Genome Editing for Genetic Enhancement – Are We Ready Yet?

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Questions to Be Addressed

- What are the intended outcome(s) of genetic enhancement?
- How is genetic enhancement achieved?
- Is the technology ready yet?

What are the intended outcome(s) of genetic enhancement?

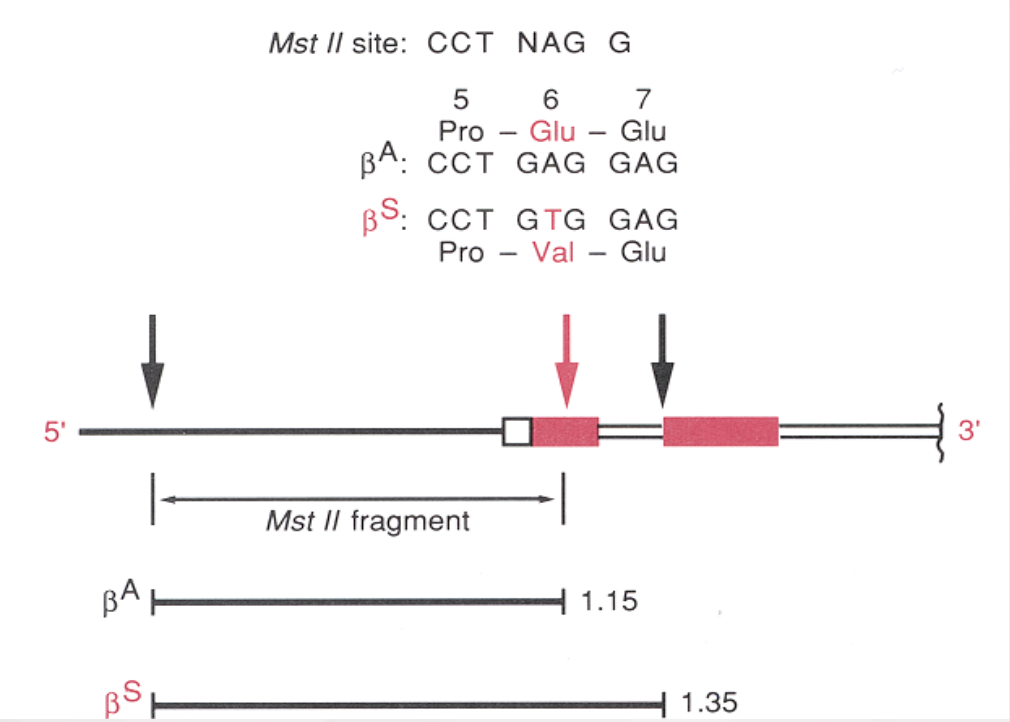
Definition of Genetic Enhancement:

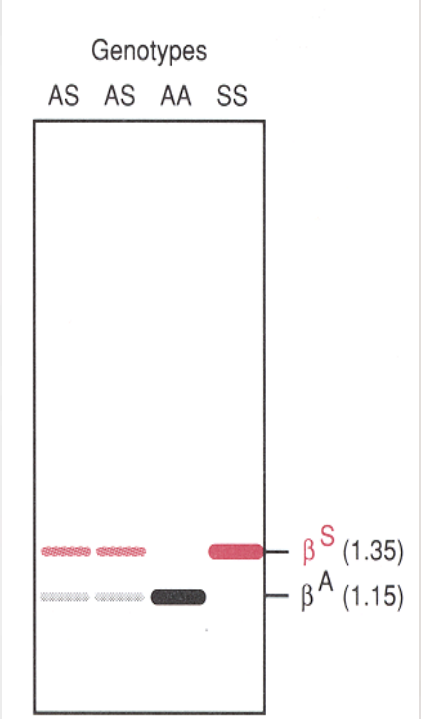
"Genetic Enhancement …….. intended to modify ….. human traits. The term commonly is used to describe efforts to make someone not just well, but better than well, by optimizing attributes or capabilities."

National Human Genome Research Institute, NIH, USA, *Genetic Enhancement* archived page.

Sickle Cell Trait

A-to-T Mutation of the β-globin gene

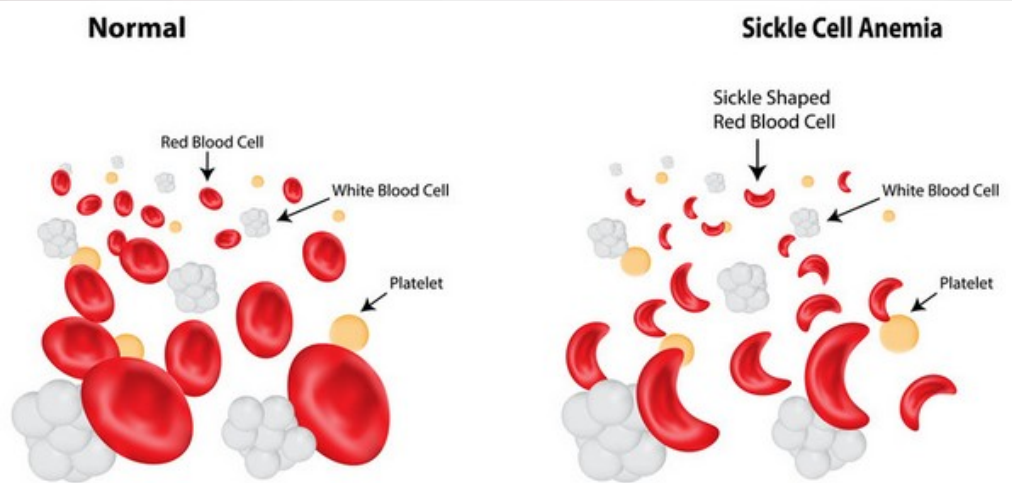




Thompson, McInnes, and Willard, Genetics in Medicine, 5th Ed., WB Saunders, p. 253.

Presence of the mutated β-globin proteins causes the red blood cells to assume a sickle shape.

Sickle Cell Trait



Crossley M, http://theconversation.com/explainer-one-day-science-may-cure-sickle-cell-anaemia-28153

Sickle cell trait originated from the Sub-Saharan region

http://www.sicklecellinfo.net/who_suffers.htm

Sickle Cell Trait: A Friend (Enhancement)

Protective effects of the sickle cell gene against malaria morbidity and mortality

Michael Aidoo, Dianne J Terlouw, Margarette S Kolczak, Peter D McElroy, Feiko O ter Kuile, Simon Kariuki, Bernard L Nahlen, Altaf A Lal, Venkatachalam Udhayakumar

Lancet. 2002 Apr 13;359(9314):1311-2

PROTECTION AFFORDED BY SICKLE-CELL TRAIT AGAINST SUBTERTIAN MALARIAL INFECTION

BY

A. C. ALLISON, D.Phil., B.M.*

(From the Clinical Pathology Laboratory, the Radcliffe Infirmary, Oxford)

Br Med J. 1954 Feb 6; 1(4857): 290–294

Persons heterozygous with the **sickle cell trait:**

- Have a **relative resistance to falciparum malaria**;
- Are **less likely** to **get the disease**;
- Run **lower parasite counts**, and
- Are **less likely to die** from malaria

The Sickle trait serves as a protective mechanism against malaria and were believed to be naturally selected in sub-Saharan Africa

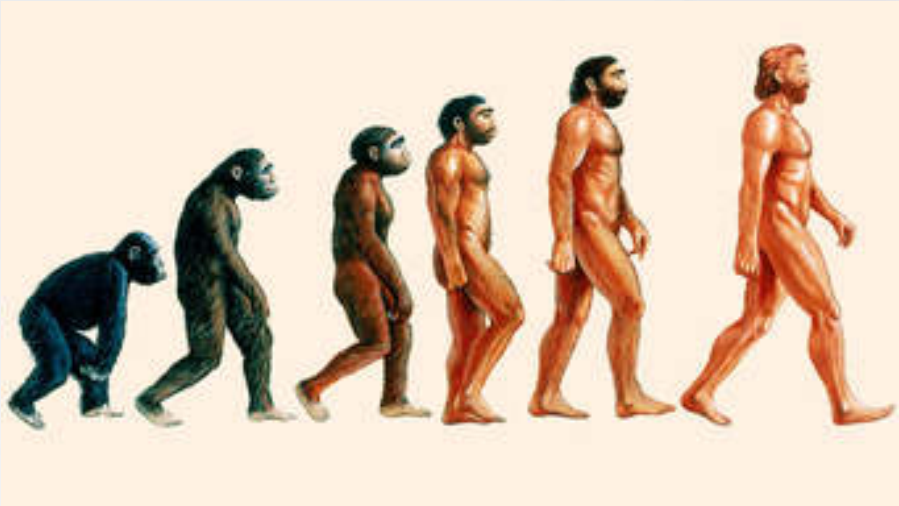
Sickle Cell Anemia: A Foe

Individuals homozygous of the Sickle Cell trait:

- **Pain:** Sickle cells blocking off the blood causes pain.
- **Anemia:** The sickle-shaped red blood cells die quickly resulting in insufficient red blood cells to carry oxygen giving rise to fatigue and paleness.
- **Delayed growth:** Anemia slows the rate of growth.
- **Eye problem:** The eye can be damaged by the lack of oxygen; it can be serious enough to cause blindness.
- **Infections:** Affected individuals are more vulnerable to infections because of damage to their immune systems from the disease.
- **Stroke:** If the blood flow to a part of the brain is blocked by the sickled cells, a stroke can occur.

Outcome of the genetic change can be beneficial or harmful

Evolution - Genetic Enhancement by Nature



http://media3.picsearch.com/is?d43ohlN-TCN0pTSbthVqnMG_MqQepCTdE3AETDbv9rI&height=192

Darwin's theory of evolution states that evolution happens by natural selection.

- Genes that allow a species to thrive are passed down from parent to offspring.
- Any individuals that possess the characteristics that will help the species thrive and survive in a particular environment will be the ones to reproduce and the genes those individuals possess will be passed down or even enhanced.
- Only the strongest survive long enough to reproduce. Over the years, those species that reproduce will continue to evolve.

Genetic Enhancement by Manipulation of the Genome – Genome Editing

Evolution	Genome Editing
Natural process	Artificial process
Long drawn out (centuries)	Quick (days/years)
Seldom involves exogenous agents	Employ non-human agents and chemicals
Outcome tested by time	Outcome not tested
Affect the entire species	Affect selected individuals

Genome Editing

- In Genome editing, or genome editing with engineered nucleases (GEEN) DNA is inserted, deleted or replaced in the genome of an organism using engineered nucleases, or "molecular scissors."

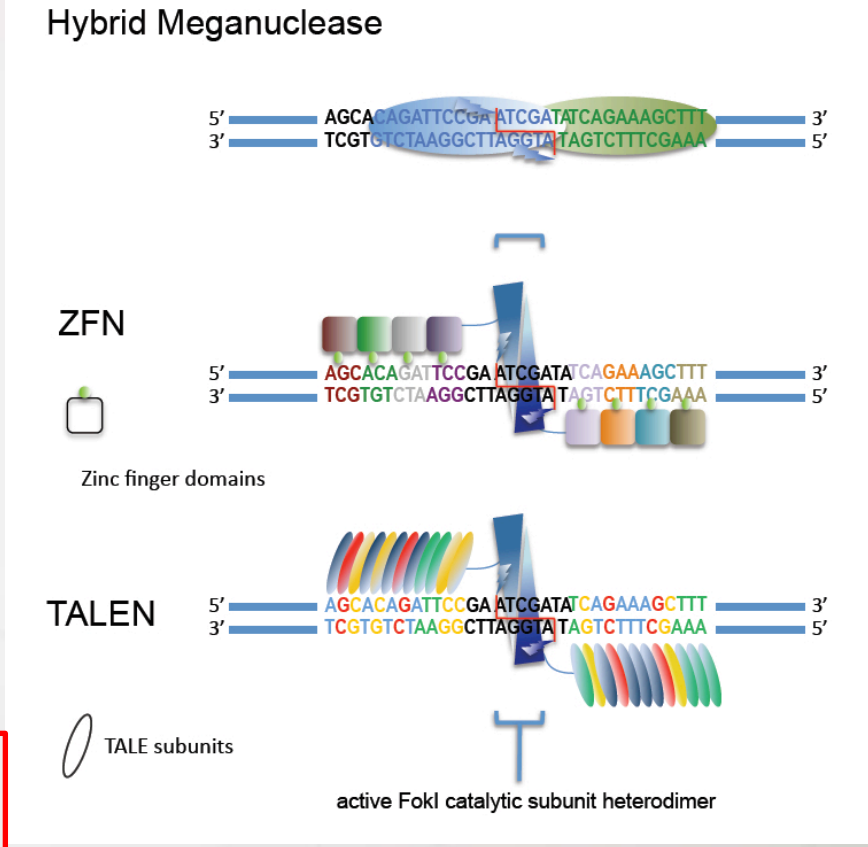
- These nucleases create site-specific double-strand breaks (DSBs) at desired locations in the genome.

- The induced double-strand breaks are repaired through nonhomologous end-joining (NHEJ) or homology directed repair (HDR), resulting in targeted mutations ('edits').

Different Genome Editing Technologies Based on Engineered Nucleases

There are currently four families of engineered nucleases being used:

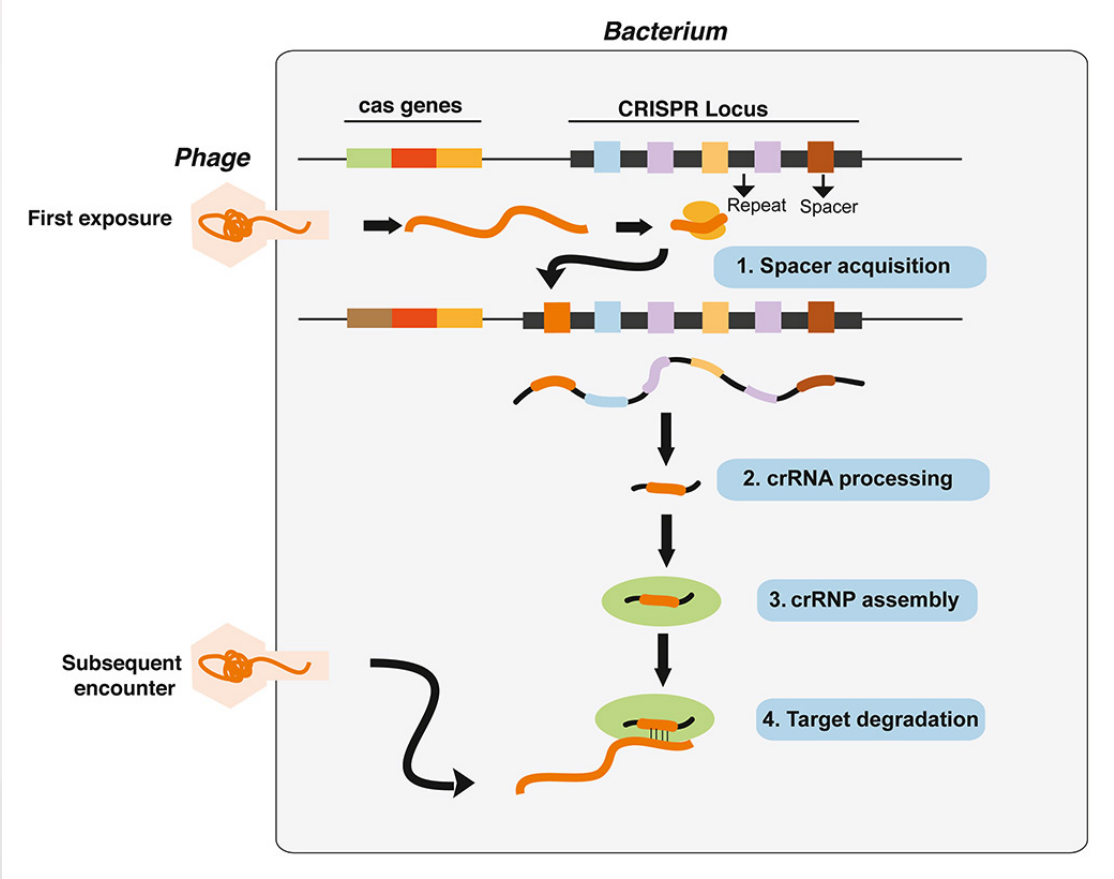
1. Meganucleases – not enough known;
2. Zinc finger nucleases (ZFNs) – more cytotoxicity;
3. Transcription activator-like effector-based nucleases (TALEN) – TALE subunits more expensive to synthesize, and
4. The CRISPR/Cas9 system - simpler, cheaper, and can be highly efficient



https://en.wikipedia.org/wiki/Genome_editing

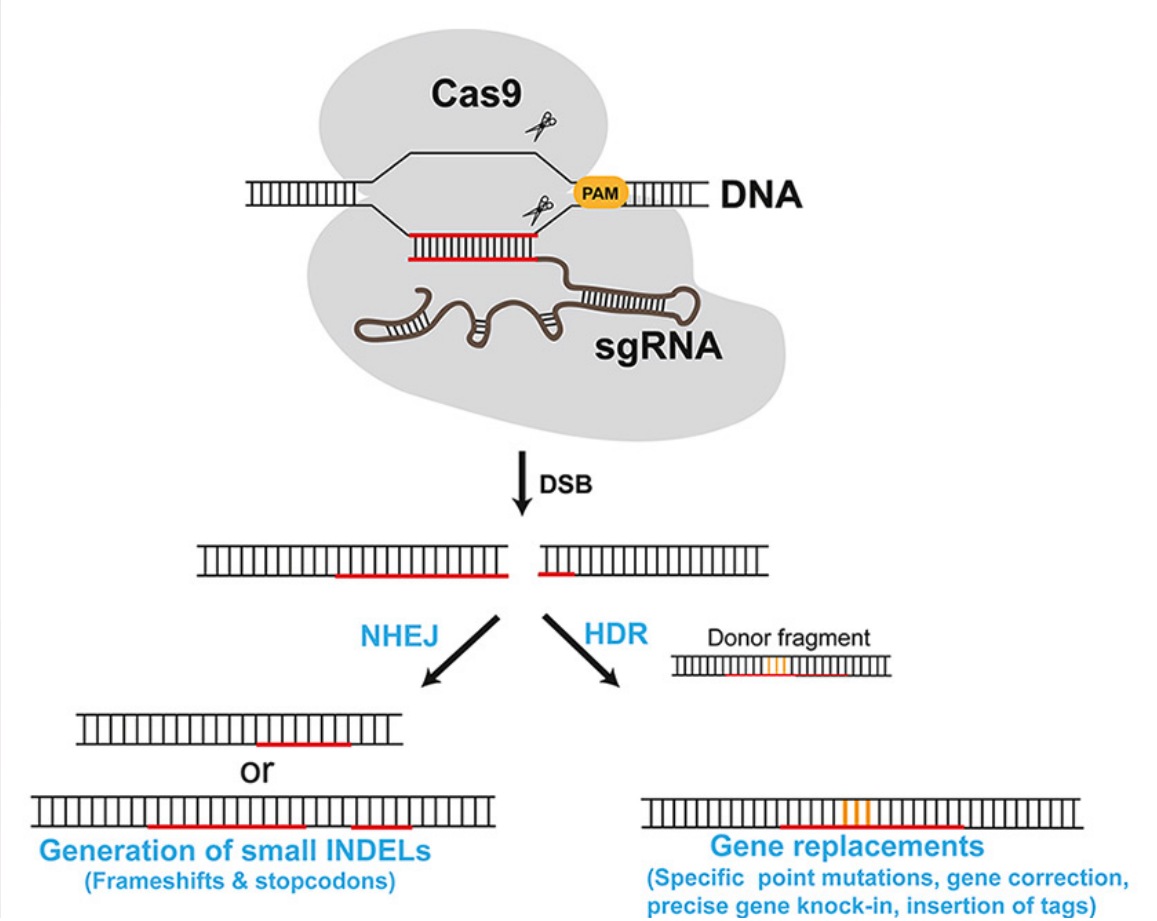
CRISPR/Cas9 System
derived from *Streptococcus pyogenes*

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat)
Cas (CRISPR-associated genes: polymerase, nuclease, helicase)
Cas9: endonuclease



Swiss Med Wkly. 2015;145:w14230

Genome Editing by CRISPR/Cas9



Swiss Med Wkly. 2015;145:w14230

The Cas9 enzyme cleaves chromosomal DNA, whose sequence is complementary to guide RNA in a targeted manner, producing site specific DNA double-stranded breaks, the repair of which give rise to targeted genome modification.

First Report of CRISPR/Cas9-mediated Genome Editing in Human Embryos

Protein Cell
DOI 10.1007/s13238-015-0153-5

April, 2015

CrossMark Protein & Cell

RESEARCH ARTICLE

CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes

Puping Liang, Yanwen Xu, Xiya Zhang, Chenhui Ding, Rui Huang, Zhen Zhang, Jie Lv, Xiaowei Xie, Yuxi Chen, Yujing Li, Ying Sun, Yaofu Bai, Zhou Songyang, Wenbin Ma, Canquan Zhou✉, Junjiu Huang✉

NATURE | NEWS

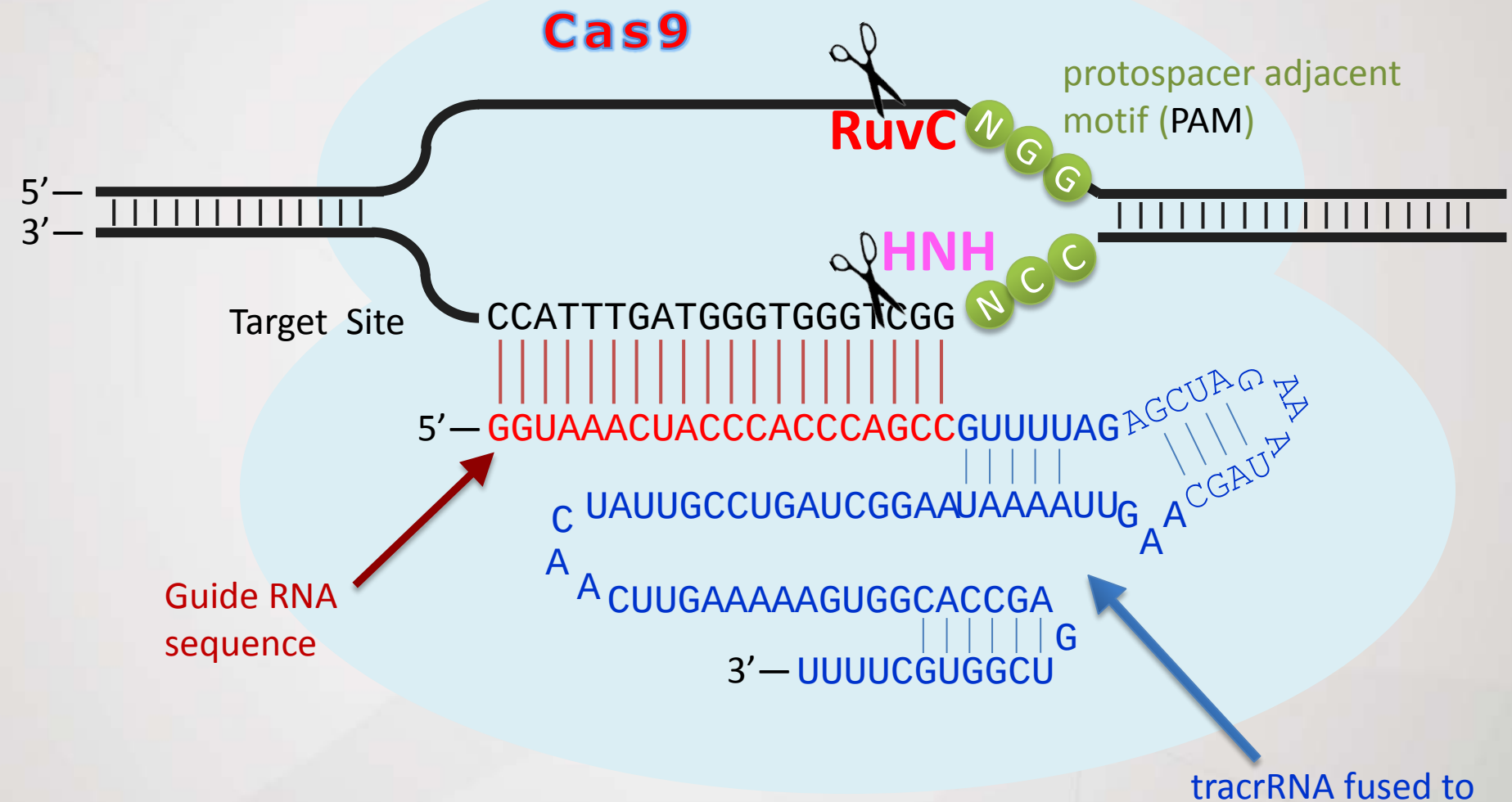
Chinese scientists genetically modify human embryos

Rumours of germline modification prove true — and look set to reignite an ethical debate.

David Cyranoski & Sara Reardon

22 April 2015

CRISPR/Cas9 System
derived from *Streptococcus pyogenes*



CRISPR – Clusters of regularly interspaced palindromic repeats
Cas9 – CRISPR-associated system protein derived from Streptococcus pyogenes

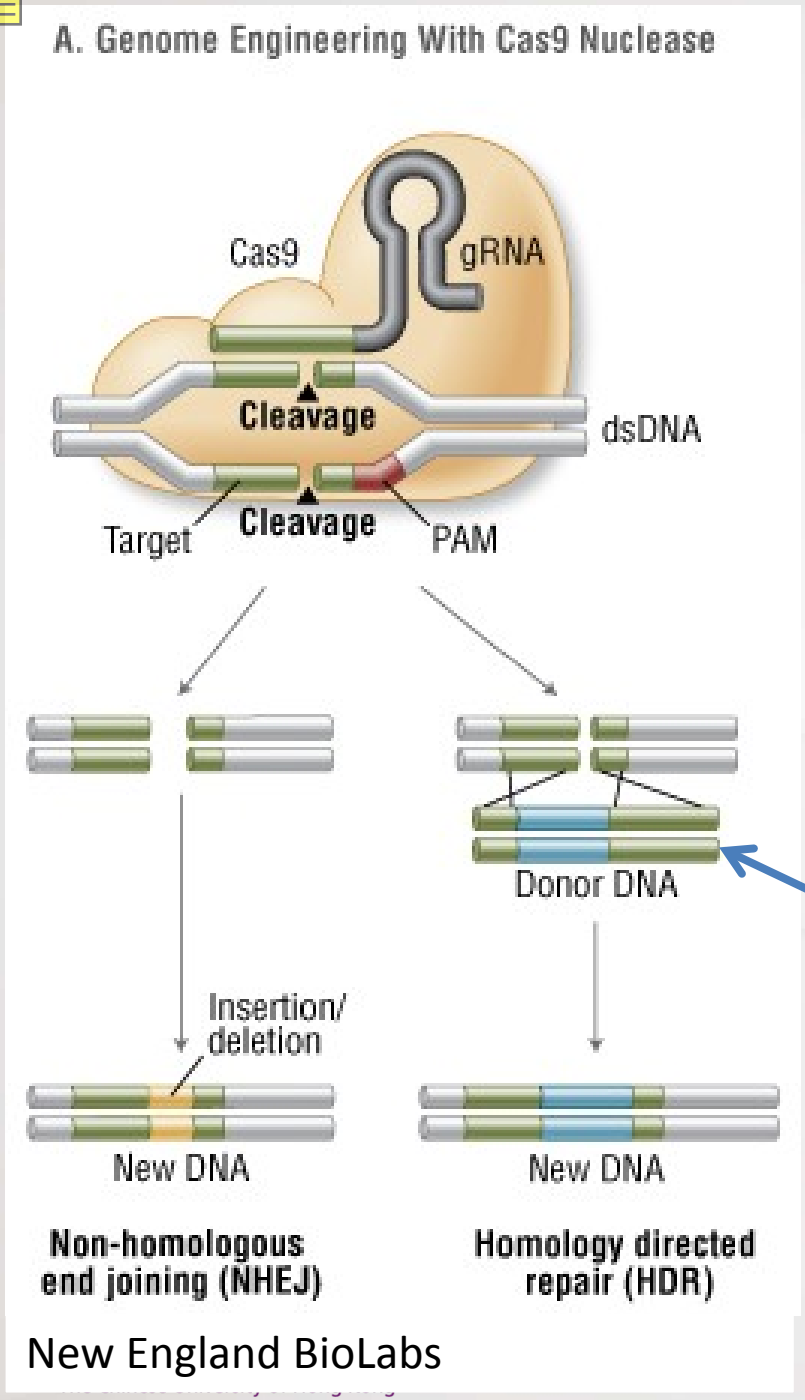
Off-target Cleavages Induced by Cas9 in Mammalian Cells

- DNA targeting specificity of RNA-guided Cas9 nucleases.
  Nat Biotechnol. 2013 31(9):827-832

- High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells.
  Nat Biotechnol. 2013 31(9):822-826

- CRISPR/Cas9 systems targeting β-globin and CCR5 genes have substantial off-target activity.
  Nucleic Acids Res. 2013 (20):9584-9592

Genome Editing with CRISPR/Cas9



New England BioLabs

- Introduce genetic materials by repair mechanism:
  Non-homologous end joining (NHEH) or
  Homology directed repair (HDR)
- Potential error caused by insertion/deletion during repair
- Error rate unknown

Intended additional copy of gene

A new approach is to used inactivated Cas9 to give the delivery of transcription factors to the promoter of desired genes so that the expression of the genes can be increased

Technical Issues of CRISPR/Cas9 Systems

- Instead of the desired edit, the target site might gain an unwanted new mutation
- Off-target mutations are sometimes observed - difficult to assess true risks (e.g., safety) and unintended impacts
- Not 100% efficient
  For cells - how many need to be altered?
  For embryos – incomplete targeting, mosaicism

Mortlock D and Ormond K. Workgroup on the Implications of Genome Editing. 2015 Policy Forum ASHG.

Other Considerations

for Genetic Enhancement by Genome Editing

- Knowledge of the human genome is incomplete – unexplored function of genes
- Most behavioral features are combined results of multigenic traits – multigene editing technology is not available
- Somatic versus germline genome editing

Unintended Health Consequences

- Off-target alterations: rates, impacts?
- Can we minimize off-target effects without sacrificing efficiency?
- Are edited cells still otherwise normal?
- Consequences of mosaicism on health?
- Gene-environment interactions, pathway interactions and systems biology impacts
- Unknowns

Mortlock D and Ormond K. Workgroup on the Implications of Genome Editing. 2015 Policy Forum ASHG.

Unintended Adverse Effects

- Inadvertent impact on germline through somatic applications
- Is germline editing an ethical alternative, given available prenatal testing options?
- Risk for misuse of gametes and embryos
- Unintended consequences in a liveborn child
- Unintended consequences in the population - propagation of undesirable alleles, creation of new illnesses or susceptibilities
- Unknowns

Mortlock D and Ormond K. Workgroup on the Implications of Genome Editing. 2015 Policy Forum ASHG.

Conclusion

Technological concerns:

A number of issues need to resolved before the current technology can be safely applied to human genome editing, namely:

- Less than 100% efficiency - mosaicism
- Off target effect
- Unintended mutation
- Incomplete understanding of the human genome
- Unknown physiological consequence

The technology of genome editing as a tool for gene enhancement is not mature and has to await further studies

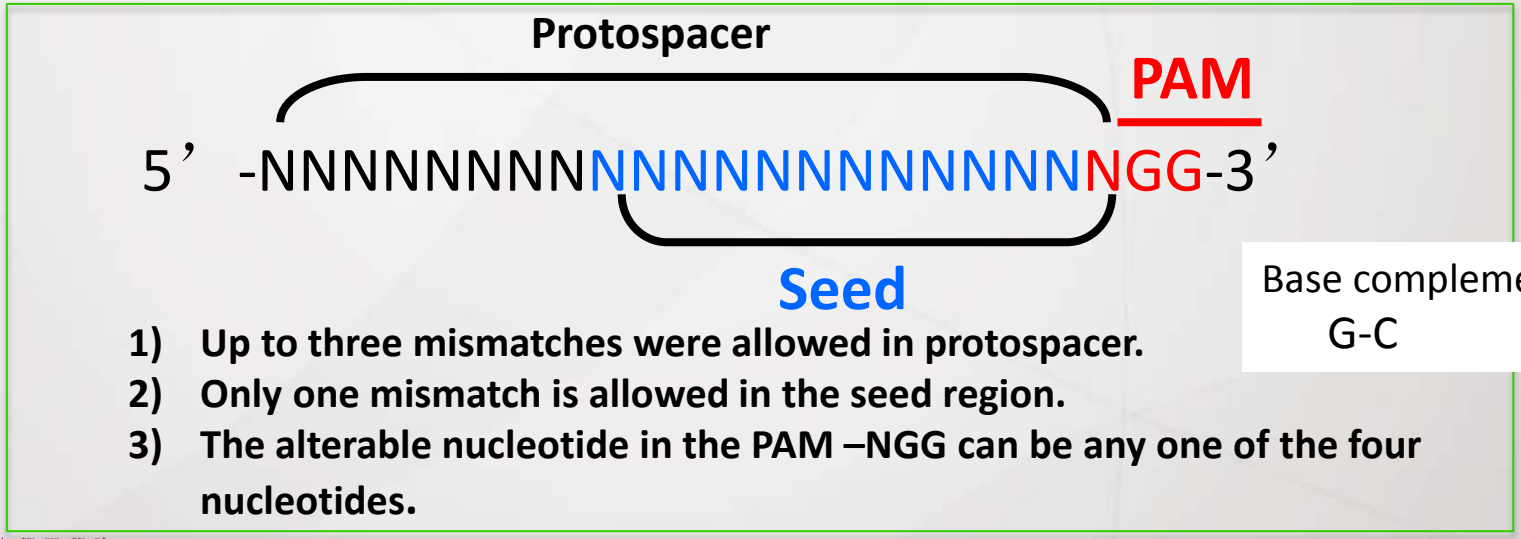
Other ethical issues:

- Somatic versus germline genome editing – change of future gene pool
- Accessibility of the technology
- Consent of unborn child
- Eugenics

Thank You for Your Attention

CRISPR/Cas9 System
derived from *Streptococcus pyogenes*

- CRISPR – bacteria RNA-mediated adaptive defense system that protect organisms from invading viruses and plasmids.
- CRISPR/Cas9 consists of guide RNA fused to CRISPR array and Cas9, a protein component originated form Streptococcus pyogenes.
- The Cas9 enzyme cleaves chromosomal DNA, whose sequence is complementary to guide RNA in a targeted manner, producing site specific DNA double-stranded breaks, the repair of which give rise to targeted genome modification.



1) **Up to three mismatches were allowed in protospacer.**
2) **Only one mismatch is allowed in the seed region.**
3) **The alterable nucleotide in the PAM –NGG can be any one of the four nucleotides.**

Base complementarity:
G-C A-T