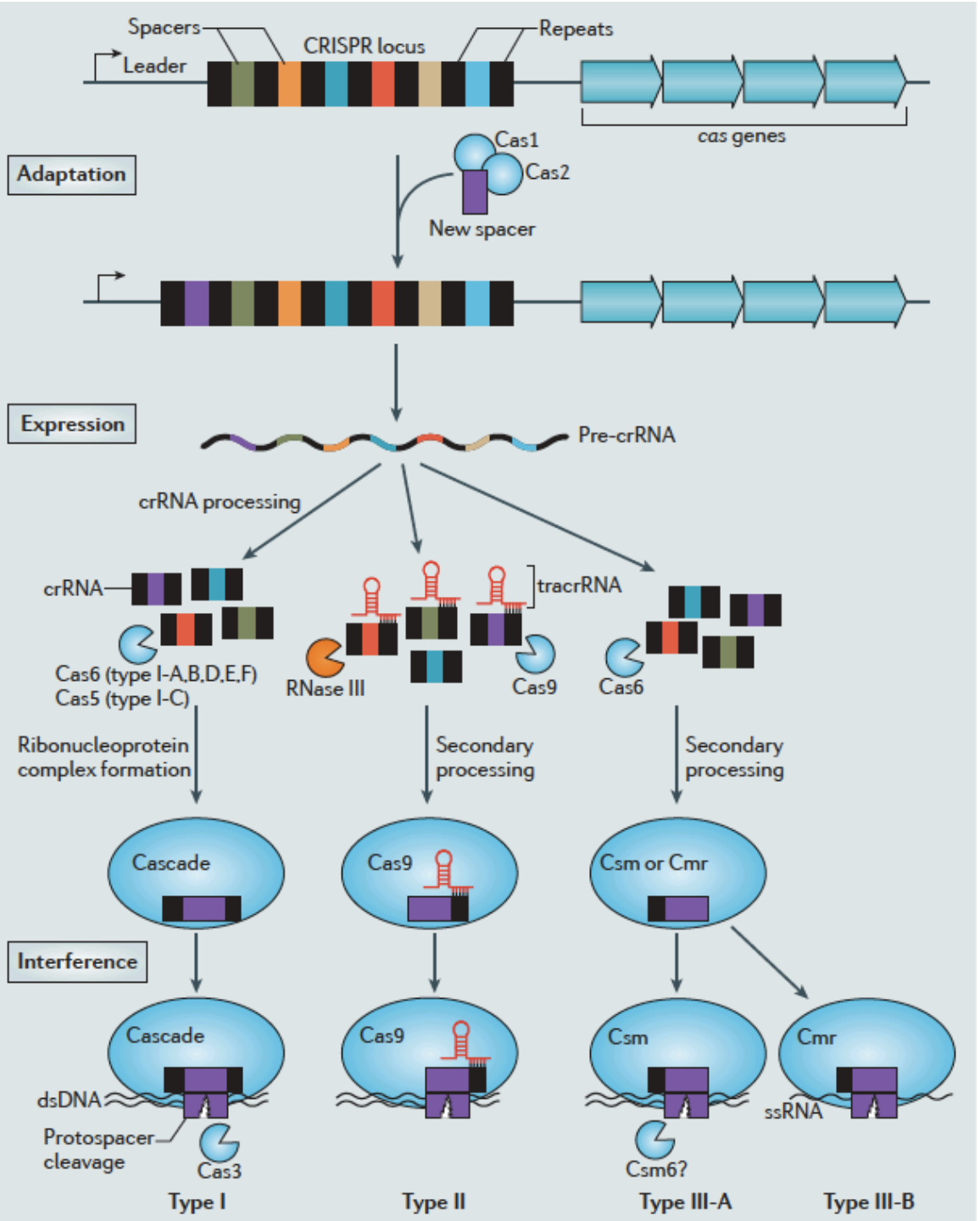
Other roles for CRISPR system

- 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

In the laboratory…

1. BE Communication lab workshop
   - Delivering effective presentation
   - Crafting clear slides
2. Prepare oligo for gRNA ‘cloning’
3. Complete gRNA insertion / amplification reaction
4. Journal article discussion
   - Contribution is expected!


a
Leader
1
2
3
4
n
cas
Transcription
b
Precursor crRNA
Processing
crRNAs
c
Effector complex
Invasive phage or plasmid DNA
Infection blocked


Spacers
Repeats
Leader
CRISPR locus
cas genes
Adaptation
Cas1
Cas2
New spacer
Expression
Pre-crRNA
crRNA processing
crRNA
tracrRNA
Cas6 (type I-A,B,D,E,F)
Cas5 (type I-C)
RNase III
Cas9
Cas6
Ribonucleoprotein complex formation
Secondary processing
Secondary processing
Cascade
Cas9
Csm or Cmr
Interference
Cascade
Cas9
Csm
Cmr
dsDNA
Protospacer cleavage
Cas3
ssRNA
Csm6?
Type I
Type II
Type III-A
Type III-B