Session 1 - June 3rd

Preconditions for using CRISPR systems to enhance cognitive traits

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Definitions

Bostrom, Sandberg (2009: 311-312). https://doi.org/10.1007/s11948-009-9142-5

Cognition

- Processes that an organism uses to organize information
  - perception, selection (attention), representation (understanding) and retention (memory);
  - basis for guiding behavior and motor response (Sandberg 2011: 71).
- Interventions to improve cognitive function may be directed at any one of these core faculties, or specific traits in their subsystems.

Cognitive enhancement

- Amplification or extension of the basic capabilities of the mind, by increasing or optimizing its information processing subsystems.
- Enhancement is relative to the initial stage of non-pathological functionality/perform.
  - **External**: Hardware and software support that gives human beings effective cognitive abilities, in many respects far outstrip those of biological brains.
  - **Internal**: A growing list of potential biological enhancements (genes, tissues, organs, brain funct. networks…).

Cognitive functions and traits

Cognitive functions

- Those involved in knowing
  - **concrete and tangible** [tactile perception]
  - or **abstract** [reasoning]
  - **innate** [not acquired intentionally or declaratively]
  - occur in the course of a non-pathological neurodevelopment and in the normal interaction with a normal environment.
- **Cognitive traits**
  - Identifiable/discrete elements involved in (regulating) brain functions (neurophysiology, organs, cellular networks, cell, genetic, molecular)
  - Injury, damage or anomalous functioning causes effects on cognitive, psychological, emotional processes and individual behavior (structures of CNS). Targets for interventions directed to improve **core faculties**.

Cognitive enhancement

Targets

- Attention
- Perception
- Understanding
- Memory / working memory
- Language
- Processing speed
- Orientation
- Reasoning
- Learning
- Calculation
- Executive control
- Inhibition

Internal cognitive enhancement

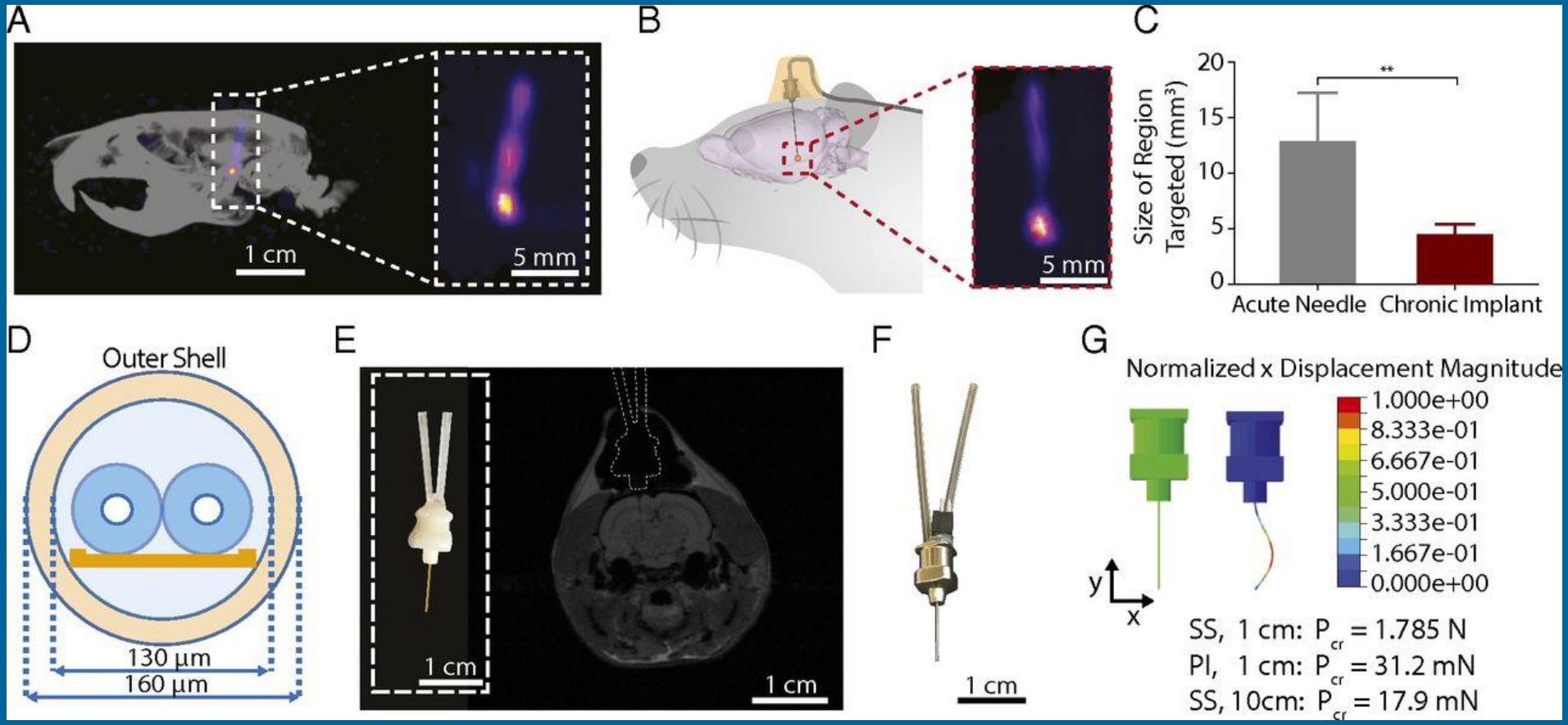
- **Growing list of potential biological enhancers**
  - **Nootropics** (C. E. Giurgea, 1972)
    - Drugs, supplements, and other substances that may improve cognitive function → executive functions, memory, creativity, or motivation, in healthy individuals.
    - Lack of research (still at a preliminary stage) about the effects or causal interaction of the majority of these agents (Ginseng, Bacopa monnieri, Salvia…)
    - More than 100 substances, from amino acids to botanical preparations are advertised on websites as having the ability to improve cognitive performance.
    - High risk of advertising fraud and marketing scams (FDA/FTC warnings in Dec. 2018 and Feb. 2019)
    - Their safety and efficacy have not been systematically examined (Ilieva, Hook, Farah 2015)
    - Often used without medical indication (to treat Alzheimer's disease, Parkinson…).
  - **American Medical Association, 2016**:
    - The nonmedical use of these drugs should be discouraged given potential for substance misuse and other adverse consequences
    - The cognitive effects of prescription stimulants appear to be highly variable among individuals, are dose-dependent, and limited or modest at best in healthy individuals.

Internal cognitive enhancement

* Pham, 2018; Ramadi, 2018.

- **Growing list of potential biological enhancers of cognition**
  - **Neural implants** (Friedlander, 2012)
    - "Neural implants, or prosthetics, are a class of devices that communicate with the nervous system. An electronics package in each device activates an array of tiny electrodes that interface directly with healthy neurons in the body." → Synchron Stentrode, through jugular vein | Microprobes for drug delivery (*)
    - Used to stimulate parts and structures of the nervous system with implanted electrical circuitry or record the electrical activity of nerve cells [EEG arrays allow interface mind- machine without direct implantation of a device].
      - Neural interfaces enable a two-way exchange of information with the nervous system.
      - Connections at multiple levels, including with peripheral nerves -in the spinal cord -, or with the brain.
    - Brain implants are a specific kind of neural device placed on the surface or the cortex of the brain that create an interface between the nervous system and microchips in order to treat damaged parts of the brain.
  - **Applied mainly to restore cognitive function**: a neural implant must gather data from one area of the brain, process this information correctly, and then deliver the resulting signal to another brain region, bypassing any damaged tissue (after a stroke or head injuries; epilepsy, dystonia, depression…)
    - Many people **regain control** over their bladder, their senses, their limbs, and their memory (cochlear implant).
    - Peripheral or spinal cord nerve interfaces gives amputees **fine motor control over artificial limbs'**, allowing people to walk and move like an average healthy human.
    - Beneficial for people with fatal conditions, but also for those whom would like to enhance their senses and brain functionality (senses, physical movement, and memory). Still under development, also for military uses.

Ramadi et al. 2018. “Focal, Remote-Controlled, Chronic Chemical Modulation of Brain Microstructures.” Proceedings of the National Academy of Sciences 115 (28): 7254–59. https://doi.org/10.1073/pnas.1804372115.



Chronic MiNDS probes for focal deep-brain interfacing. (*A*) PET/computed tomography (PET/CT) scans of rat head following 2-µL acute injection of Cu-64 in vivo. (*B*) Illustration of implanted short, minimally invasive drug delivery system (S-MiNDS) probe in a rat. (*Inset*) PET images of 2-µL infusion of Cu-64 through chronically implanted probe. (*C*) Size of brain region targeted using infusion through acutely inserted needle and chronic implant.

Stentrode™ is a minimally invasive implantable brain device (the first endovascular neural interface) that can interpret signals from the brain for patients with paralysis. Implanted via the jugular vein, the Stentrode is placed inside the brain in the command-control center -motor cortex- without the need for open brain surgery.
The signals are captured and sent to a wireless unit implanted in the chest, which sends them to an external receiver (12/06/2018). https://youtu.be/NZlIL0iI1Sg

Slow progress in somatic gene therapy

Steffin, David H M, Emily M Hsieh, and Rayne H Rouce. 2019. "Gene Therapy: Current Applications and Future Possibilities." *Advances in Pediatrics*. https://doi.org/https://doi.org/10.1016/j.yapd.2019.04.001

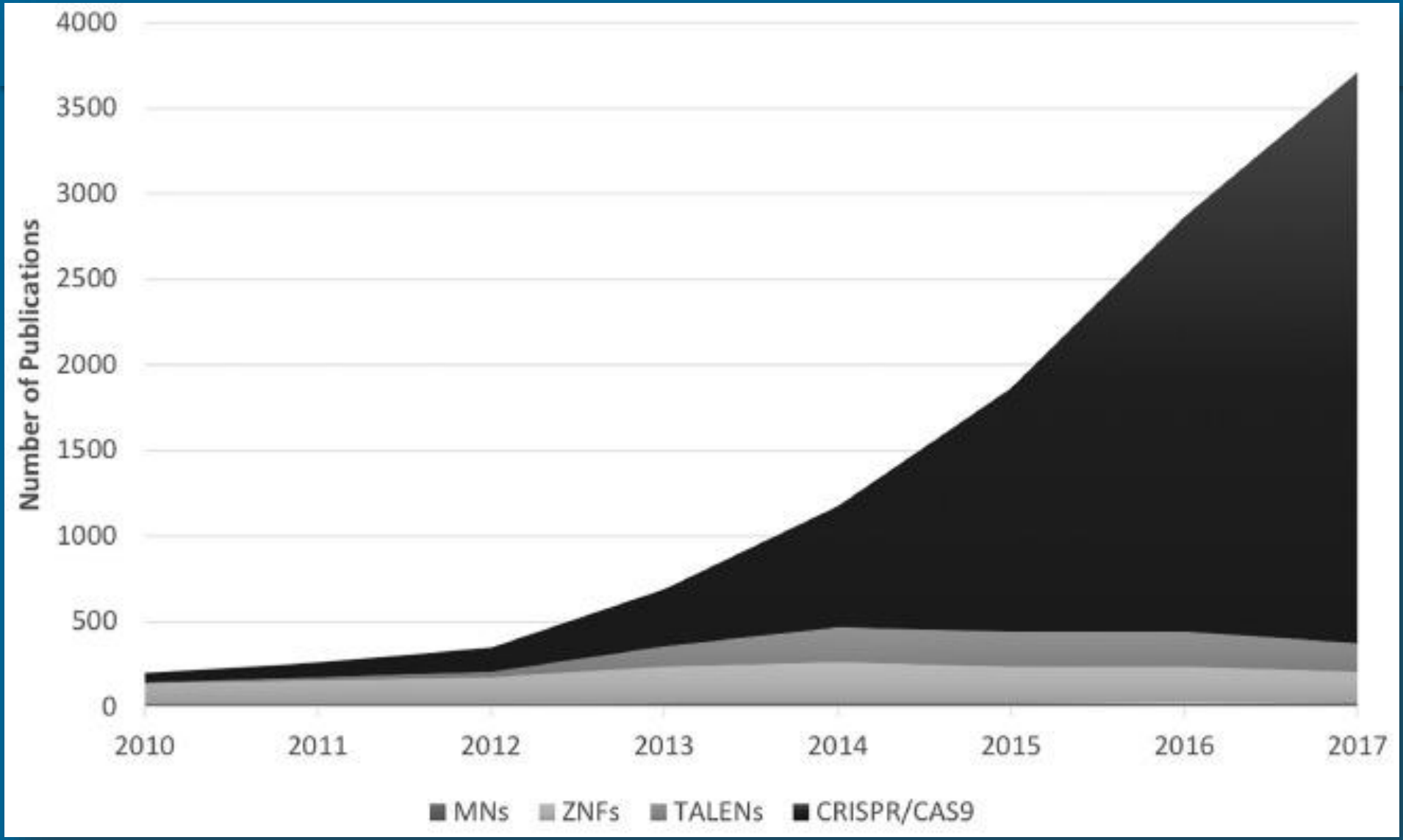
- GT includes multiple approaches to manipulate genetic material in an effort to treat specific diseases:
  - replacing a mutated or defective gene with a healthy one,
  - introducing a new gene to help fight disease,
  - or editing an existing gene to change its function.
- Demonstrated success in inherited and acquired pediatric diseases
  - immunodeficiencies, inherited retinal disorders, blood disorders, neurologic disorders, and cancer.
- Early clinical trials of gene therapy, despite mixed outcomes, paved the way for current clinical applications
  - Highly dependant of viral vectors to transmit genetic material.
- Upcoming clinical trials
  - CRISPR-Cas9 is a novel gene-editing technique that offers precise mechanisms of human genome modification.

Zhang, Z. et a. 2017. "CRISPR/Cas9 Genome-Editing System in Human Stem Cells: Current Status and Future Prospects." *Molecular Therapy - Nucleic Acids* 9: 230–41. https://doi.org/10.1016/j.omtn.2017.09.009

Clinical Translational Studies

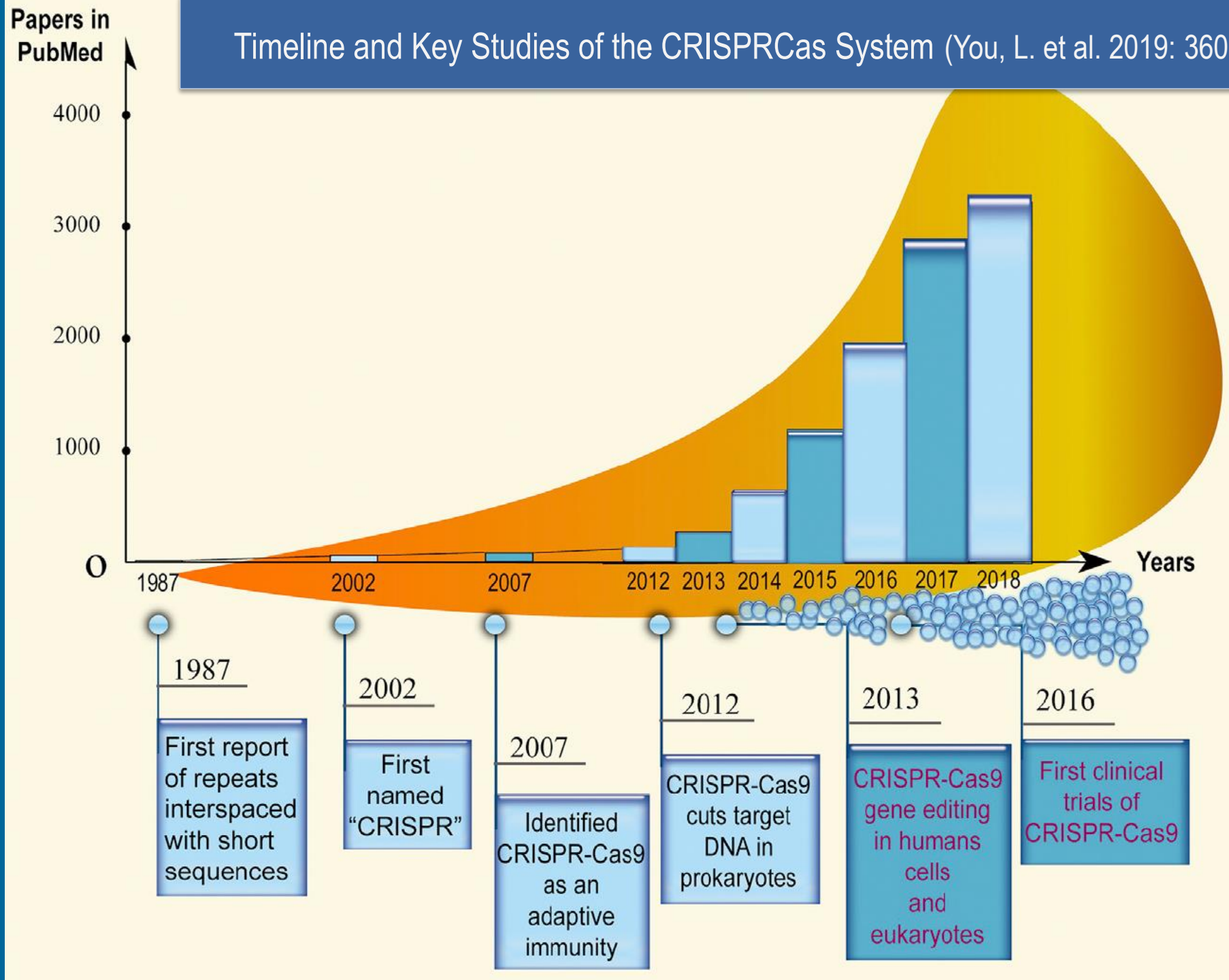
Disease model and drug screening	ESCs	*RB1*-null cells showed aberrant mitochondria and were sensitive to carboplatin (retinoblastoma)	2017	40
	iPSCs	isogenic iPSCs model for pathomechanism and drug screening (myelodysplastic syndrome)	2016	42
	iPSCs	point mutation iPSCs model for studying individual difference in hypoglycemia	2017	43
	ESCs	WRN-null hESCs model Werner syndrome (WS-iPSCs showed abnormal karyotypes seriously)	2015	44
	organoids	gene knockout kidney organoids showed cyst of tubules	2015	45
	organoids	modeling dyskeratosis congenita reveals the therapeutic functions of Wnt agonists	2016	49
	organoids	gene knockout intestinal organoids form tumors in mice kidney after subcapsule injection	2015	51
	organoids	organoids model reveals the function of *APC* and *P53* in intestinal neoplasia	2015	52
	organoids	organoids model reveals the function of TGF-β in colorectal cancer (CRC) formation	2016	53
	organoids	combinatorial drug responses in organoids model (colorectal cancer [CRC])	2016	54
Gene correction therapy	HSCs	gene-corrected patient HSCs showed functional recovery in X-linked chronic granulomatous	2017	65
	iPSCs	gene-corrected patient iPSCs recovered β-globin (HBB) expression	2015	59
	organoids	gene-corrected patient organoids showed functional recovery in cystic fibrosis	2013	50
	HSCs	gene-corrected patient HSCs recovered β-globin (HBB) expression in β-thalassemia	2016	61
	iPSCs	gene-corrected patient iPSCs showed functional recovery in hemophilia A	2015	63
	iPSCs	gene-corrected patient iPSCs showed normal phenotypes in Huntington's disease	2017	64
Anti-virus therapy	T cells	CXCR4-disrupted T cells showed HIV resistance	2015	69
	HSCs	CCR5-disrupted HSCs presented HIV resistance	2017	70
	iPSCs	CCR5-disrupted iPSCs and its derived blood cells showed HIV resistance	2015	71
	iPSCs	CCR5Δ32 iPSCs and its derived cells showed HIV resistance	2014	72
	iPSCs	CRISPR/Cas9-expressed iPSCs showed HIV resistance by elimination of virus RNA	2015	74
Anti-tumor therapy	CAR T cells	the anti-tumor efficacy of CAR T cells was enhanced through disrupting the PD-1 gene	2017	77
	CAR T cells	the anti-tumor efficacy of CAR T cells was improved by fusing CD19 CAR to the TRAC gene	2017	78
	iPSCs	NK cells derived from ADAM17-disrupted iPSCs presented higher HIV resistance	2016	79

PubMed publications on Meganucleases (MNs), Zinc Finger Nucleases (ZNFs), Transcription Activator-Like Effector Nucleases (TALENs) or CRISPR/Cas9, between 2010 and 2017. https://www.ncbi.nlm.nih.gov/pubmed



Memi, F. et al. 2018. "CRISPR/Cas9 Gene-Editing: Research Technologies, Clinical Applications and Ethical Considerations." *Seminars in Perinatology* 42 (8): 487–500. https://doi.org/https://doi.org/10.1053/j.semperi.2018.09.003

Timeline and Key Studies of the CRISPRCas System (You, L. et al. 2019: 360)



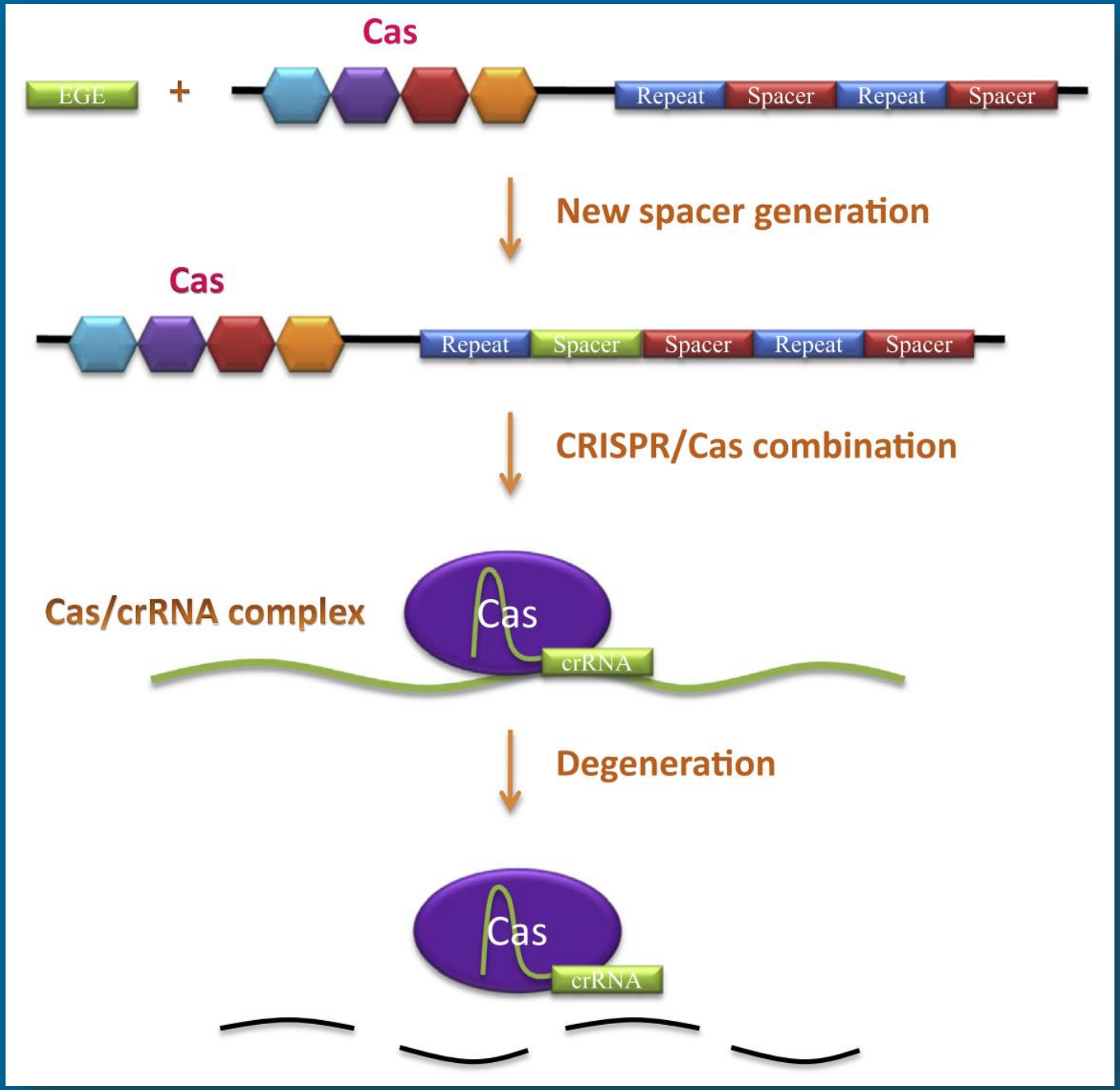
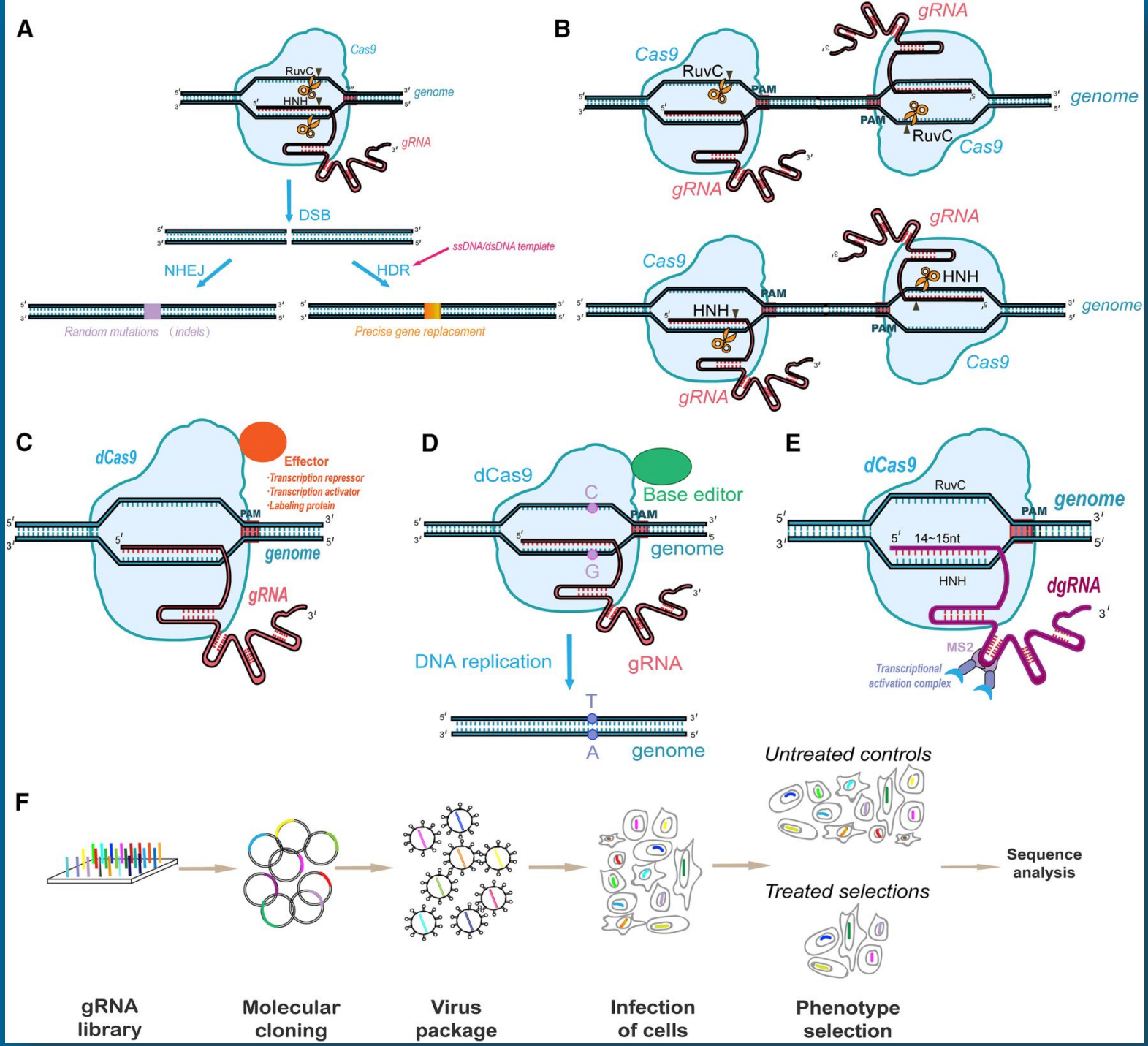


Figure 1.
Figure 1. CRISPR-Cas Immune System

When EGEs invade the host, some fragments will integrate into the CRISPR loci as a new spacer casually that is co- expressed with Cas nucleases to form Cas/crRNA complexes.

These complexes can identify and bind with the same EGEs during a subsequent invasion following the base complementation pairing rule and then finally break the EGEs.

Source:
www.moleculartherapy.org


A
Cas9
RuvC
HNH
genome
gRNA
DSB
NHEJ
HDR
ssDNA/dsDNA template
Random mutations (indels)
Precise gene replacement
B
Cas9
RuvC
PAM
gRNA
genome
HNH
C
dCas9
Effector
·Transcription repressor
·Transcription activator
·Labeling protein
PAM
genome
gRNA
D
dCas9
Base editor
C
G
PAM
genome
gRNA
DNA replication
T
A
genome
E
dCas9
RuvC
PAM
genome
5′ 14~15nt
HNH
dgRNA
MS2
Transcriptional activation complex
F
Untreated controls
Treated selections
Sequence analysis
gRNA library
Molecular cloning
Virus package
Infection of cells
Phenotype selection

Limitations of the CRISPR/Cas9 technology

Memi, F. et al. 2018

The CRISPR/Cas9 technology, despite its superior efficiency, ease-of-use and low cost has clear limitations:

- Lack of accuracy, off-target effects, and embryo mosaicism (in germline editing).
  - Specificity of Cas9 nucleases: these proteins can tolerate mismatches on the guide sequence, leading to **off-target cleavage effects** (Fu et al. 2013).
  - New methods to improve targeting specificity under development (Tycko 2016; Gorski et al. 2017).
  - Recent work in mouse embryonic stem cells reported **high incidence of off-target deletions and complex rearrangements** following CRISPR/Cas9 gene-editing (Zhang et al. 2015).

    M Kosicki, K Tomberg, A Bradley (2018). Repair of double-strand breaks induced by CRISPR-Cas9 leads to large deletions and complex rearrangements. *Nat Biotechnol* (2018)

- Insertion of DNA sequences through CRISPR/Cas9 or the alteration of existing sequences have proven considerably more challenging than the introduction of stochastic mutations.
  - NHEJ-mediated repair is error-prone and leads to small insertions or deletions (indels) at the target sites, while the HDR mechanism utilizes a DNA template for precise repair.
    - CRISPR/Cas9-mediated DNA cleavage results in Double Stranded Breaks (DSBs), which can be repaired by Non-Homologous End Joining (NHEJ) or Homology-Directed Repair (HDR).
  - **Shrock & Guell 2017**: Homology-Directed Repair (HDR) mechanism remains much less efficient than Non-Homologous End Joining (NHEJ). Activation of the P53 pathway is a possible side effect.

Off-target cleavage of CRISPR/Cas9 in human 3PN embryos (Liang, 2015)

(A) Off-target cleavage in human embryos was summarized here. PAM sequence are labeled in green. *HBB*, on-target cleavage of the *HBB* locus. OT1–7, the top 7 predicted off-target sites. HBD, the predicted off-target site in the HBD locus. Mismatched nucleotides compared to the *HBB* locus are labeled in red. Some of the off-target sites failed to be amplified by PCR in this experim.

A

Sites	Sequence	Indel frequency (mutant embryos/total embryos)		Locus
		20 ng/μL gRNA 100 ng/μL Cas9	40 ng/μL gRNA 200 ng/μL Cas9	
HBB	GTAACGGCAGACTTCTCCTCAGG	11/11	17/17	chr11:5248231
G1-OT1	TTAAAGGAAGACTTCTCCTCAGG	0/11	0/12	chr3:181783903
G1-OT2	GTAATGGCATATTTCTCCTCAGG	0/11	0/12	chr1:227894389
G1-OT3	GTGACGGCACACTTCTTCTCCAG	0/10	0/10	chrX:149810034
G1-OT4	GAAAAGGCAGACTTCTCCCCTAG	4/10	2/10	chr11:132762118
G1-OT5	GGAGGGGCAGGCTTCTCCTCTGG	7/11	3/12	chr6:158896257
G1-OT6	GAAATGGCCAACTTCTCCTCAAG	0/11	0/10	chr1:204671648
G1-OT7	GAGAGGGCAGCCTTCTCCTCCAG	0/11	0/9	chr20:30590029
HBD	TTGACAGCAGTCTTCTCCTCAGG	0/11	0/12	chr14:5234413

B

CRISPR/Cas9 induced on- and off-target indels in exomes of human 3PN embryos

Cas9/gRNA (ng/μL)	100/20			200/40		
Sample No.	A	B	C	D	E	F
On-target indels	1	1	1	1	2	4
Candidate off-target sites	1	0	1	0	0	0
T7E1 assay confirmed off-target sites	1	0	1	0	0	0

Box 1: List of indications and potential ethical considerations for heritable genome editing.

(Steffin et al. 2019: 14)

Regulatory Framework for Clinical Trials That Use Heritable Genome Editing

1. Absence of reasonable alternatives
2. Restriction to preventing a serious disease or condition
3. Restriction to editing genes that have been convincingly demonstrated to cause or strongly predispose to the disease or condition
4. Restriction to converting such genes to version that are prevalent in the population and are known to be associated with ordinary health with little or no evidence of adverse effects
5. Availability of credible preclinical and/or clinical data on risks and potential health benefits of the procedures
6. Ongoing, rigorous oversight during clinical trials of the effects of the procedure on the health and safety of the research participants
7. Comprehensive plans for long-term, multigenerational follow-up that still respect personal autonomy
8. Maximum transparency consistent with patient privacy
9. Continued reassessment of health and societal benefits and risks
10. Reliable oversight mechanisms to prevent extension to uses other than preventing a serious disease or condition

Conclusion

- **CRISPR/Cas systems are now the most promising tool for precise gene editing, but still experimental**
  - "this technology has not fundamentally changed the ethical issues surrounding gene therapy and genetic engineering" (Memi et al. 2018)
    - Ethically acceptable on somatic cells, under usual clinical trials
  - Three types of ethical controversy of germline gene-editing:
    - **Necessity**? - IVF in combination with pre-implantation genetic diagnosis (PGD) to select for healthy embryos is sufficient to manage the vast majority of monogenic diseases
      - » Exception: parents being both homozygous for a recessive disease-causing gene (i.e. Cystic Fibrosis, or Tay-Sachs disease), or when one parent is homozygous for a dominant disease-causing gene (i.e. Huntington's or Polycystic Kidney disease)
      - » desire for genetic relation, when adoption, donor gametes and same-sex marriage are becoming increasingly common family schemes?
      - » mitochondrial replacement therapy (MRT), the only example of a legalized germline editing application, is only legal in the UK.
    - **Unforeseeable/unidentified risks**, because of the limitations of the CRISPR/Cas9 technology in targeting efficiency, off-target effects and immunogenicity. **Reversibility**?
    - **Eugenics /social plausibility** of germline gene-editing? Gen. enhanc. in farm, pets, research..

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