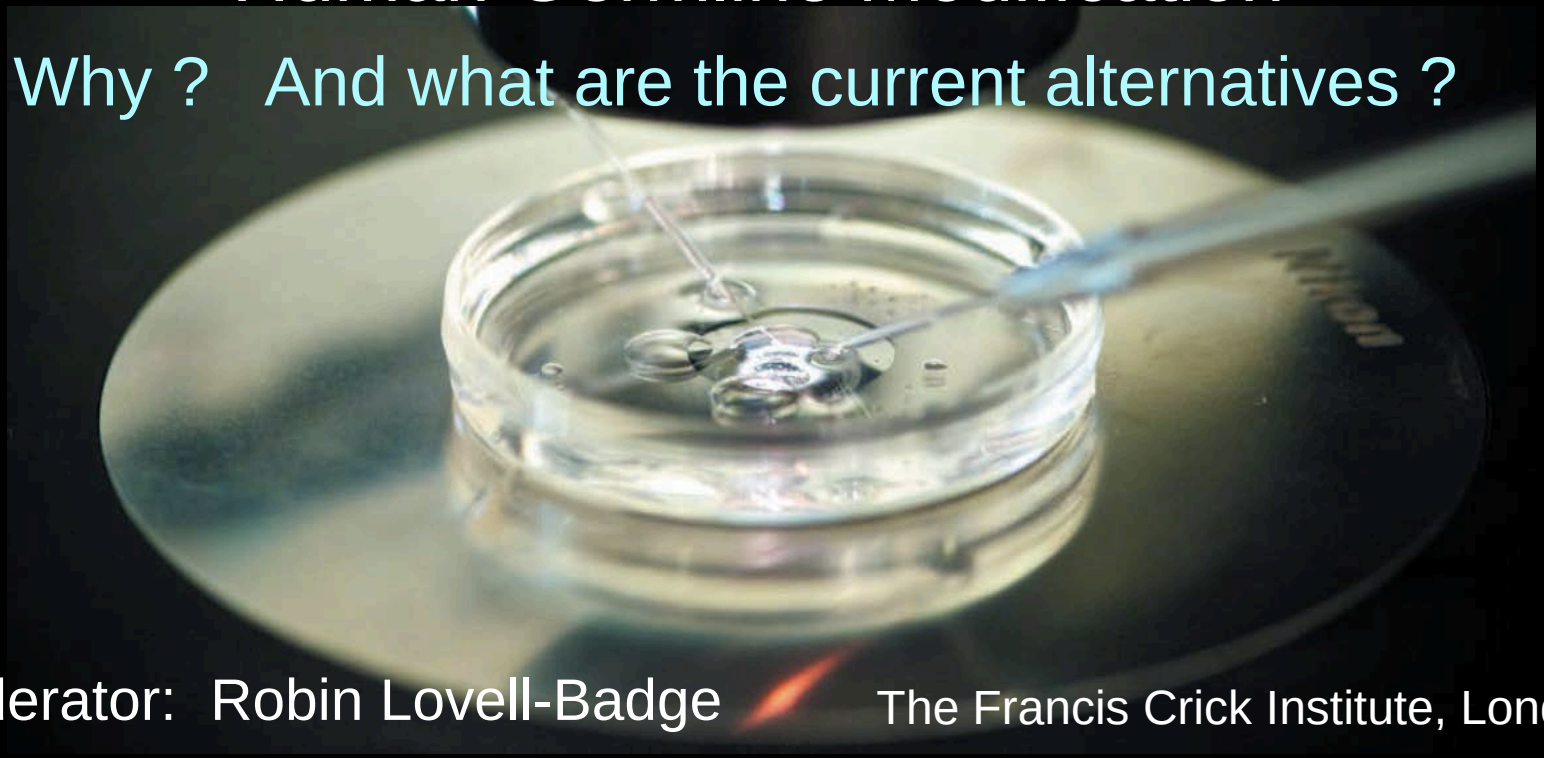
Applications of Gene Editing Technology: Human Germline Modification



Moderator: Robin Lovell-Badge — The Francis Crick Institute, London, UK

Speakers:	Peter Braude	King's College, London, UK
	George Q. Daley	Boston Children's Hospital, USA
	Kyle Orwig	University of Pittsburg, USA
Discussants:	George Church	Harvard Medical School, USA
	Azim Surani	The Gurdon Institute, Cambridge, UK

Why this debate now ?

- CRISPR/Cas9: Simple to make components: guide RNAs, Cas9, and DNA templates
- Relatively simple to introduce these into cells and early embryos.
- Highly specific (off-target events ?)
- Highly efficient (mosaicism ?)
- Ability to “multiplex”
- Versatile:

  - Alterations to DNA: including “indels” and deletions, insertions or substitutions from single base-pairs up to many kilobases.
  - Cas9 DNAse activity can be mutated and other proteins linked to it: including transcriptional activators or repressors or chromatin modifiers, to manipulate specific gene activity without altering DNA.

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Some potential reasons for genome editing human cells, including those of the germ line and early embryos

1. Basic understanding of human biology: the role of specific genes and processes.

2. To create and study models of human genetic disease in vitro.

3. To treat disease (somatic cells).

4. Germline changes to avoid/prevent genetic disease.

5. Germline alterations to give “genetic enhancement”.

Experiments in vitro to provide understanding of human biology

- To study the role and mechanism of action of specific genes or gene pathways.
- To understand specific processes, such as cell-cell interactions, cell movement, cell lineages and how these are specified, etc.
- The use of stem cells in vitro to screen for molecules that can either influence these processes in a beneficial way, or which are harmful.

Experiments in vitro to understand human biology

Such work already takes place with a variety of human cell culture systems in vitro, for example:

- Organ-specific stem cells, e.g. neural stem cells, gut stem cells.
- Embryonic Stem (ES) cells and induced pluripotent stem (iPS) cells, which can be differentiated in vitro to:
    - Complex tissues:

      cortical brain structures, optic cups, pituitaries, kidney-like structures, etc.
    - Specific cell types:

      neurons, primordial germ cells, etc.

Why not use the techniques to study preimplantation embryos and other germline cells ……

Research	Possible applications
1. Better understanding of the biology of early human embryos, including how cell types are specified in the early human embryo, and of the genes involved.	1. Improved techniques for culturing embryos following IVF, better implantation rates, fewer miscarriages.
2. The ability to derive and study stem-cell lines representing cell lineages thought to exist in the early human embryo; including progenitors of the placenta and yolk sac.	2. Improved ability to establish stem-cell lines for research, screens drugs for embryo/placenta toxicity or beneficial effects to prevent miscarriage. Reduction in embryos needed for research.
3. Better understanding of the role of specific genes in human germ-cell development, including the differentiation of sperm and eggs.	3. Fertility enhancement and the development of novel contraceptives.
4. Genome editing techniques.	4. Improved efficiency and versatility of genome editing in early embryos and germline cells. Knowledge relevant as to whether and how the techniques could be applied for clinical applications.

Stages at which genome editing could be used to modify the human germline

- At fertilisation: coincident with intracytoplasmic sperm injection (ICSI)
- In zygotes: injection into the cytoplasm of 1-cell fertilised eggs.
- 2-cell to blastocyst stage embryos: likely to give mosaics, unless have an efficient delivery method, such as viral vectors
- Postimplantation stages: In theory, a viral vector could be used to infect germ cells in the embryonic gonads.
- Postnatally: (a) maturing eggs in the ovary. Probably inefficient.
  (b) spermatogonial stem cells: in vitro or in vivo
- Via induced-pluripotent stem cells and in vitro-derived gametes

To avoid/prevent/treat genetic disease

Through correcting genetic defects in early embryos, or via germline cells, hopefully with beneficial consequences for the child born and subsequent generations. For example:

- Correcting infertility due to Y chromosome defects.
- Correcting dominant mutations (leading to congenital or late onset disease).
- Correcting recessive mutations (including where loss of heterozygosity of a tumor suppressor gene in somatic cells is likely to lead to cancer).
- Altering an allele associated with disease risk to one that is protective.

Genetic enhancement:

(i) Disease resistance: infectious disease, cancer

(ii) Diet: Tolerance to lactose, gluten, etc. Ability to obtain nutritional benefit from plants or parts of plants that we can't currently digest.

(iii) "Human traits":

- Physical: muscle mass or type, height, appearance/cosmetic
- Specific characteristics, such as perfect pitch
- Longevity
- Intelligence

(iv) "Non-human traits":

- Trivial (e.g. GFP)
- Sensory systems (ultraviolet, infrared, electromagnetic fields) ?
- Tolerance to drought, heat, or cold ?
- Synthetic genes ?

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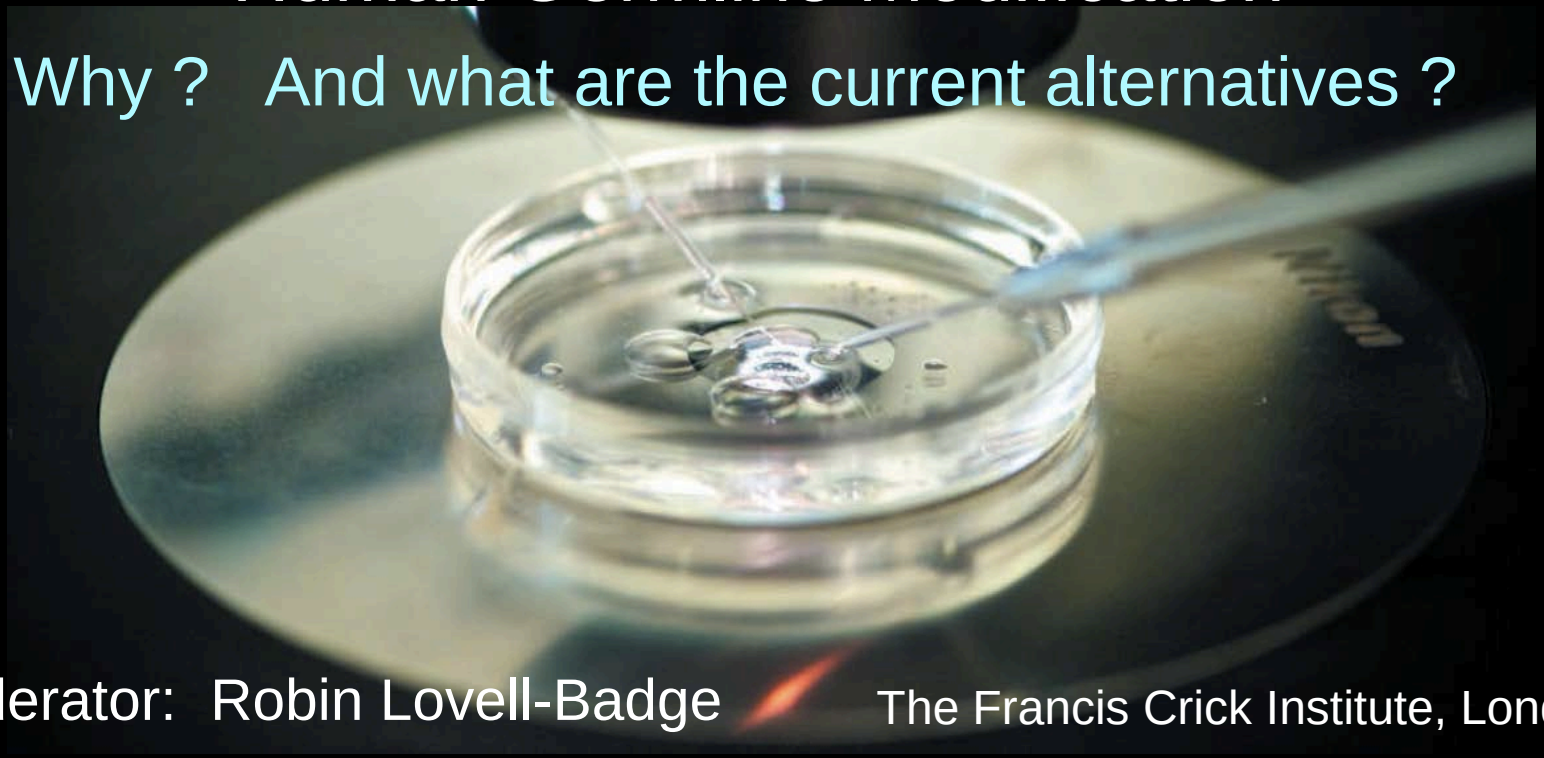

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