CRISPR/Cas9 genome editing technology

MIT CAB/ESCRO Meeting

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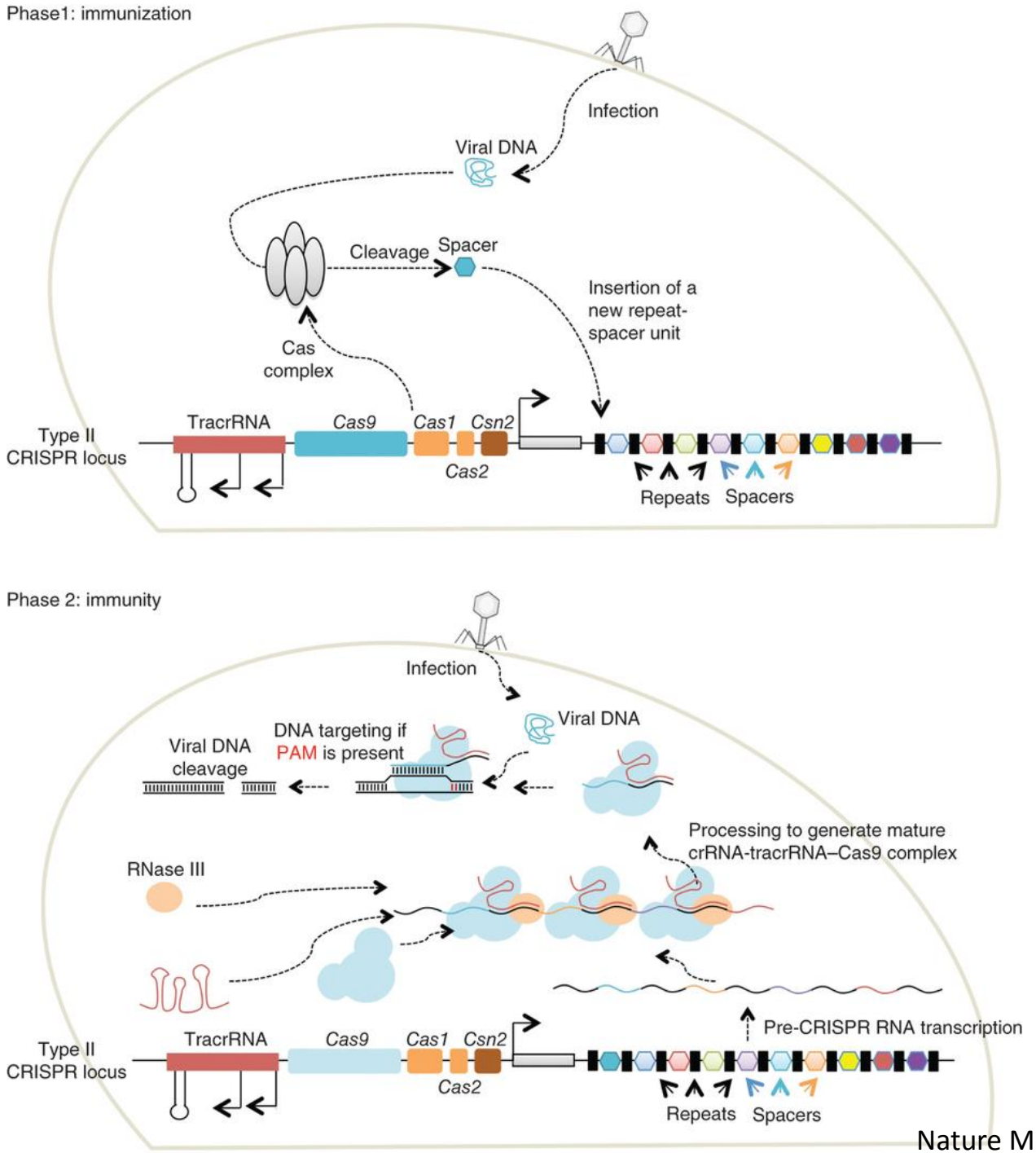
CRISPR system discovery

- **c**lustered **r**egularly **i**nterspaced, **s**hort **p**alindromic **r**epeat
- Repeat sequences first observed in 1987
- System Present in 40% of bacteria and 90% of archea
- Natural function
  - Bacterial “vaccination” observed in 2007 when repeated exposure of bacterium to phage lead to incorporation of phage sequence into bacterial genome repeats
    - Science. 2007 Mar 23;315(5819):1709-12.
  - Bacterial immune mechanism based on RNA guided nuclease activity
- *Streptococcus pyogenes* system adapted for genome editing



Nature Methods 10, 957–963 (2013)

Key Components

- Guide RNA
  - crRNA – sequence homology to target
  - tracrRNA – fixed sequence
  - PAM sequence – required for

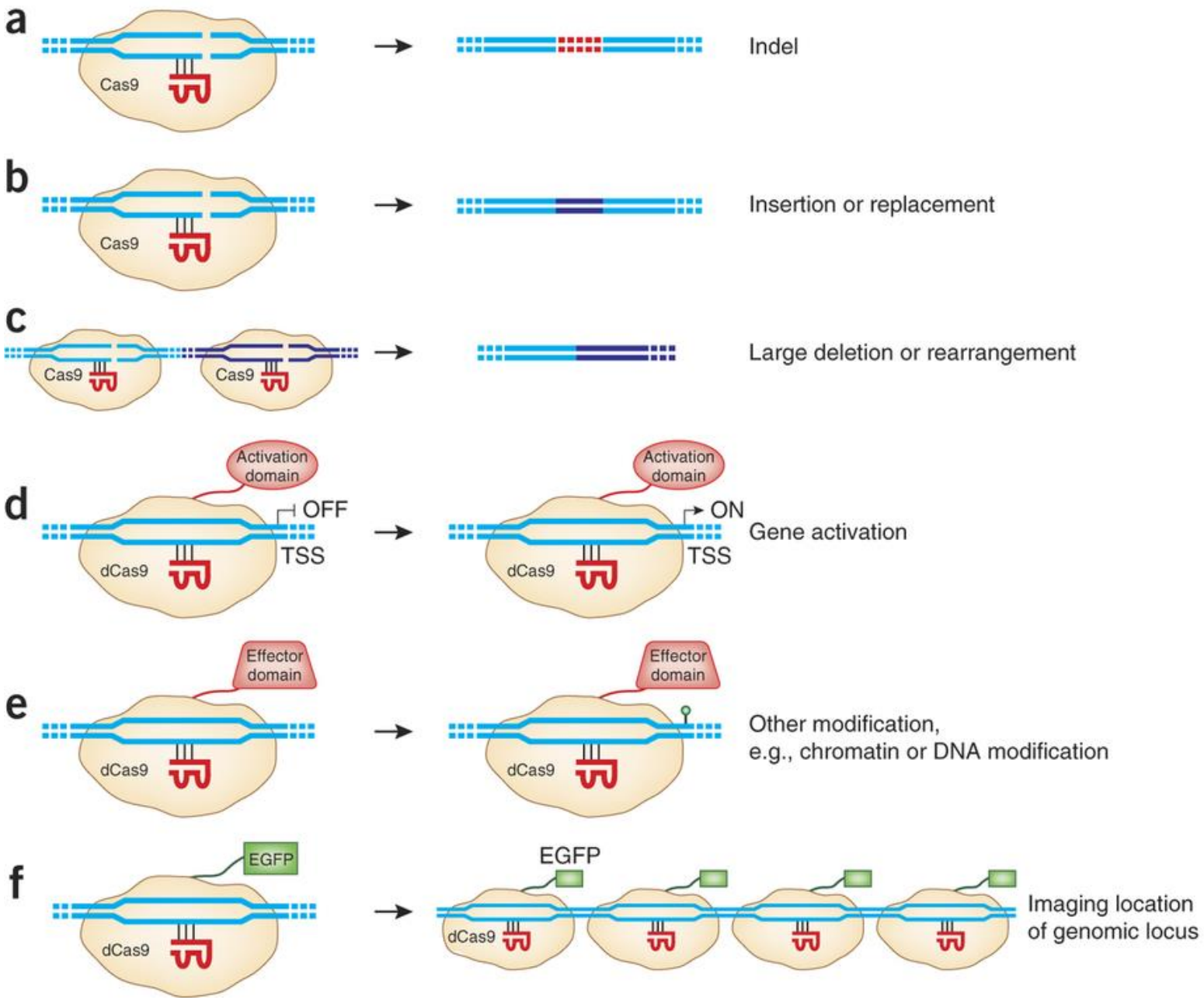
- Endonuclease
  - Cas9

Adaptation for Gene Editing

- Only two components needed
  - RNA and Cas9 (protein)
- crRNA and tracrRNA combined
  - ‘single guide’ sgRNA

- Modified endonuclease activity of Cas9
  - DSB
  - Nickase
  - Catalytically dead – retains ability to bind but cannot cleave target

Nature Biotechnology 32, 347–355 (2014)



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Benefit over other genome editing technology

- Previously developed systems:
  - Protein-based with customizable DNA-binding specificities to create DSB-inducing nucleases
    - ZFN
    - TALEN
- RNA-guided nucleases (RGNs)
  - Rely on simple base-pairing rules to engineer specificity
  - Multiple targets can be edited at once
  - High throughput adaptability based on sgRNA libraries

Risks

- Off-target effects
  - Data varies on showing number of mismatches tolerated leading to off-target mutations (anywhere from 1 to 5 based on specific location in sgRNA)
    - Reported rate of mutation at off-target sites as high as 2-5% in human cells
    - *In vitro* Cas9 digestion of whole genome followed by sequencing yielded mutation rate of 0.1% (Nature Methods 12, 237–243 (2015)
    - Evidence that binding of Cas9 is promiscuous but nuclease activity is not (Nat Biotechnol. 2014 Jul;32(7):670-6)
- Presence of Cas9 alone
  - Studies have shown nickase Cas9 alone can induce indel mutations
- Delivery mechanisms
  - Potential for researcher exposure

Delivery technology

- Both RNA and Cas9 must be present in a cell to edit the genome
  - RNA forms:
    - *In vitro* transcribed
    - Encoded on plasmid or viral vector genome
  - Cas9 forms:
    - Purified protein
    - Encoded on plasmid or viral vector genome
- Mechanical (electroporation, microfluidics)
- Transfection
- Viral vector
- Microinjection

Mechanisms to reduce risk

- Reduced concentration of sgRNA and Cas9
  - Inducible expression of CRISPR components
  - Reduced copies of plasmid for electroporation
  - Results have shown either no change in off-target effects relative to on-target or some decrease
- Paired nickases or Cas9 dimerization
- Increased specificity of sgRNA
  - Changes in sgRNA length
  - Promoter sequence requirements
- Separation of delivery of sgRNA and Cas9

Other Considerations

- DURC
  - Introduction of CRISPR/Cas9 system into bacteria to alter its susceptibility to natural or engineered phage
- Ethics of use in humans
  - Edit genes in somatic cells
  - Edit genes in embryo or germ line leading to heritable genetic modifications

Risk Assessment

- Delivery method
- Target and hypothesized outcome
- Temporal expression of CRISPR components
- Dosage of CRISPR components