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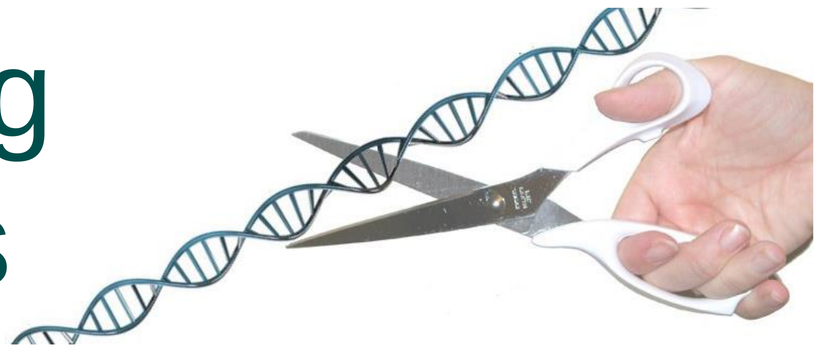
Genome editing in human cells



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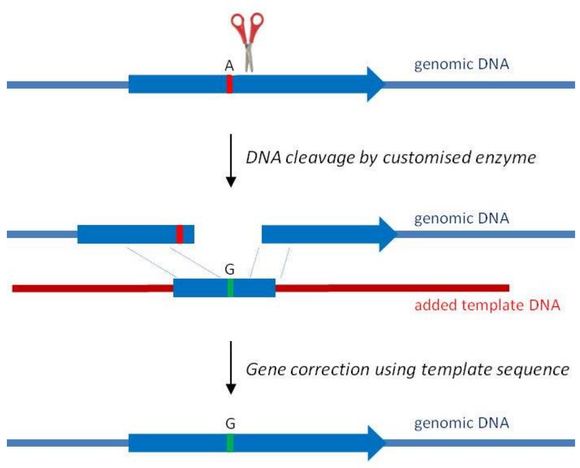
Correcting genetic mutations in human cells

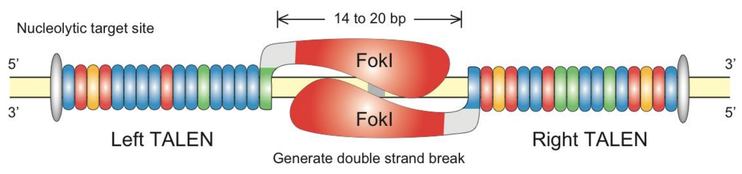
Many genetic diseases are caused by the mutation of a single DNA base in a particular gene.

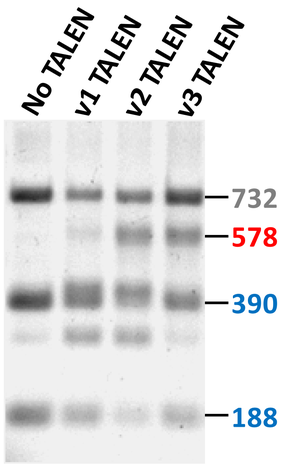
Genome editing uses a customised enzyme that cuts DNA at a single site in the genome, close to the site of the mutation.

The natural repair processes inside the cell then repair the damaged DNA, and if an additional DNA 'template' is added to the cell, the repair processes will often replace a short piece of the cell's original DNA with the new sequence from the template.



TALEN proteins can be designed to bind and cut virtually any sequence in the genome



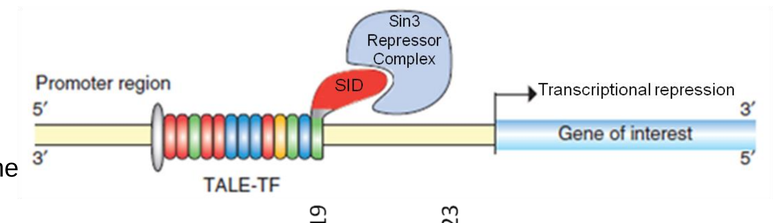


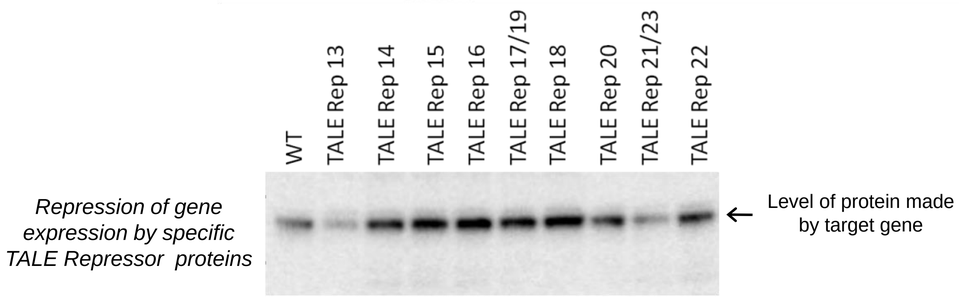
We have optimised versions of TALENs (v2, v3) with improved cutting efficiency and improved specificity

Genome editing resulting from TALEN activity is shown by increased amounts of the red 578 band.

Version 2 and 3 TALENs have greatly increased performance compared to standard TALENs

We have also created TALE repressor proteins that can turn off the expression of a target gene



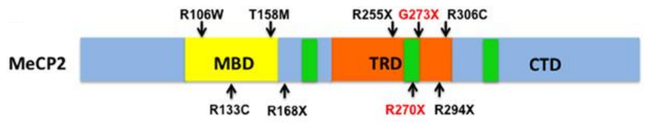


Repression of gene expression by specific TALE Repressor proteins

Genome editing to correct Rett syndrome mutations

Picture from Rett Syndrome Research Trust

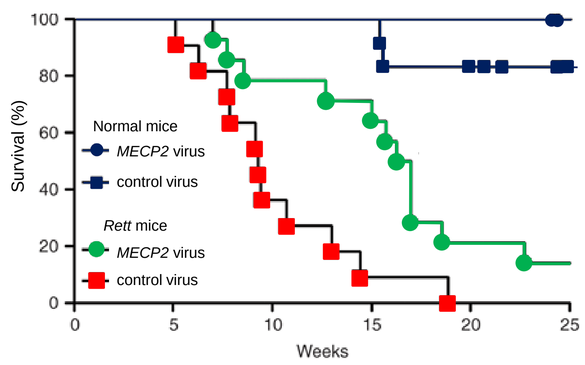
- Rett syndrome is a genetic disorder caused by mutations in the gene *MECP2*.
- It affects 1 in 10,000 girls, and becomes apparent at age 1 – 2 . Average female life expectancy ~40's
- Features include loss of movement control, loss of speech, compulsive hand movements, and problems with breathing, sleeping and digestion.

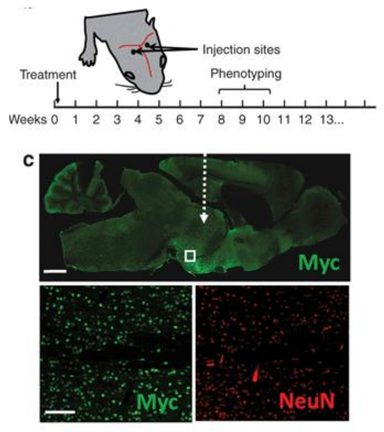


Common MECP2 mutations

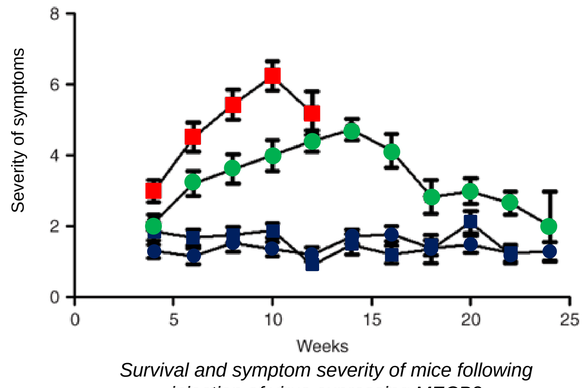
Our collaborators, Stuart Cobb and Mark Bailey, have shown that Rett syndrome can be reversed in adult mice by expressing the correct form of MECP2.

A virus was used to deliver MECP2 to Rett mutant mice, and significant improvements in survival were observed, along with a reduction in the severity of symptoms.





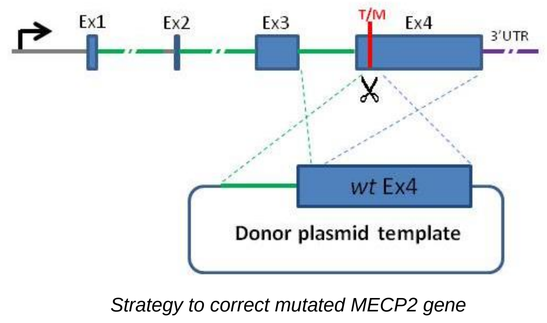
Expression of MECP2 protein (green) from the viral vector injected into mouse brain



Survival and symptom severity of mice following injection of virus expressing MECP2

Gadalla et al, 2013. Mol. Therapy 21:18-30

We are developing genome editing strategies to correct mutations in the endogenous MECP2 gene. This would remove the risk of expressing too much MECP2 protein from the viral vector, which could cause mental and physical disabilities.



Strategy to correct mutated MECP2 gene

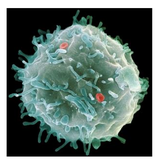
Genetic engineering of red blood cells for transfusion

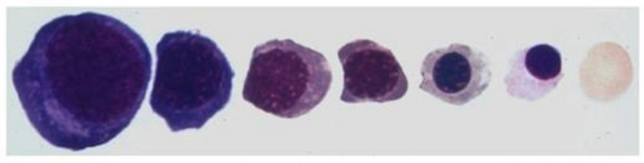
Regular blood transfusions are essential for those with genetic disorders affecting red blood cells (thalassaemias).

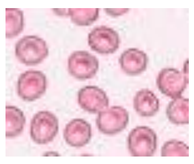
However, donated blood is not available in all countries, and carries the risk of transmitting infections between people such as hepatitis or HIV.

Jo Mountford's group at the University of Glasgow have developed a method for generating high volumes of red blood cells from pluripotent stem cells grown in the lab.

Red blood cells made in the lab produce foetal haemoglobin, which binds oxygen more tightly than adult haemoglobin. This blood is not ideal for transfusion, particularly for weak and vulnerable patients.

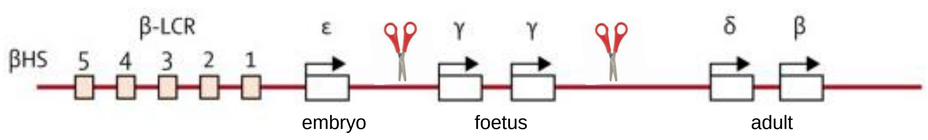






Growth media 1 → Growth media 2 → Growth media 3 → Growth media 4 → Growth media 5 → Growth media 6

Pluripotent stem cells → Mesodermal cells → Hemangioblast cells → Blood progenitors → Red blood cell progenitors → Mature red blood cells



We are developing genome editing technology to remove the foetal β-globin genes and force the cells to produce adult β-globin. This will mean the synthetic red blood cells are more efficient at taking oxygen to where it's needed in the body.

The red blood cells that will be given to patients have no DNA and are irradiated, removing any risk of infection or cancer.