GENETIC ENGINEERING OF HUMANS

Tapani Ronni, PhD

Hello!

I am Tapani Ronni

I am here because I love to give scientific presentations.

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About the Speaker

- PhD in Genetics, University of Helsinki, Finland
- Postdoctoral fellow, University of California, Los Angeles
- Scientific interests: gene therapy, microbiology, immunology
- A full time medical translator since 2007 (English-Finnish)

Contents of This Talk

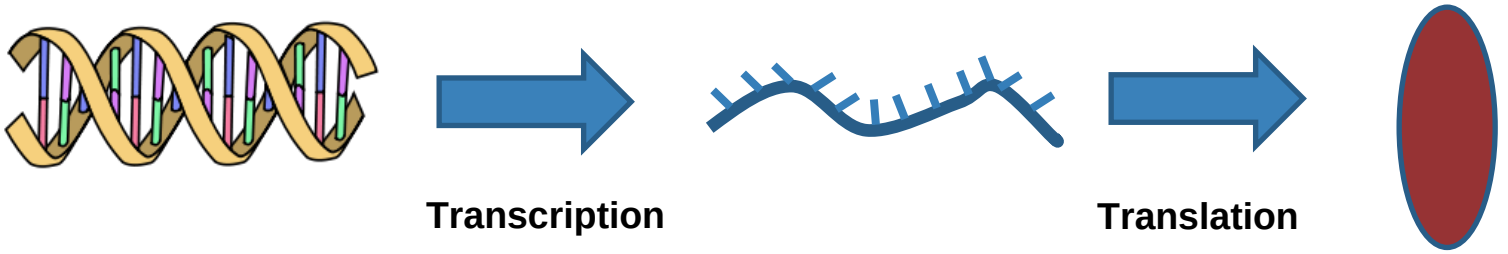
- From gene to RNA to protein
- Gene editing with CRISPR
- Somatic vs. germline gene therapy
- Two CRISPR babies in China
- Where do we go from here?

Genetic Engineering

- Scientific alteration of the structure of genetic materials in a living organism[1]
- First achieved in bacteria and viruses
- Genetic engineering of mammals (mice) started in the 80s
- Genetic engineering of humans?

1 American Heritage Dictionary, 5th Edition

From Gene to RNA to Protein



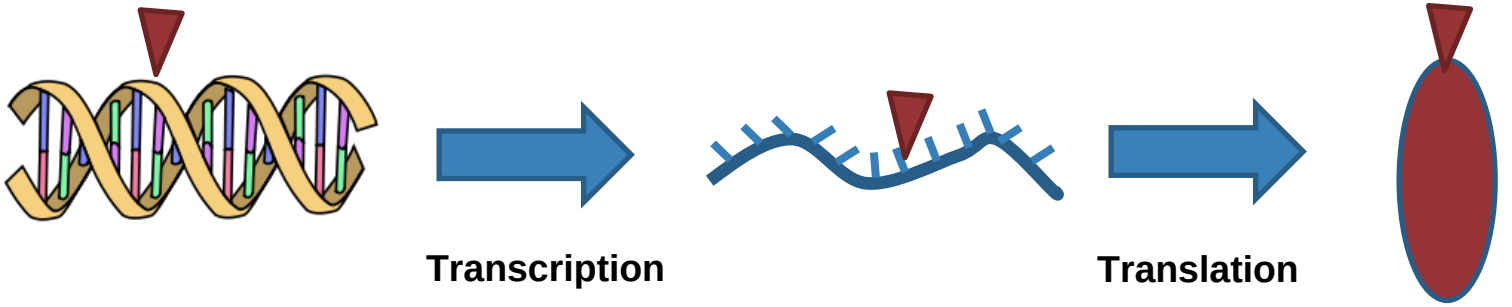
Genomic, double stranded DNA

Single-stranded RNA (*messenger RNA*)

Protein

Public Domain image, https://commons.wikimedia.org/w/index.php?curid=7876890

From Altered Gene to Altered RNA to Altered Protein



Genomic, double stranded DNA

Single-stranded RNA (*messenger RNA*)

Protein

Gene Therapy

- **The insertion of usually genetically altered genes into cells especially to replace defective genes in the treatment of genetic disorders or to provide a specialized disease-fighting condition[1]**

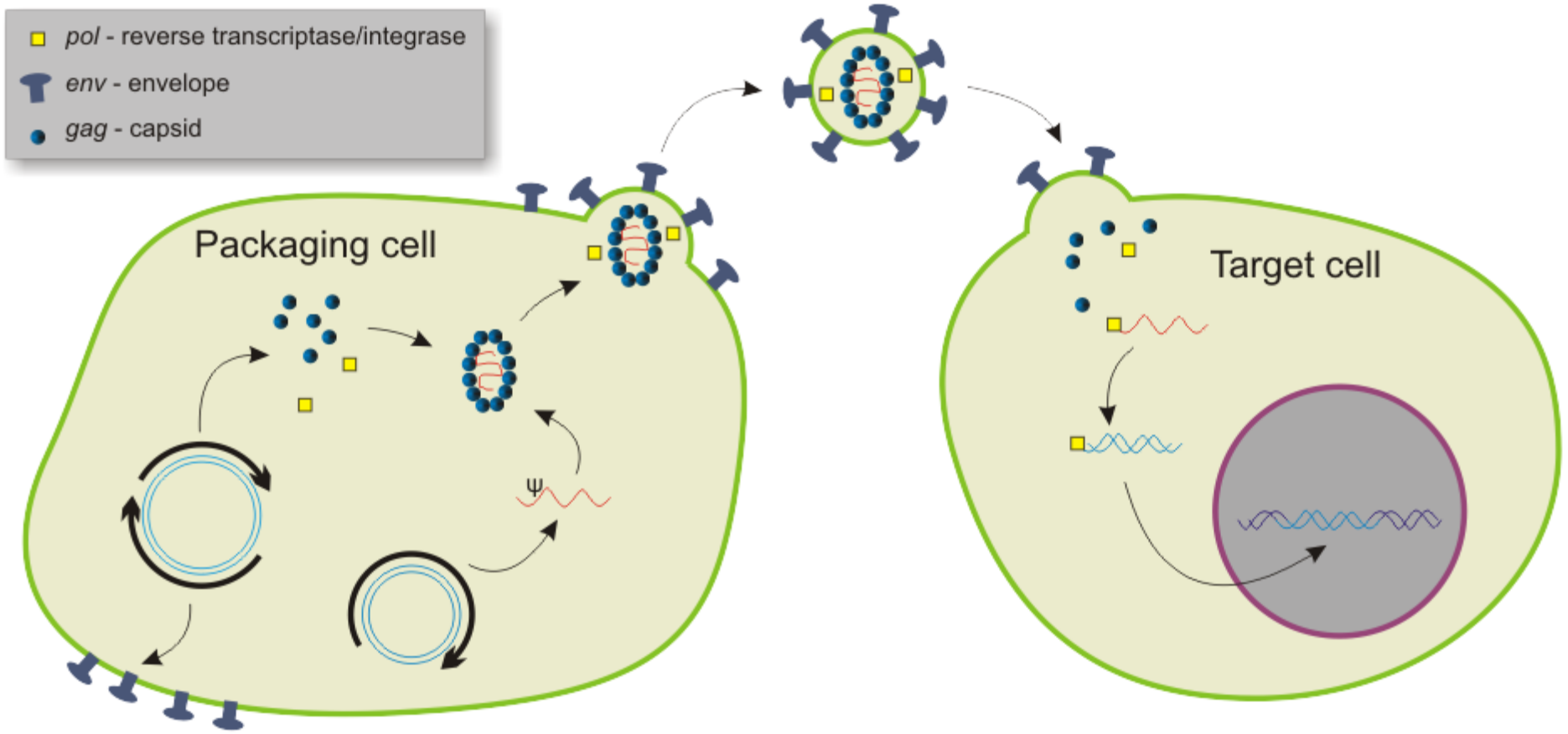
1 Merriam-Webster.com

Gene Therapy Vectors

- **Viral and non-viral (liposomes)**
- **Most promising vectors are lentiviral vectors (related to HIV)**
- **Deleted to be safe (replication deficient)**
- **Able to infect non-dividing cells and dividing cells**

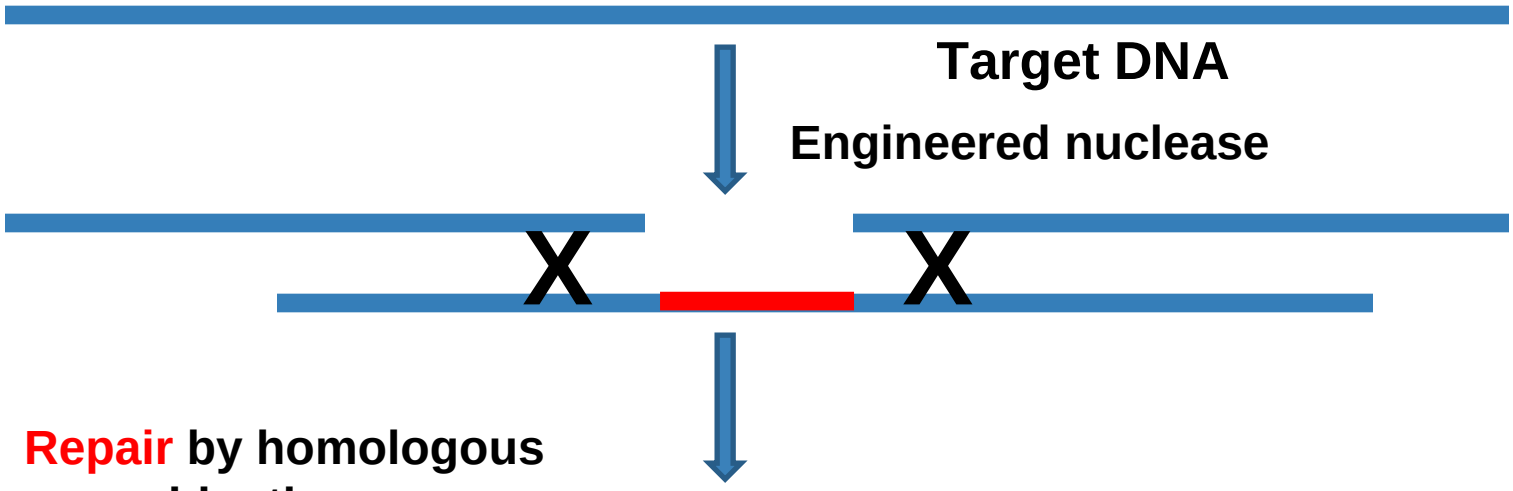
1 Merriam-Webster.com

How Lentiviral Vectors Are Made



Wikipedia Commons image

Gene Editing with CRISPR



Corrected point mutation
(e.g. with Sickle Cell Anemia)

Functional gene correction
(e.g. with SCID-X1)

Safe harbor gene addition
(e.g. with SCID-X1)

Somatic vs. Germline Gene Therapy

- Germline cells = eggs and sperm
- The rest are somatic cells
- Somatic DNA alterations are not inherited
- Germline DNA alterations are

Somatic Gene Therapy

- Already on the market for some conditions
- Usually uses virus vectors to deliver new DNA / CRISPR mutation
- Fairly uncontroversial, safety much improved since the early days
- Treatments are one shot and very expensive

Somatic Gene Therapy

- **BlueBird Pharma got approval in EU for a gene therapy called ZYNTEGLO for beta-thalassemia**
- **Proposed price tag: 1.5 million dollars over 5 years**
- **315,000 euros first payment, next payments only if effective**

Germline Gene Therapy

- Germline gene therapy is currently not allowed for ethical reasons

- A rogue Chinese scientist Dr He Jiankui claimed to have done it anyway last year

Two CRISPR babies in China

- “Lulu” and “Nana” were created with in vitro fertilization and gene editing of the fertilized egg

- Whereabouts unknown, health unknown
- Off-target mutations caused by CRISPR?

- They would be around 1 year old now

Two CRISPR babies in China (cont'd)

- **"Lulu" and "Nana" have a full or partial deletion of CCR5 gene, which:**
  - **May protect from HIV infection later**
  - **May weaken the immune system in other ways**

Two CRISPR babies in China (cont'd)

- After his explosive public talk in Hong Kong, Dr Jiankui lost his job
- Will not talk to press
- Chinese government has not volunteered much information

Two CRISPR babies in China (cont'd)

- Problems in the Informed Consent process
- Problems in Ethics Committee review, if any
- Chinese regulations ignored
- Who knew what and when did they know it?

CRISPR babies in Russia?

Russian biologist Denis Rebrikov also wants to created CCR5 deleted babies if he gets regulatory approval

Also plans to “cure deafness” with gene editing

The Russian Ministry of Health won’t let him proceed for now

Where do we go from here?

- Germ line editing is still illegal
- Lots of ethical questions remain
- International co-operation needed

Deletions are diluted over generations

- **Deletion of a wild type gene (e.g. CCR5del) likely to get diluted out in a few generations**
- **XdelXdel Parent generation is homozygous for deletion: eggs or sperm have always Xdel**
- **F1 generation is heterozygous XdelX (if the other parent is wild type) / phenotype depends on penetrance of the deletion**
- **F2 generation should have 50% XdelX, 50% XX**
- **F3 generation should have 25% XdelX, 75% XX**
  - **(these are statistical expectations)**

Corrections do not dilute

- Correction of a defective gene has a much better chance of being permanent
- Reason: F1 generation gets a corrected gene from the patient and a wild type gene from the spouse, so the F1 has wild type phenotype. Each subsequent generation is also wild type.

The Field Is Moving Fast

ARTICLE

https://doi.org/10.1038/s41586-019-1711-4

Search-and-replace genome editing without double-strand breaks or donor DNA

Andrew V. Anzalone[1,2,3], Peyton B. Randolph[1,2,3], Jessie R. Davis[1,2,3], Alexander A. Sousa[1,2,3], Luke W. Koblan[1,2,3], Jonathan M. Levy[1,2,3], Peter J. Chen[1,2,3], Christopher Wilson[1,2,3], Gregory A. Newby[1,2,3], Aditya Raguram[1,2,3] & David R. Liu[1,2,3]*

Most genetic variants that contribute to disease[1] are challenging to correct efficiently and without excess byproducts[2–5]. Here we describe prime editing, a versatile and precise genome editing method that directly writes new genetic information into a specified DNA site using a catalytically impaired Cas9 fused to an engineered reverse transcriptase, programmed with a prime editing guide RNA (pegRNA) that both specifies the target site and encodes the desired edit. We performed more than 175 edits in human cells including targeted insertions, deletions, and all 12 types of point mutation without requiring double-strand breaks or donor DNA templates. We applied prime editing in human cells to correct efficiently and with few byproducts the primary genetic causes of sickle cell disease (requiring a transversion in *HBB*) and Tay-Sachs disease (requiring a deletion in *HEXA*), to install a protective transversion in *PRNP*, and to insert various tags and epitopes precisely into target loci. Four human cell lines and primary post-mitotic mouse cortical neurons support prime editing with varying efficiencies. Prime editing offers efficiency and product purity advantages over homology-directed repair, complementary strengths and weaknesses compared to base editing, and much lower off-target editing than Cas9 nuclease at known Cas9 off-target sites. Prime editing substantially expands the scope and capabilities of genome editing, and in principle could correct about 89% of known pathogenic human genetic variants.

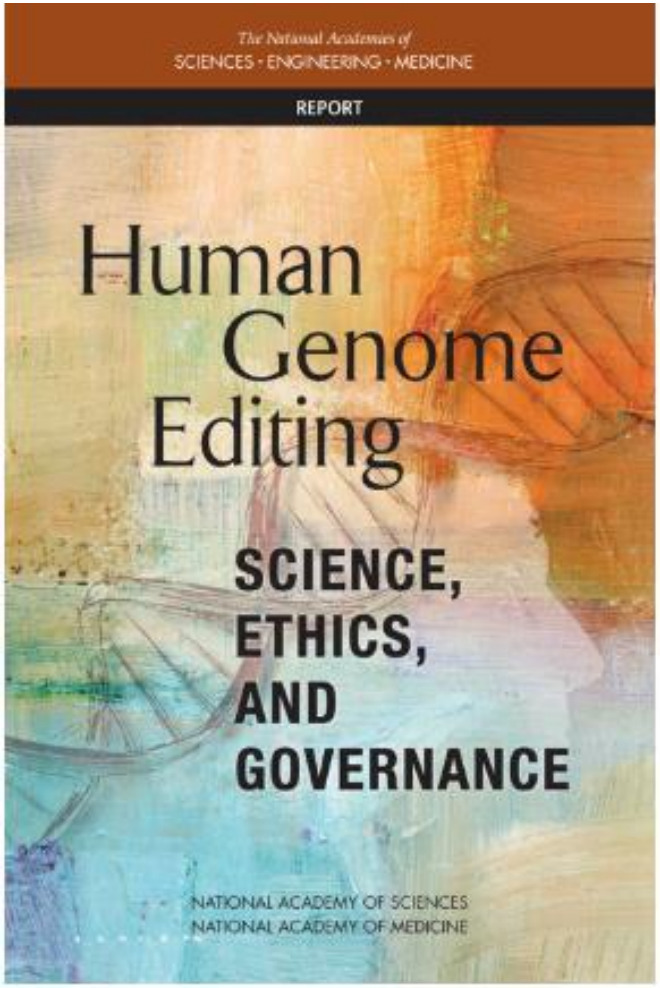
The ability to make virtually any targeted change in the genome of any living cell or organism is a longstanding aspiration of the life sciences. Despite rapid advances in genome editing technologies, the majority of the >75,000 known human genetic variants associated with diseases[1] remain difficult to correct or install in most therapeutically relevant cell types (Fig. 1a). Programmable nucleases such as CRISPR-Cas9 make double-strand DNA breaks (DSBs) that can disrupt genes by inducing mixtures of insertions and deletions (indels) at target sites[2–4]. DSBs, however, are associated with undesired outcomes including complex mixtures of products, translocations[5], and p53 activation[6,7]. Moreover, the vast majority of pathogenic alleles arise from specific insertions, deletions, or base substitutions that require more precise editing tech-

that causes Tay-Sachs disease (*HEXA* 1278+TATC), or targeted insertions, such as the 3-base insertion required to directly correct the most common cause of cystic fibrosis (*CFTR* ΔF508). Targeted transversions, insertions, and deletions thus are difficult to install or correct efficiently and without excess byproducts in most cell types, even though they collectively account for most known pathogenic alleles (Fig. 1a).

Here we describe the development of prime editing, a "search-and-replace" genome editing technology that mediates targeted insertions, deletions, all 12 possible base-to-base conversions, and combinations thereof in human cells without requiring DSBs or donor DNA templates. Prime editors (PEs), initially exemplified by PE1, use a reverse transcriptase (RT) fused to an RNA-programmable nickase and a prime

Ethical and Legal Issues

Human Genome Editing Report



Consent for Gene Therapy

- Informed consent: communication between the patient and physician where the patient **consents** to a clinical trial or procedure
- Used in all clinical trials, including in **somatic gene therapy** trials
- Unborn cannot give consent in **germline gene therapy**
- Can a parent give it?

Where do we go from here?

- **Human Genome Editing report recommendations:**
- **Somatic gene therapy only for treatment or prevention of diseases**
- **Germline gene therapy requires serious discussion, possible future uses if safety and efficacy can be worked out**
  - **Currently illegal in USA**

Alternatives

- In vitro fertilization and genetic testing of the resulting embryos on a petri dish
- Only implant a healthy embryo

Human Enhancement

- **Human Genome Editing report recommendations:**
- **Should not be done at all right now**
- **Encourage public discussion and policy debate with respect to somatic human genome editing for other uses than treatment or prevention of disease**

Human Enhancement (cont'd)

Do we want to have a society of genetic haves and have-nots?

Where do we go from here?

- Where do you stand?
- Theology / philosophy / ethics
- Liability issues of multi-generational clinical trials?

References

◎**Anzalone AV et al. Search-and-replace genome editing without double-strand breaks or donor DNA. Nature. 2019 Oct 21; https://doi.org/10.1038/s41586-019-1711-4**

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◎**Cyranoski, D. Russian Scientist Plans More CRISP-edited Babies. Nature. 2019 Jun;570(7760):145-146. doi: 10.1038/d41586-019-01770-x.**

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Thank you!

Questions? Comments?

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