# The unknowns of early human development and the impact on genome editing

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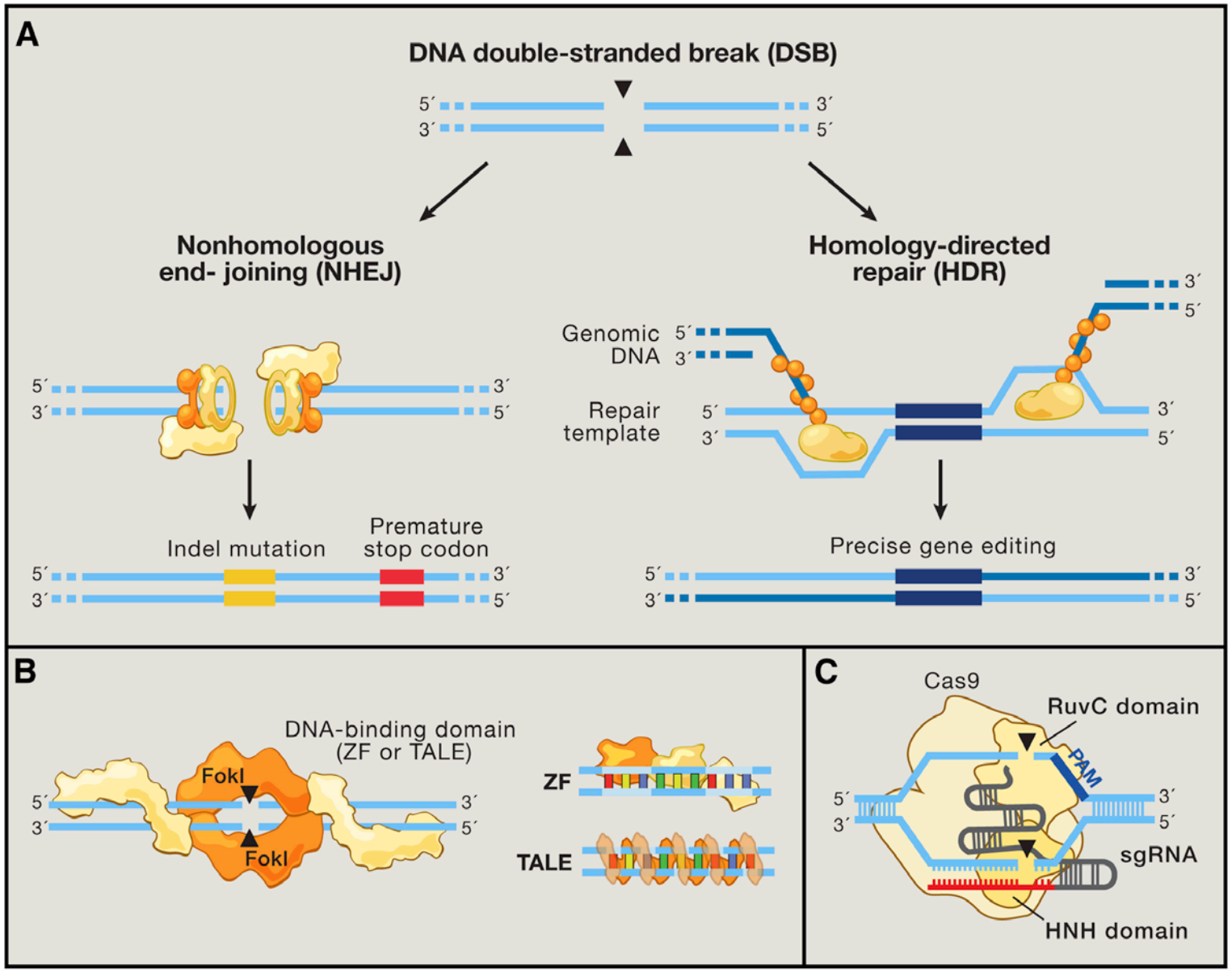
# Efficient genome editing of human embryos depends on an understanding of:

1. Cell cycle
2. Chromosome segregation
3. DNA-damage repair

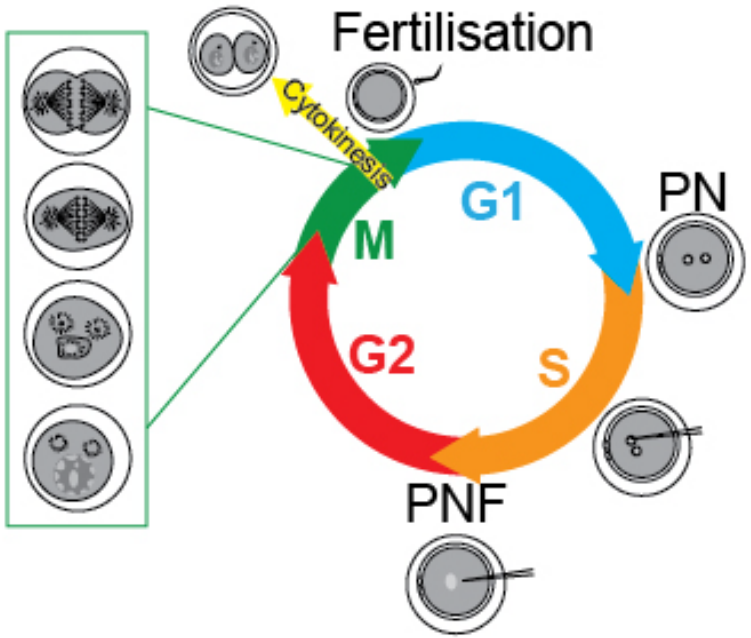
# Methods of genome editing

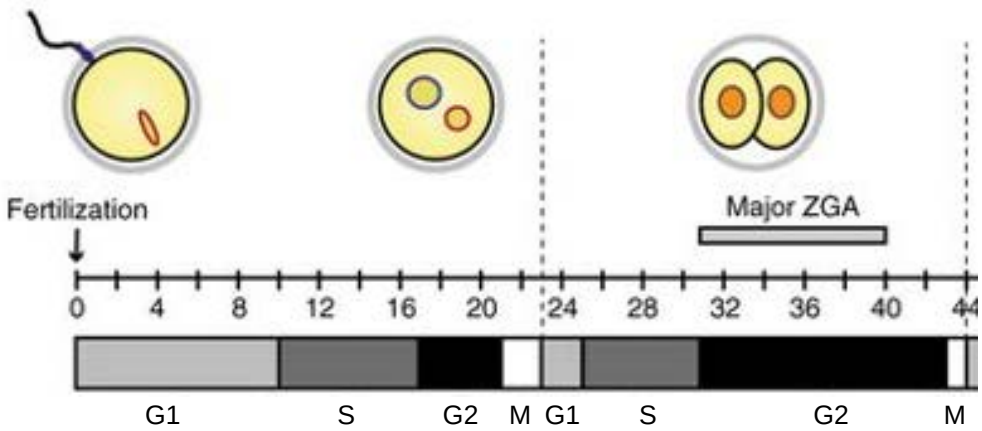



Hsu et al., *Cell* 2014

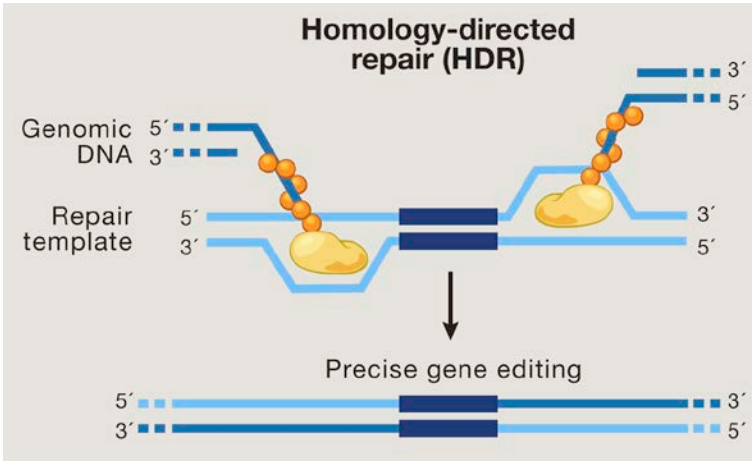
# DSB repair pathway choice depends on cell cycle

G1 phase



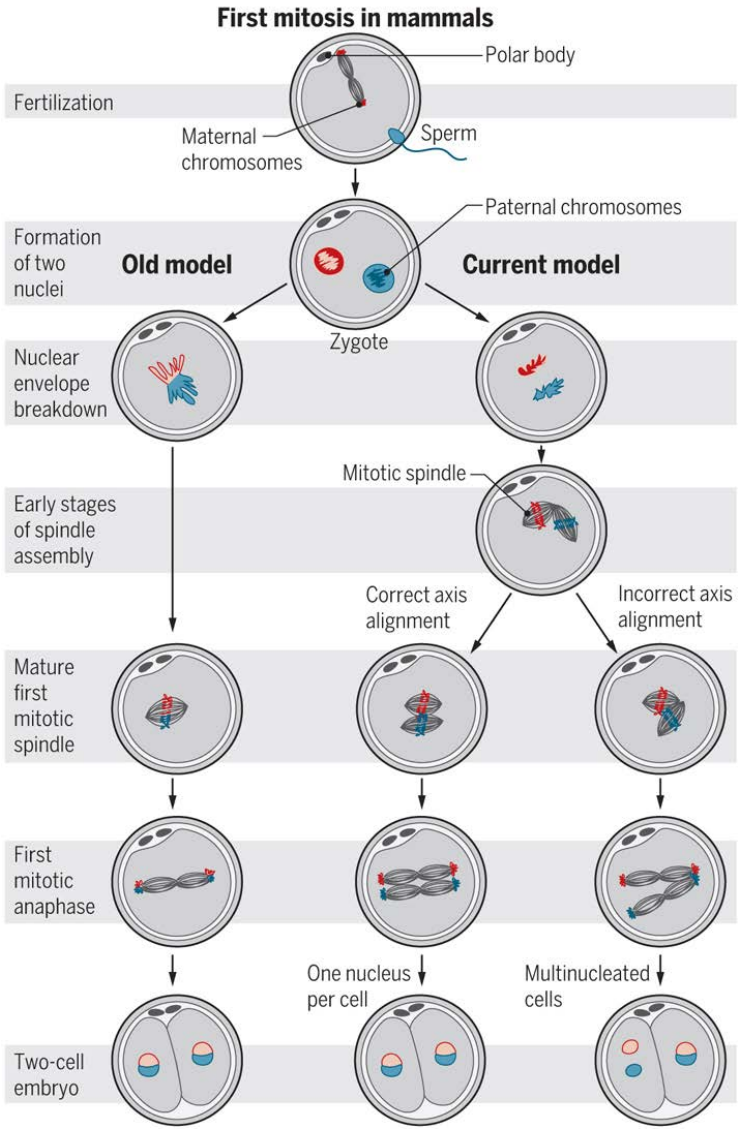

G2/S phase

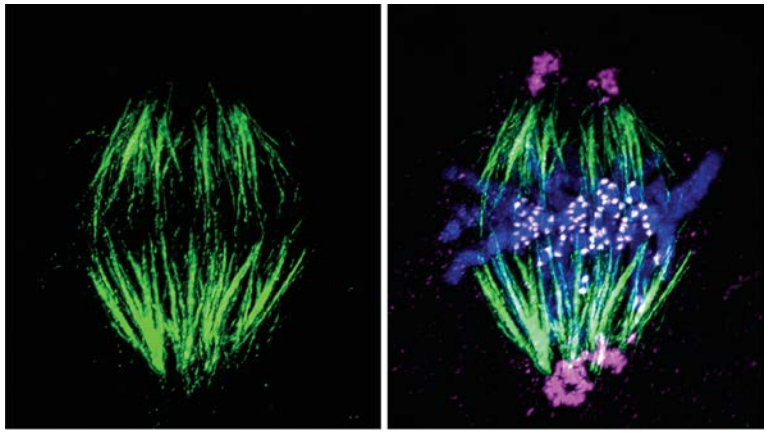


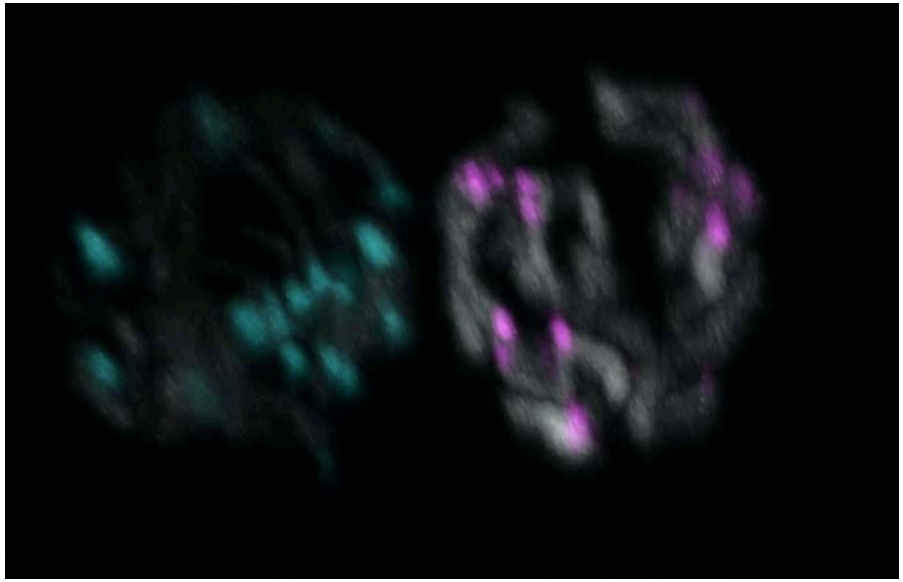

Hsu *et al., Cell* 2014

Ma *et al.. Nature Biotech* 2018

# Parental genomes are kept apart at the first cell division(s) by dual spindle formation



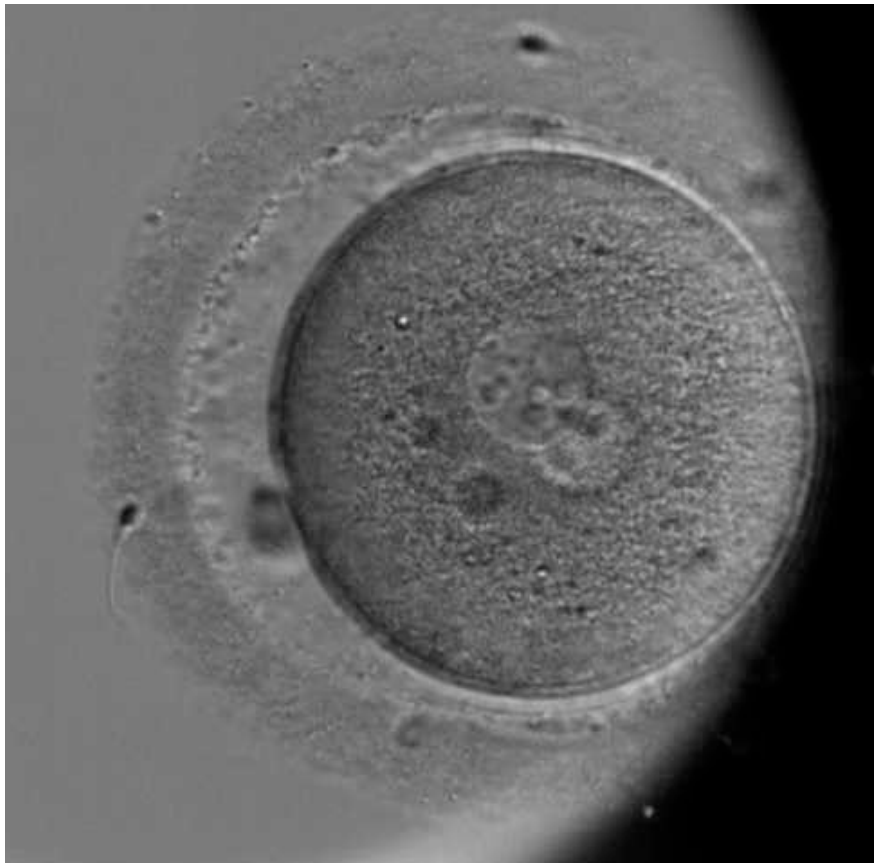





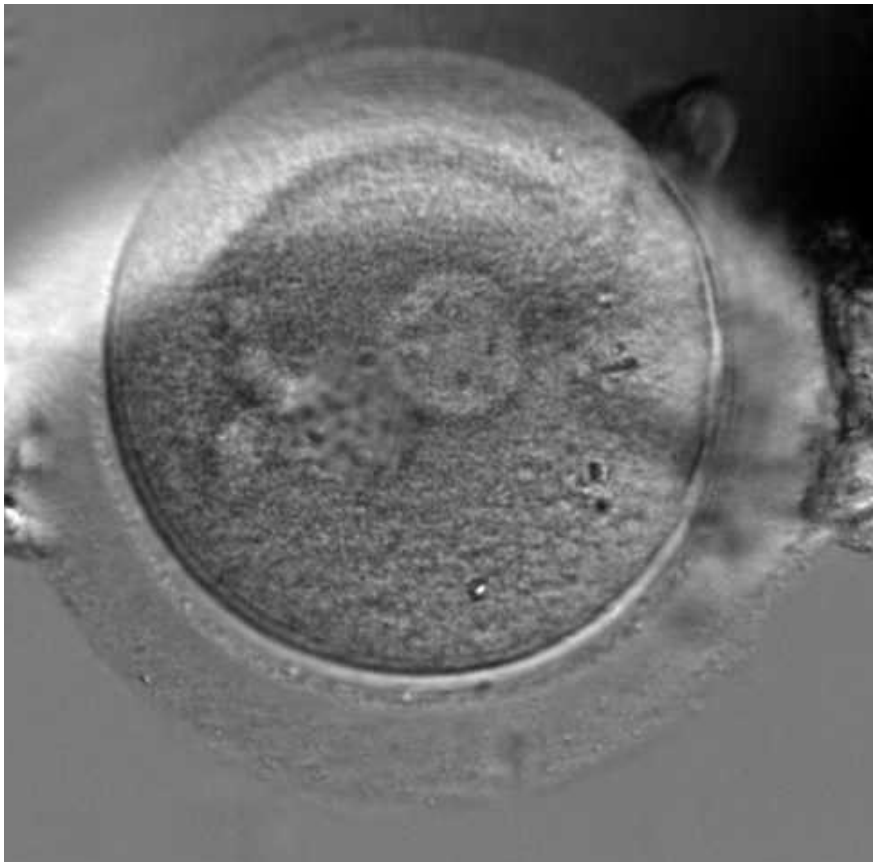
Reichmann et al., *Science* 2018
Zielinska and Schuh, *Science* 2018

# Spindle abnormalities in early human embryos

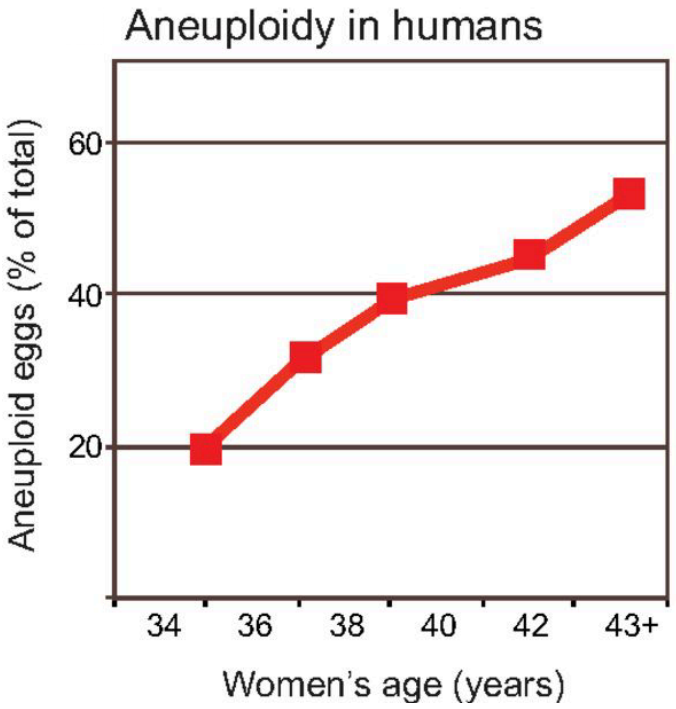
Bipolar spindle formation



Multipolar spindle formation



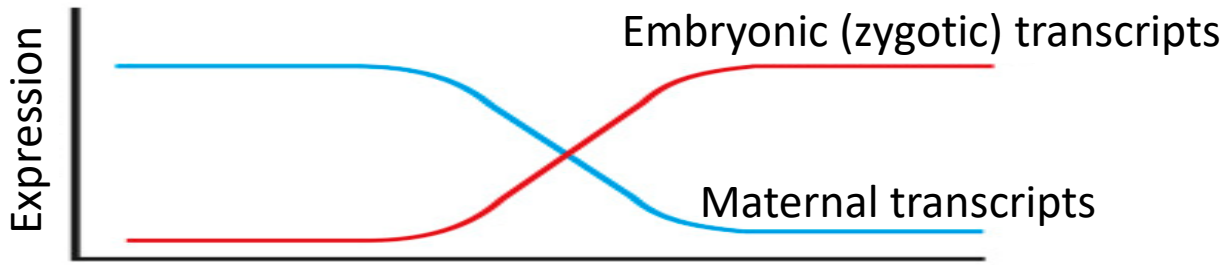
# Human embryos exhibit high rates of aneuploidy




Jones and Lane, *Development* 2013

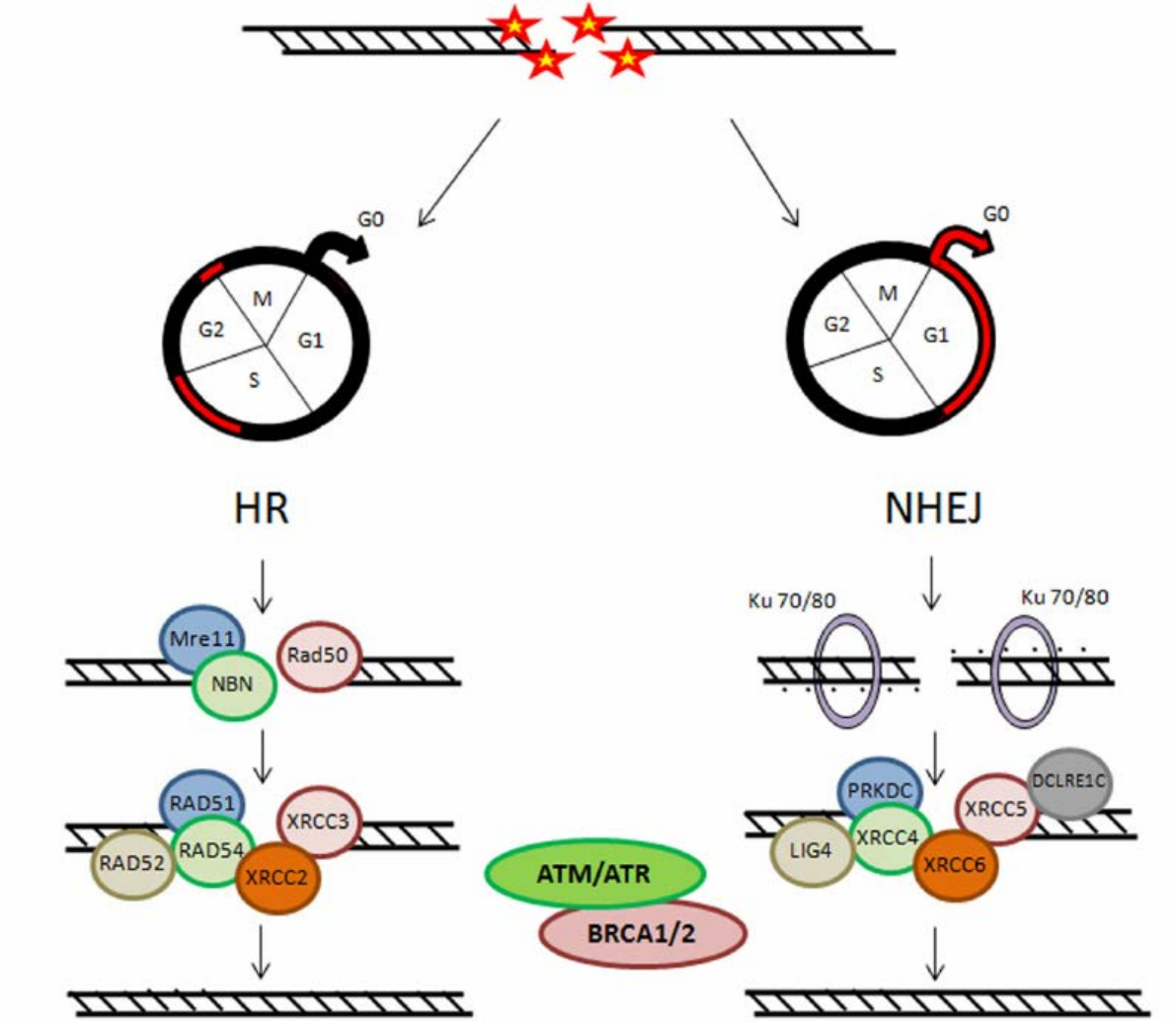
# Consequence of the timing of embryo genome activation

Embryo genome activation



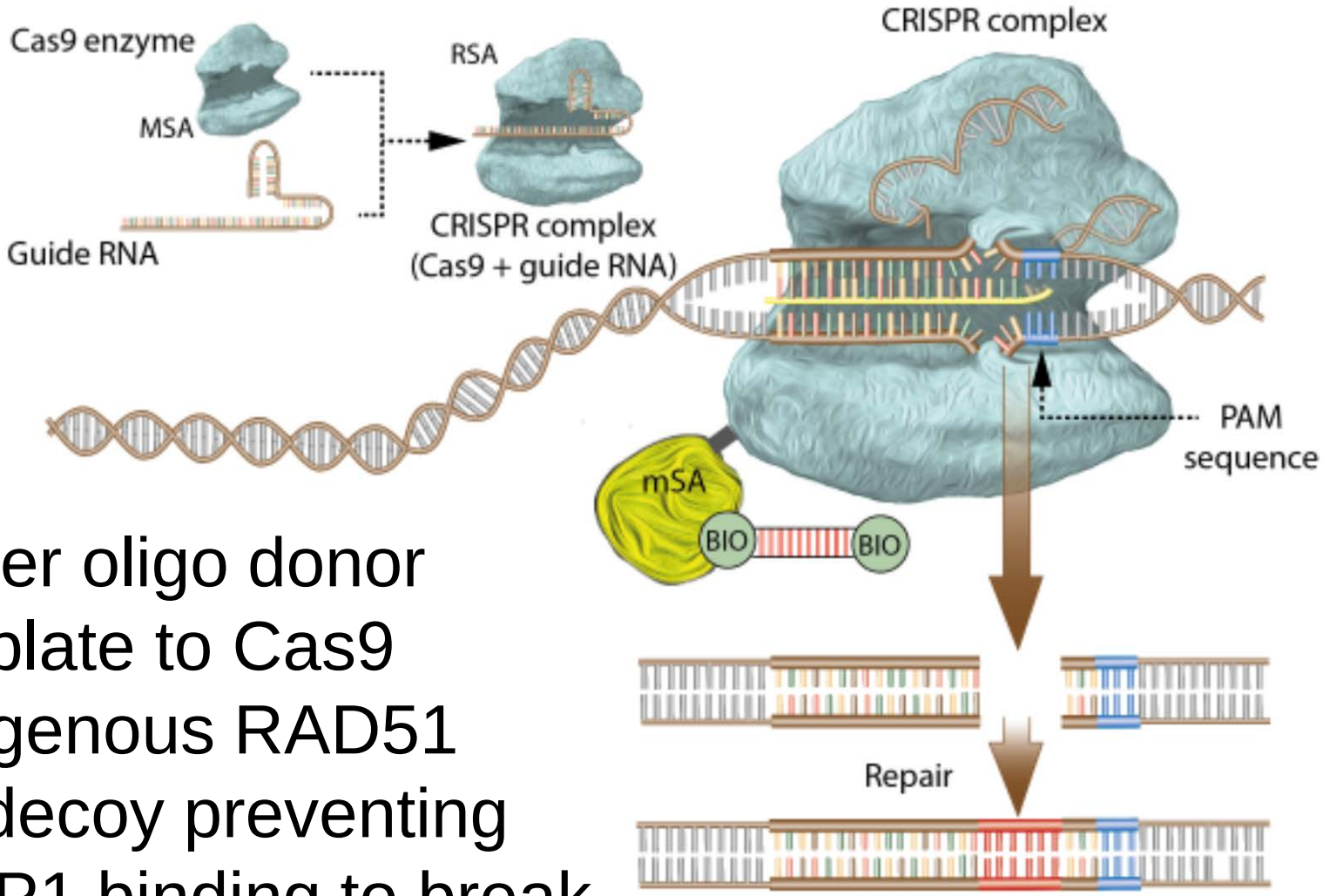

Treen *et al., Current Biology* 2018
Niakan *et al., Development* 2012

# Unclear which DDR factors are present in early human embryos



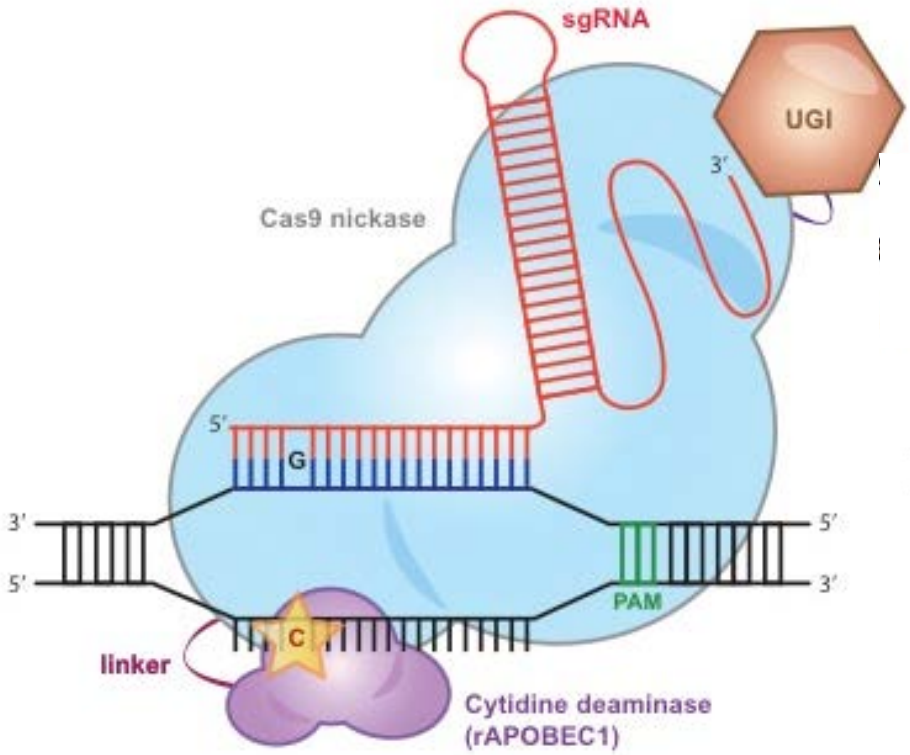

Minafra and Bravata, *Trans. Cancer Research* 2014

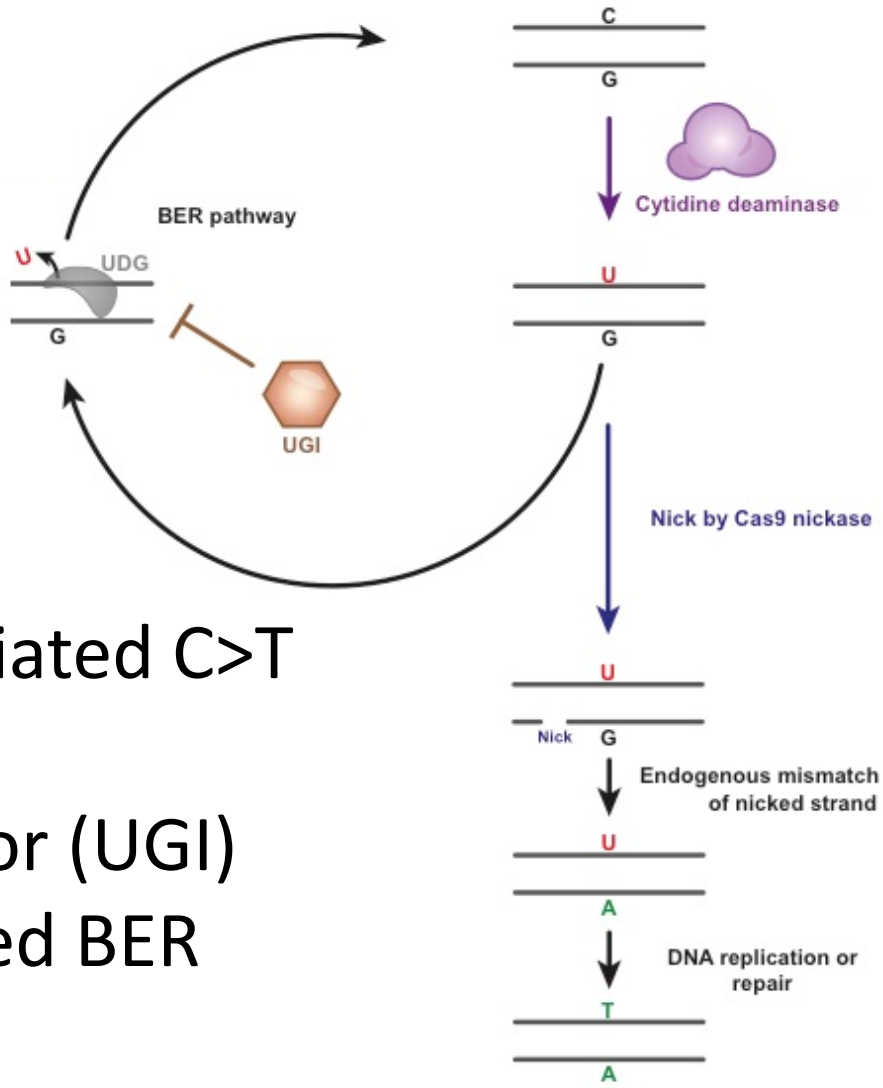
# Promoting homologous repair




- Tether oligo donor template to Cas9
- Exogenous RAD51
- i53 decoy preventing 53BP1 binding to break sites

# Base editing



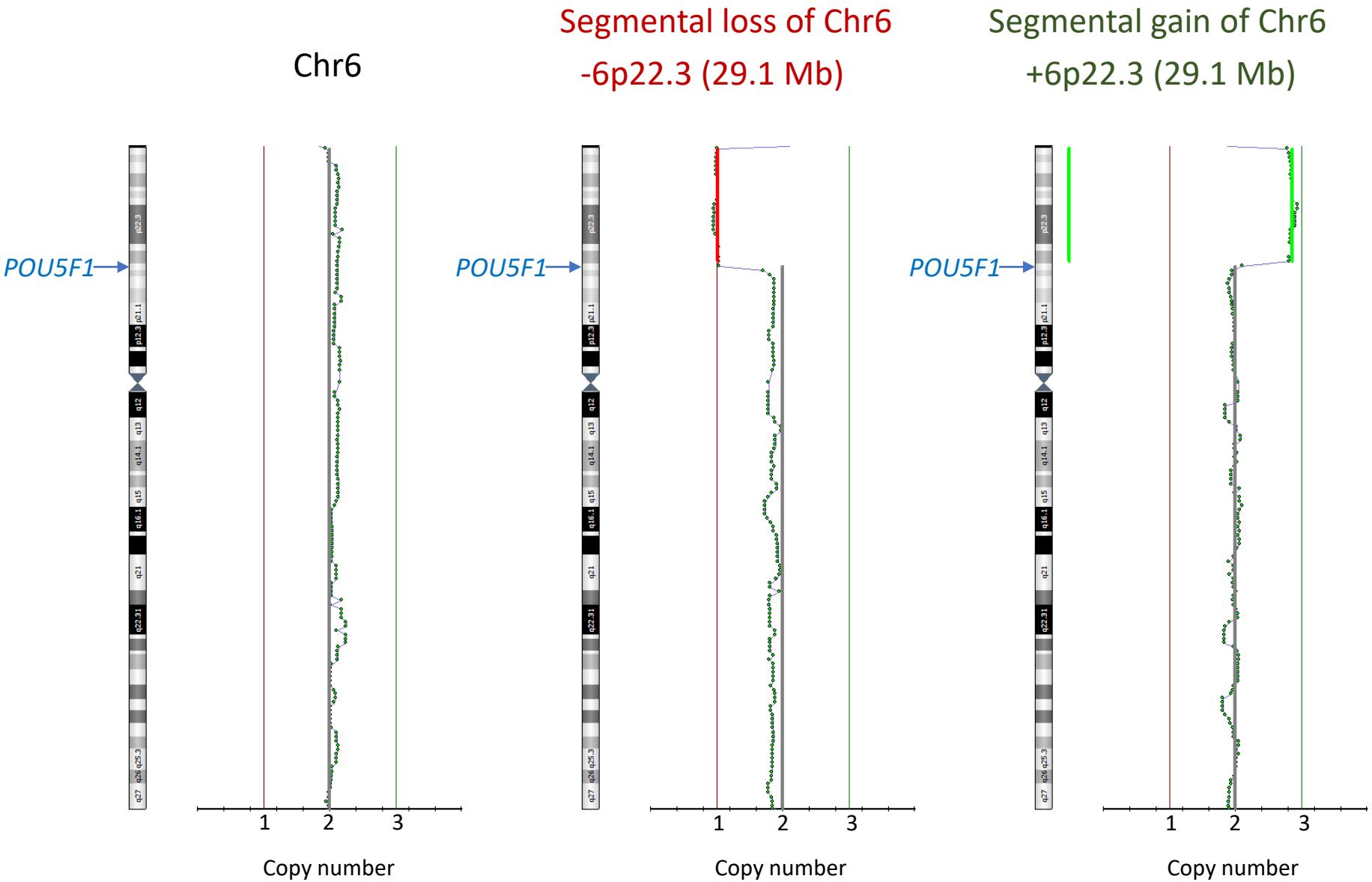

- Cytidine deaminase mediated C>T (G>A)
- Uracil glycosylase inhibitor (UGI) inhibits the UDG-mediated BER pathway
- Cas9 nickase promotes mismatch repair

# HFEA – Scientific and Clinical Advances Advisory Committee, Horizon Scanning Panel and the Ethics and Law Advisory Group

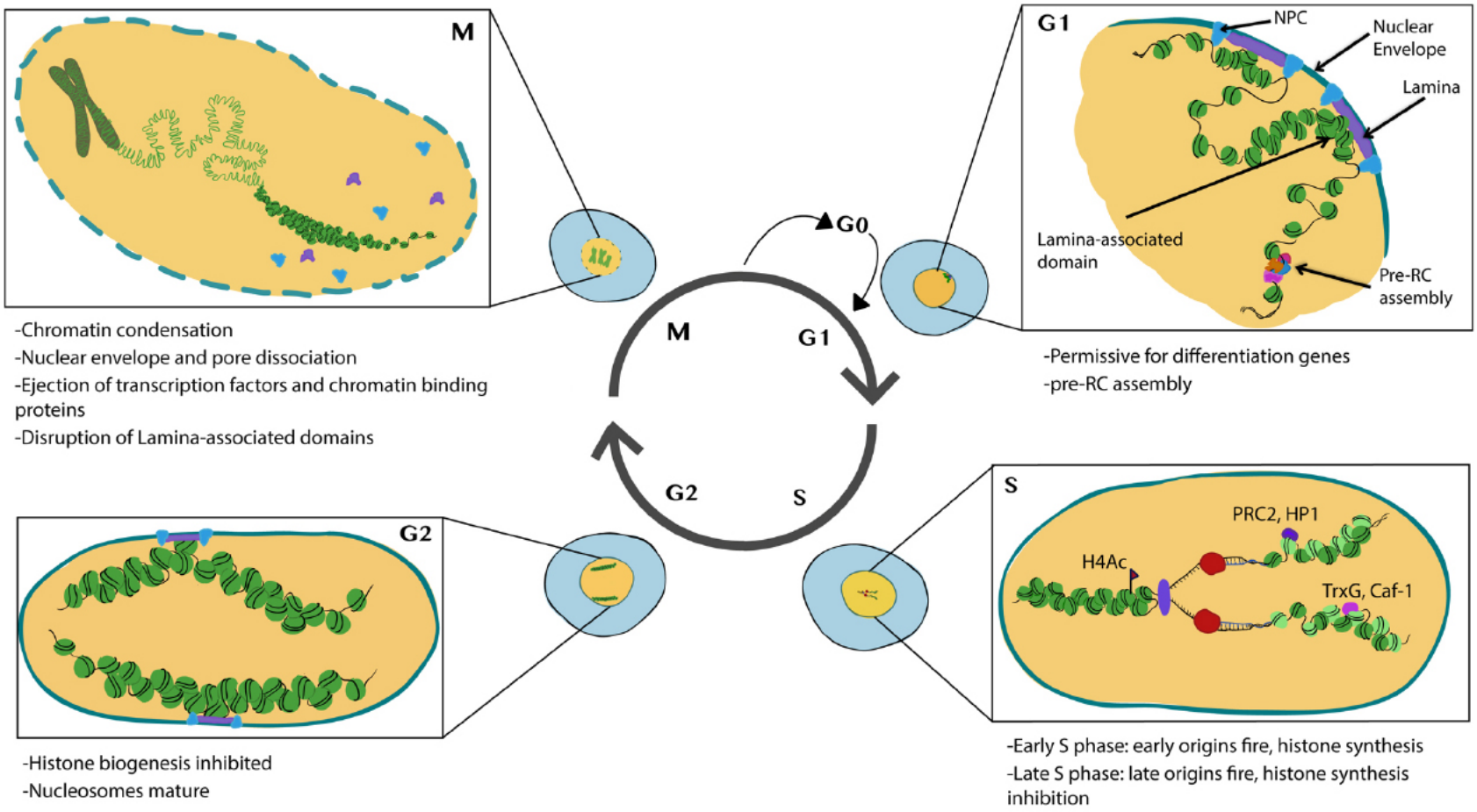
1. Research into human embryo development – specifically the roles of genes and growth factors involved in early development
2. The development of more objective criteria for embryo selection – by investigating gene function in early embryogenesis
3. Research into the genetic background of adverse medical conditions – genetically modified ES cells could be create to model medical conditions
4. Research into the fate of cells during embryo development
5. Introducing a gene to increase the efficiency of stem cell derivation

"Genetic modification of embryos: information for Research Licence Committee" paper SCAAC(01/09)04 January 27th 2009

# Segmental loss or gain of Chr6 in human embryos following CRISPR/Cas9 genome editing

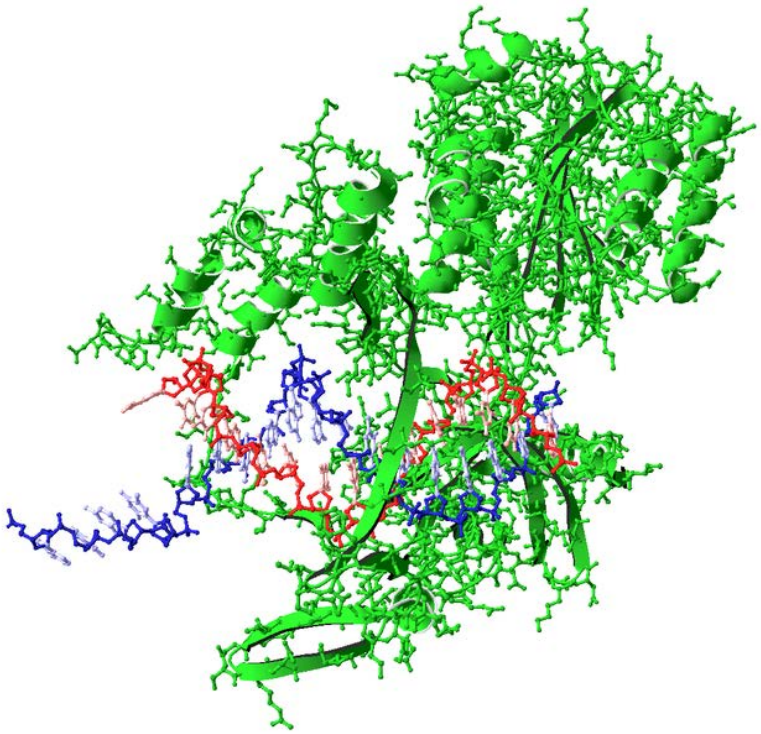



*Unpublished*



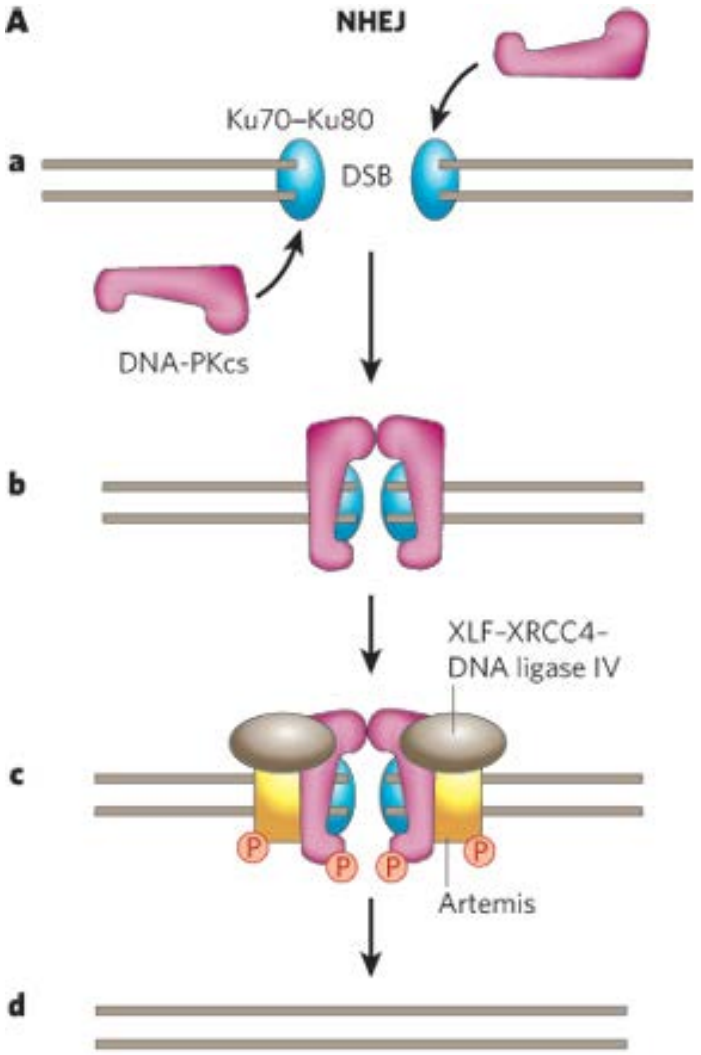

Ma et al., *Front. Genet.* 2015

# Non-homologous end joining (NHEJ)

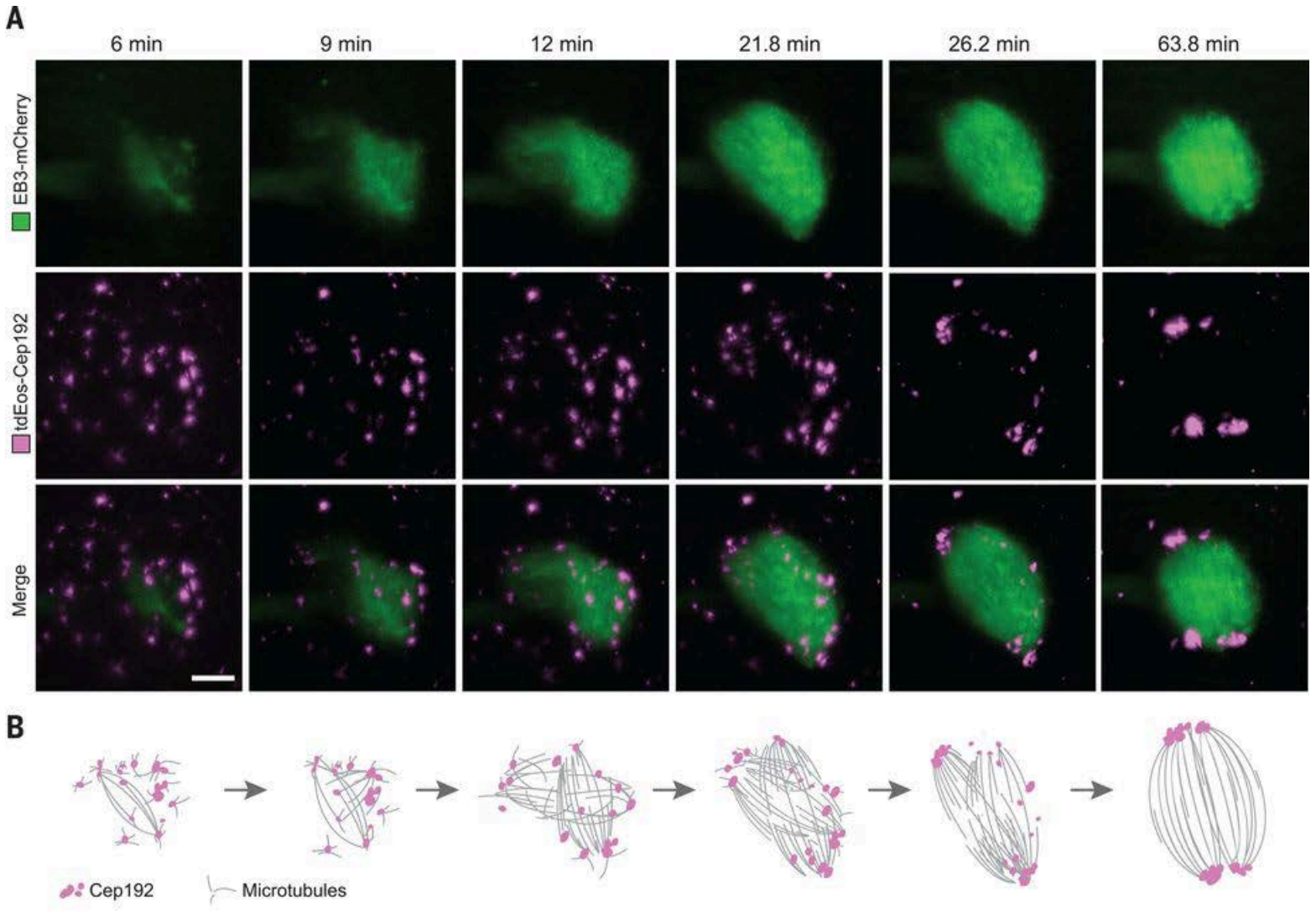


Ku70/Ku80 heterodimer

- Homology independent
- RAD51-independent
- Error prone



# Individual bipolar spindle formation around each pronucleus




Judith Reichmann et al. Science 2018;361:189-193