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CELS191 LECTURE MATERIAL

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Population-genetic Theory, Genomic Imprinting, Phylogenetics, Phenotypic Plasticity, NZ Molluscs, History of Eugenics

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CELS191 2025

Human Molecular Genetics

Lecture 25

Investigating the Function of Individual Genes

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Lecture 25 Objectives

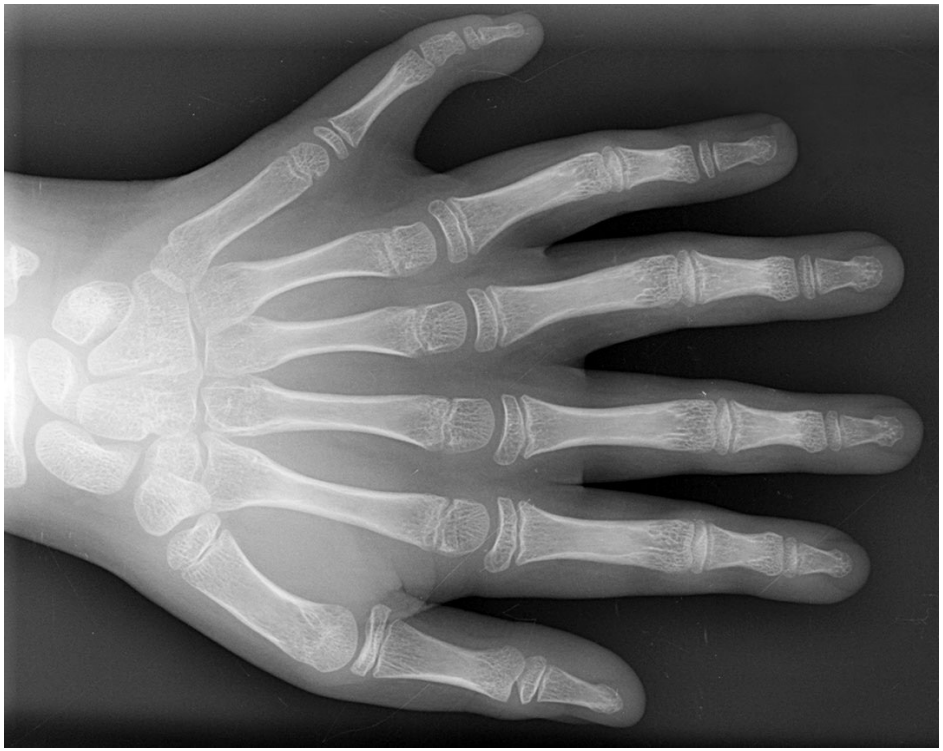
After you have revised this lecture you should be able to:

- Explain how we can get information about the function of a gene from its phenotype.
- Outline how we use genetic techniques in model organisms to find out what a gene does.
- Outline how we use genetic techniques to determine if a gene variant is pathogenic (disease causing).
- Outline how gene editing and gene therapy could be used to correct some genetic disorders.

How do we get information about the function of a gene from its phenotype?

- By studying organisms that are naturally variant for a particular gene, we can work out what that gene might do.
- Where no natural variation exists, we can make our own.
- By studying both these types of variants we can learn how particular variants lead to phenotypic changes.

Natural Variants: Where genetic change alters the phenotype - give clues to gene function



- The phenotype is what we see. Here, we see polydactyly.
- The cause of this phenotype is a change in a gene.
- The normal role of this gene is to prevent this phenotype.
- What is the normal function of this gene?

The Value of Mutants

- While *variation* in the human genome is common (**Lecture 22**), most of this does not affect phenotype.
- Mutations are rare, they are a subset of variation, but do not always affect "fitness".
- Around 4,000 (20%) human genes have unknown function.
- Luckily, many of these are conserved in other animals - even, in many cases, with fruit flies and yeast! If we can find or create mutants in these related genes, we may learn their functions in humans.

Variation is common, but phenotype-causing mutations are rare

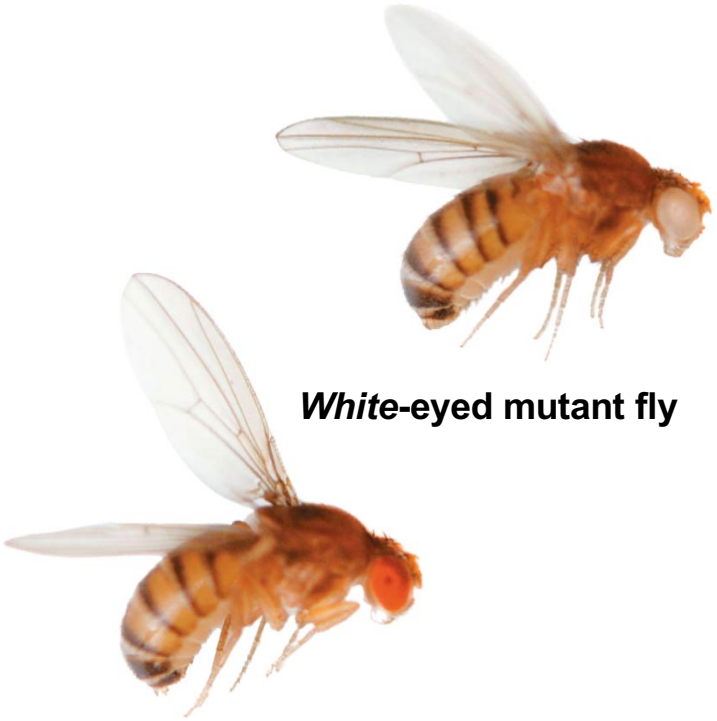
***White*-eyed mutant fly**

TH Morgan's first identified fruit fly phenotype: white eyes, took more than two years of deliberate fly breeding to discover.

Red-eyed wild type fly

- This phenotype was heritable, seen mostly in male flies, and was the first example of a sex-linked gene in an animal.
- What does the white gene do in "normal" flies?

These rare mutants point to the functions of their genes



***White*-eyed mutant fly**

Red-eyed wild type fly

- The white-eye gene *(white, w)* gives fruit flies their distinctive red eyes.
- White-eyed male flies produce a defective protein which prevents the pigment from being transported into the cells of the compound eye.
- The human version of this gene, ABCG1, makes a protein required for cholesterol and lipid transport into cells.

Myotonia congenita

Defect in the *CLCN1* gene which encodes a chloride channel receptor CLC-1.

- A similar inherited condition was known to occur in goats.
- Muscles fail to relax after contraction, due to the impairment in Cl- ion transport.

https://www.youtube.com/watch?v=mbeIlYOnWOY

How do we use genetic techniques to find out what a gene does?

- Study organisms that are naturally variant for that gene (rare!).
- Increase the rate of random mutation, select for a phenotype of interest and sequence the genome to identify the mutation (genetic screen).
- Take a gene you are interested in, copy it and insert it into another organism (transgenesis/genetic engineering).
- Deliberately break a particular gene to see what happens (targeted mutation/gene knockout/reverse genetics).
- This type of approach is called **functional molecular genetics.**

Model organisms can be used to *make* mutants

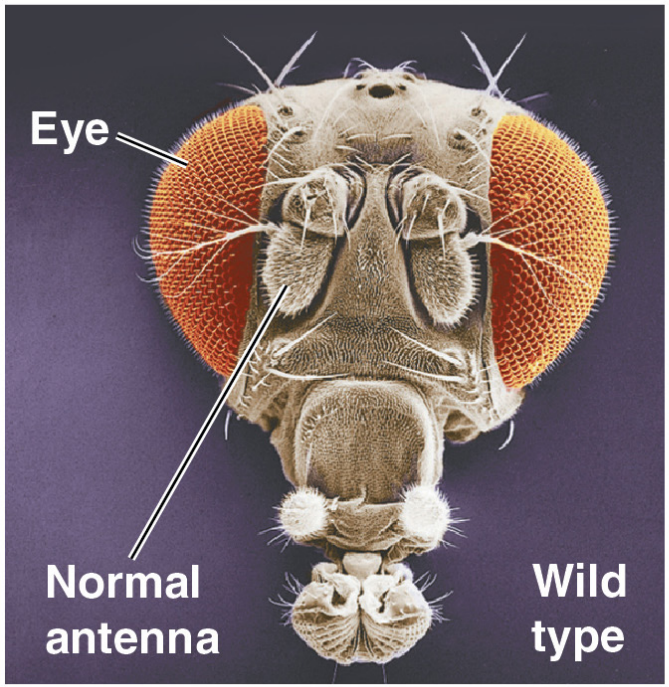
Mouse
- 80 million years diverged
- Has versions (homologues) of almost all human genes

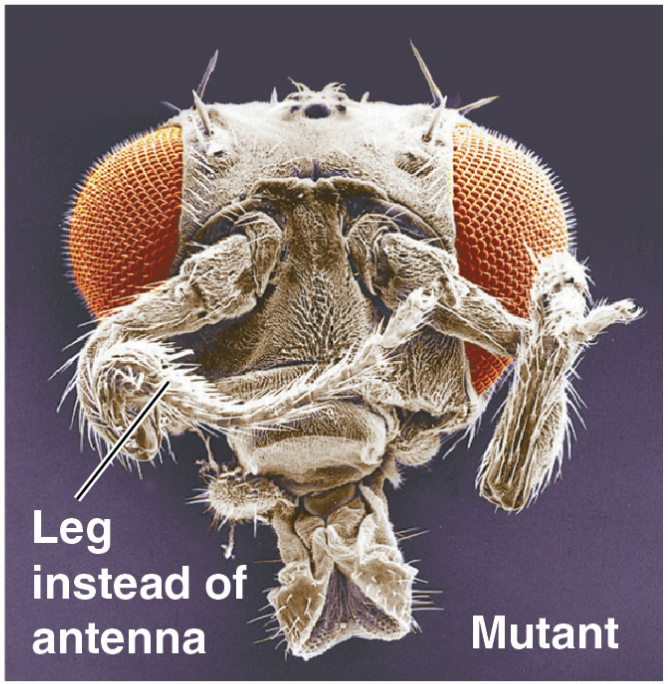
Zebrafish
- 400 million years diverged
- Has versions of most human genes

Drosophila
- 600 million years diverged
- Has versions of many human genes

- We share many of our genes with other animals.
- Model organisms are ones that can be easily raised in a controlled environment and are easy to manipulate genetically.
- Each organism has a different approach that works best for making changes to the DNA genome.

Mutants can be made by treatment of gametes with mutagens such as X-rays or chemicals





- *Antennapedia* mutation (right), discovered by Ed Lewis.
- Mutations made this way are random and can give quite dramatic phenotypes.

Transgenesis

- The DNA code is **universal**, so any DNA can be used by any organism- even synthetic DNA.
- Engineering a multicellular organism by adding in "foreign" DNA is known as transgenesis.
- We can use transgenic DNA to understand how genes work, to engineer recombinant proteins (synthetic biology), or in gene therapy approaches.

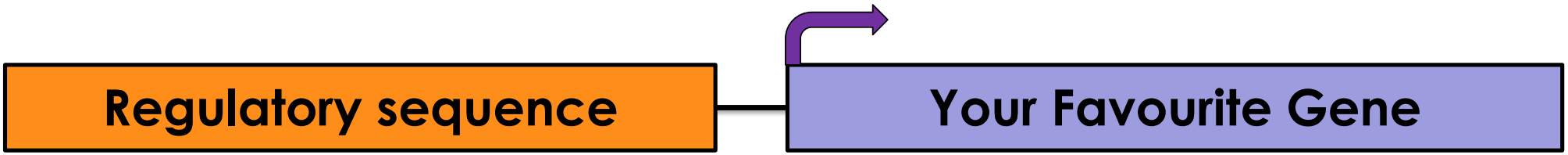
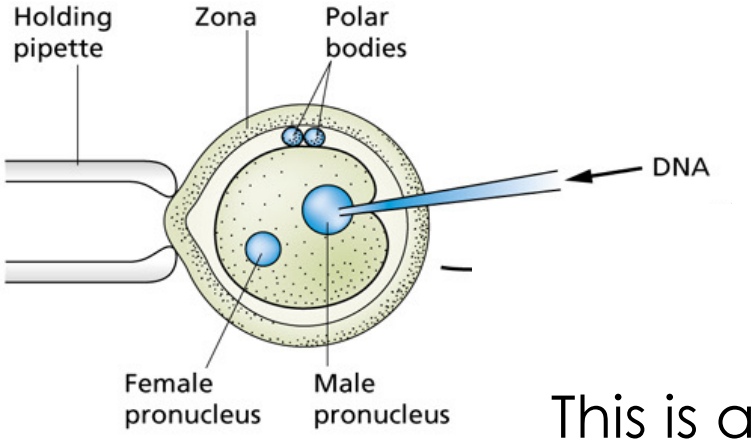


Image (Left): Fig. 10.11 Essential Developmental Biology, Slack p122b



Transgenesis in Mice

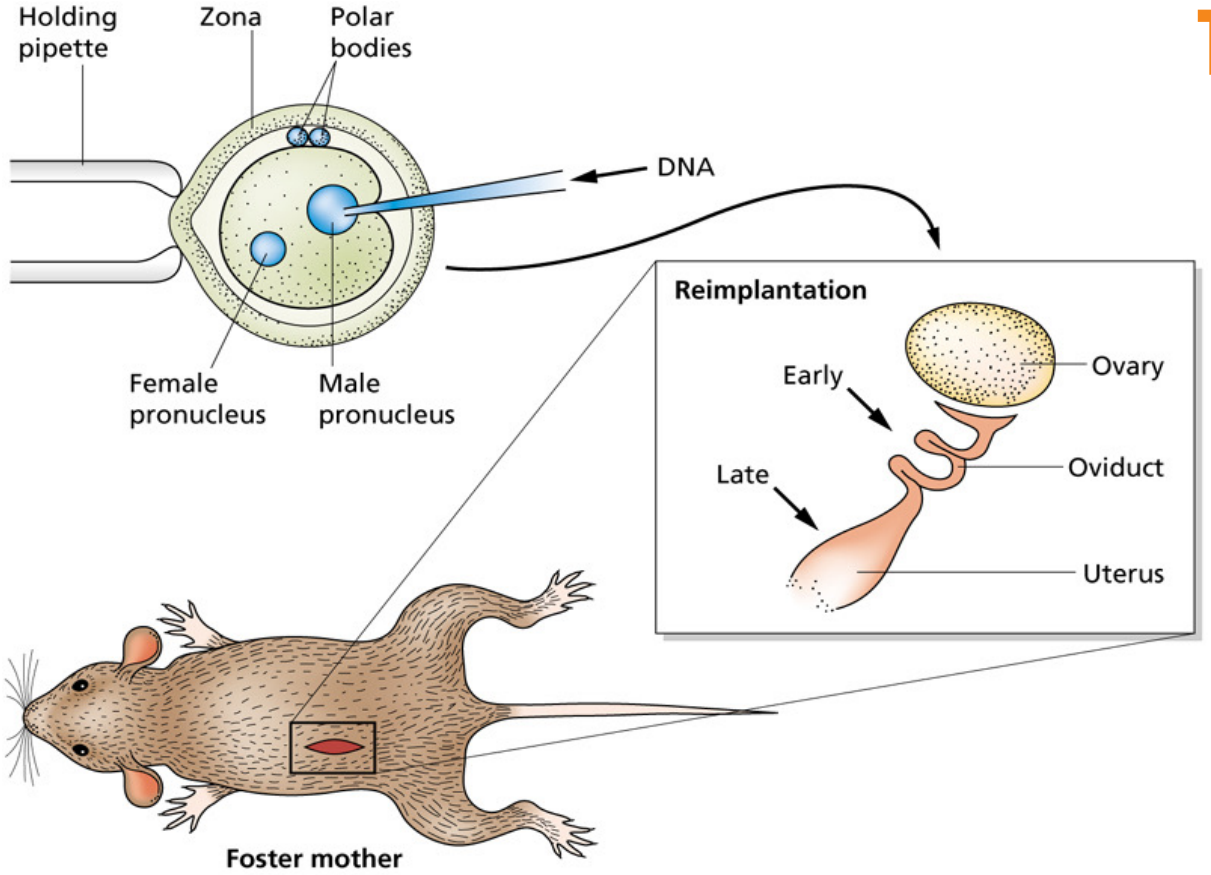
Put the jellyfish gene into mice

Regulatory sequence — **Green Fluorescent Protein**

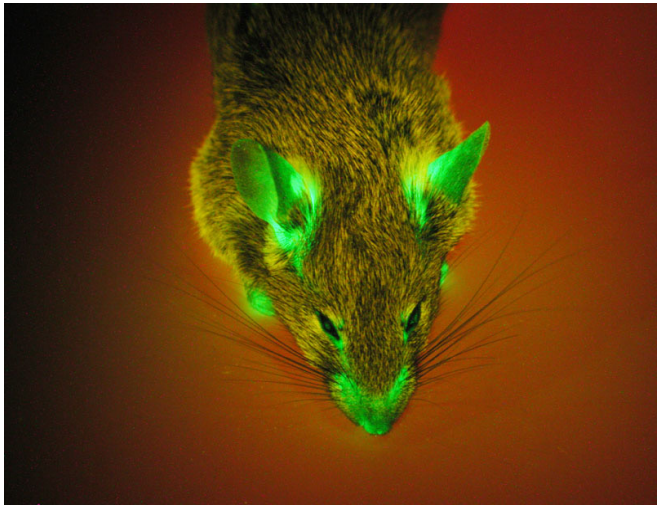
This is a ***transgene***

Image (Left): Fig. 10.11 Essential Developmental Biology, Slack p122b

Transgenesis in Mice



Transgenic mouse makes the jellyfish green fluorescent protein



Screen litter for transgene insertion

transgene insertion

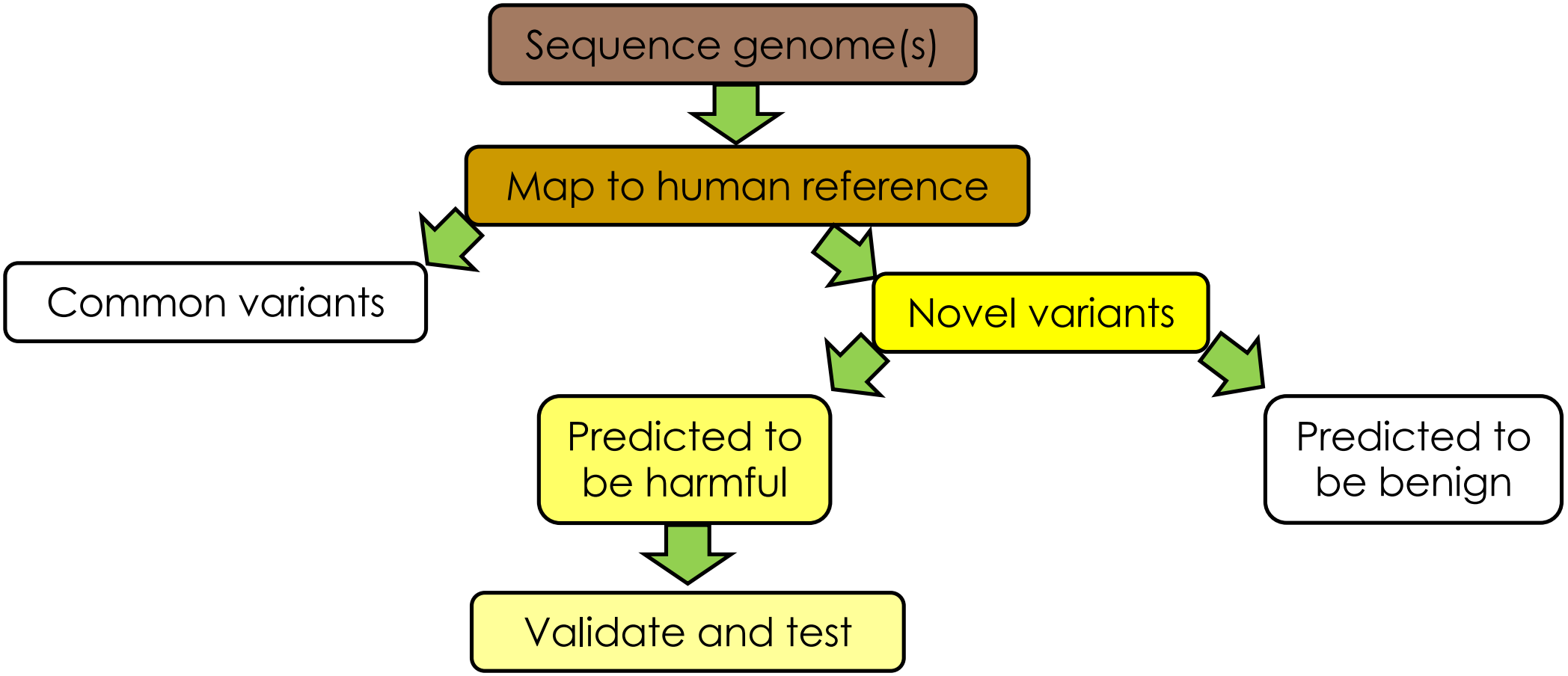
Image (Left): Concepts of Genetics 12ed Klug et al

Some examples of Transgenesis relevant to human health

- Growth hormone gene from rat inserted into mice (pictured, 1982).
- Human insulin produced by bacteria for treatment of type 1 diabetes (1982).
- Factor IX for haemophilia B in hamster cells (1998).
- Spider silk for surgical sutures produced in the milk of transgenic goats (2012).
- Brewer's yeast modified to produce cannabinoids (2019).

Image: CELS191 Supplied

How to find out if a variant is pathogenic



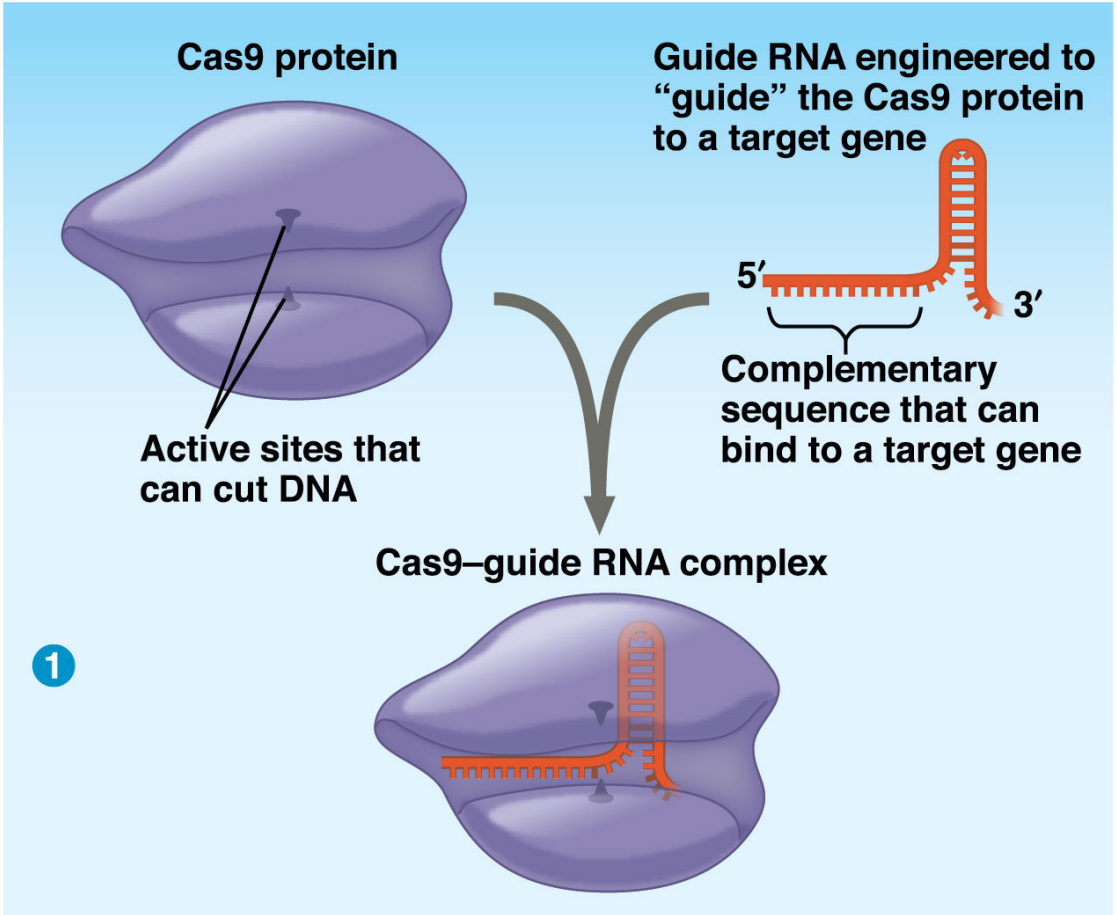
How do we know if a gene variant is pathogenic?

Modern genetics targets mutations to the DNA sequence of your choice- to "break" specific genes.

- We can damage, or modify, the gene we are interested in by genetically modifying an organism or cell line.
- By examining the organism, or its offspring, we should be able to work out what the gene normally does.
- There are many ways to do this, but we will look at one: CRISPR-Cas9.

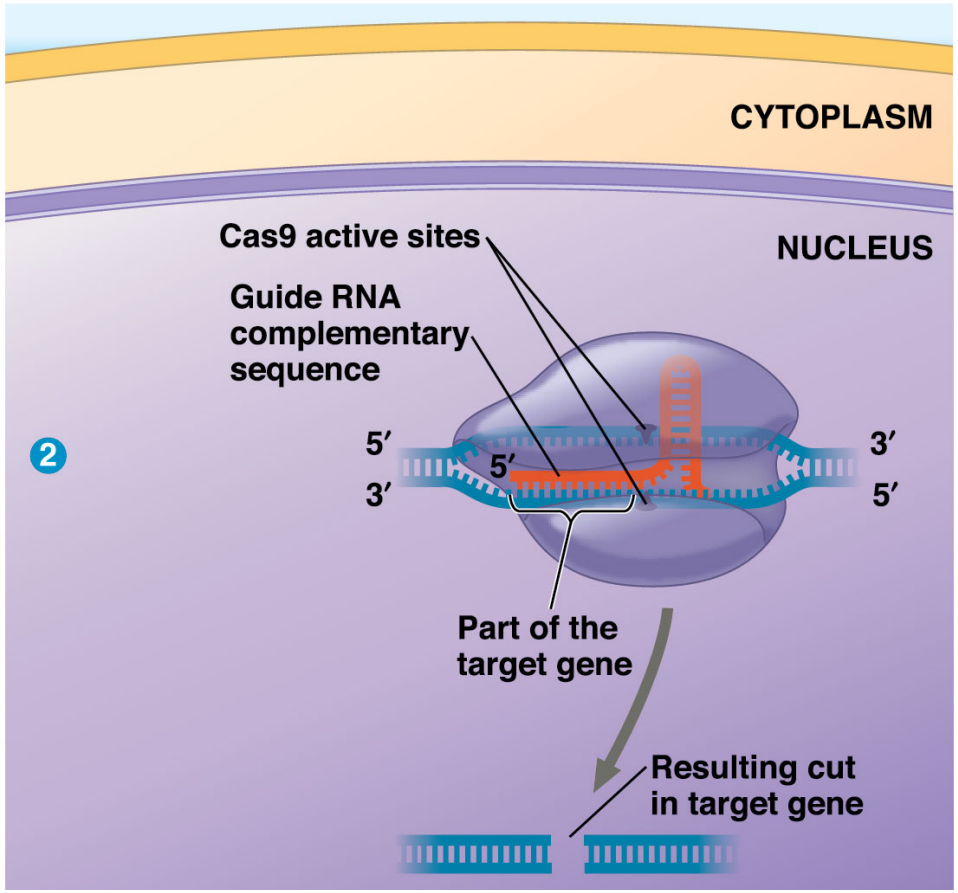
Targeted Mutation with CRISPR-Cas9

- CRISPR= clustered regularly interspaced short palindromic repeats.
- Cas9=CRISPR associated protein 9.
- Evolved in bacteria for antiviral defense.
- Decide which gene you wish to mutate.
- Design a short "guide" RNA that only binds to your gene of interest.
- Mix these together!



Targeted Mutation with CRISPR-Cas9

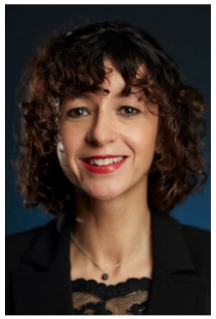
- Get this into the cells of interest in your model organism (not as easy as it sounds)
- Cas9 enters the nucleus and finds the target sequence in the genome that matches guide RNA
- Cas9 makes double stranded break in DNA at target site



Targeted Mutation with CRISPR-Cas9

- In the absence of a template, DNA repair enzymes try to patch up the cut.
- This often results in errors as there is no template to read from.
- Small InDels (**refer to lecture 22**) are created at the target site, the gene is potentially disrupted, or mutated (a).
- If repair template is provided, it is possible to use this to "edit" the DNA sequence at the cut site - "gene editing" (b).

Nobel Prize (Chemistry) 2020



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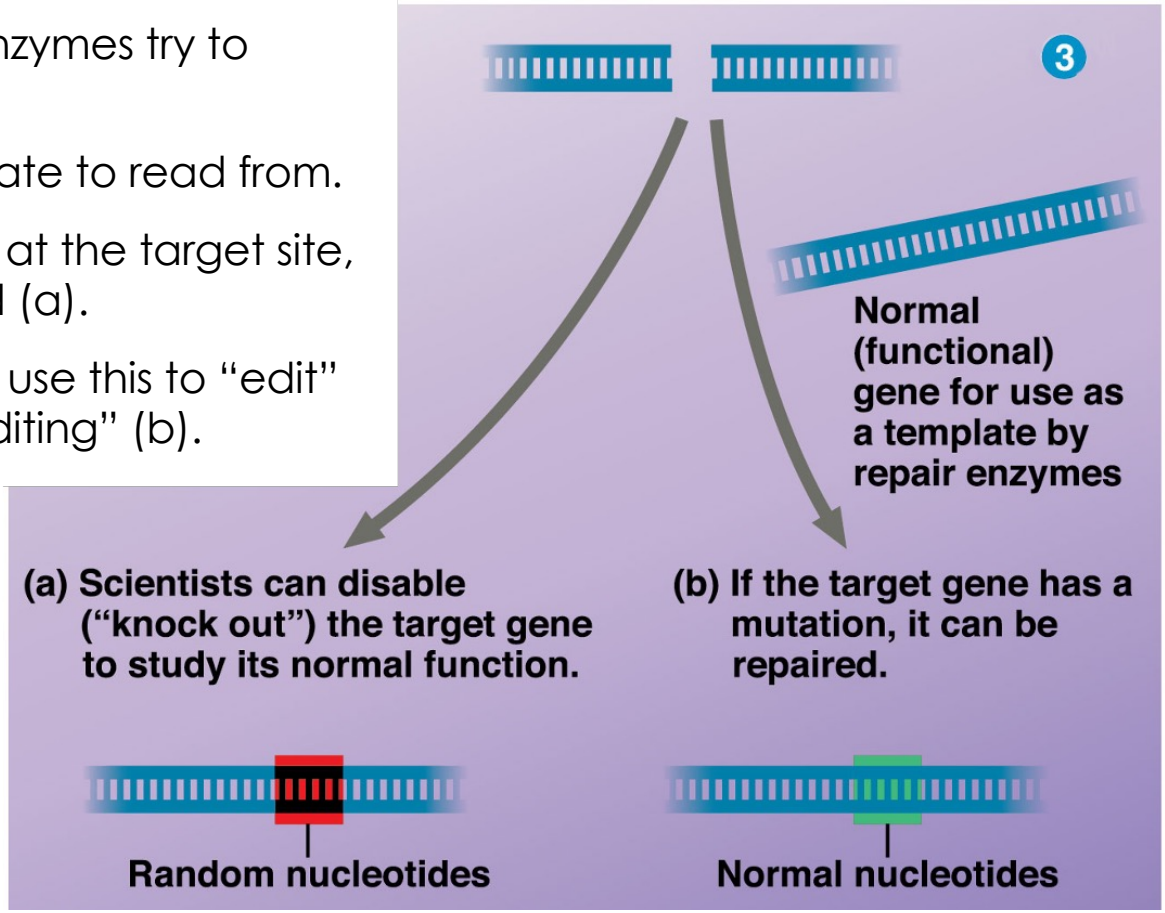


Genetic Disease: Can we fix it?

Yes we can! (but only if we know what causes it and have a way to correct the defect)

SOMATIC

- Targets the cells or organs affected.
- Does not affect the next generation (is not a change to the germline*).
- Gene Therapy Example: Cystic fibrosis: one of the most common lethal single gene genetic conditions, defect in CFTR gene, which codes for a chloride ion transporter.
- Gene Editing with CRISPR-Cas9 Example: Sickle cell disease: mutation in haemoglobin, the oxygen carrying protein in red blood cells.

***Note:** the CRISPR edited babies allegedly created in 2018 by He Jianhui are *germline* mutations.

Gene Therapy Example: *Cystic fibrosis*

- Cystic fibrosis is one of the most common single gene genetic conditions.
- Caused by mutations that disrupt the function of a chloride ion channel encoded by the CFTR gene.
- CFTRdeltaF508 is the most common disease-causing mutation.
- Lung disease, pancreatic insufficiency, reduced life expectancy.
- 1/2500 live births, autosomal recessive.
- Pig model (2008) replicates the most common deltaF508 mutation and can be used to test smart drugs and gene therapy options.

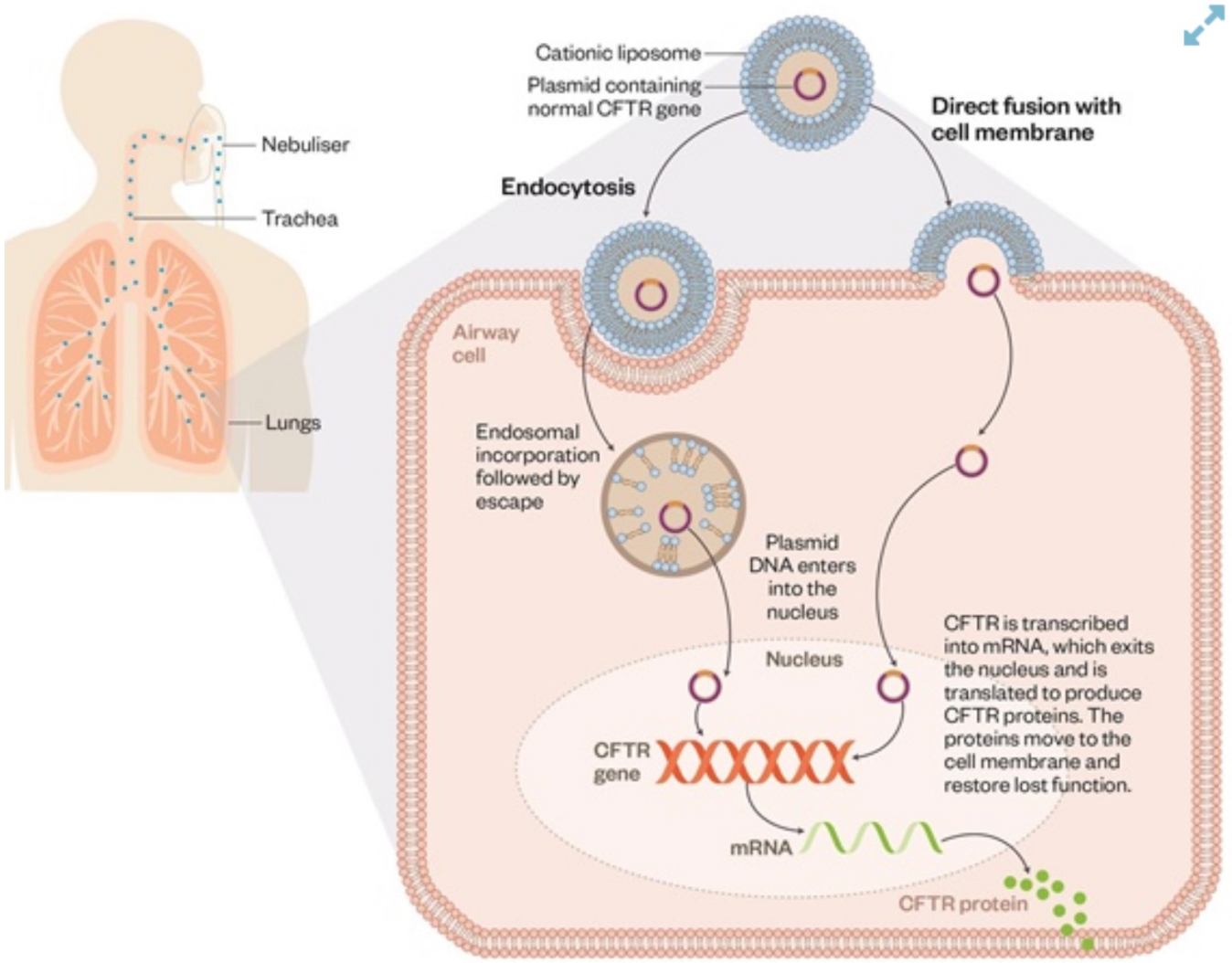
Context Slide

Image: The Pharmaceutical Journal 10 JUN 2016 By Sarah DeWeerdt
(Developing gene therapy to treat cystic fibrosis: challenges and successes)

Gene Therapy:
Cystic fibrosis

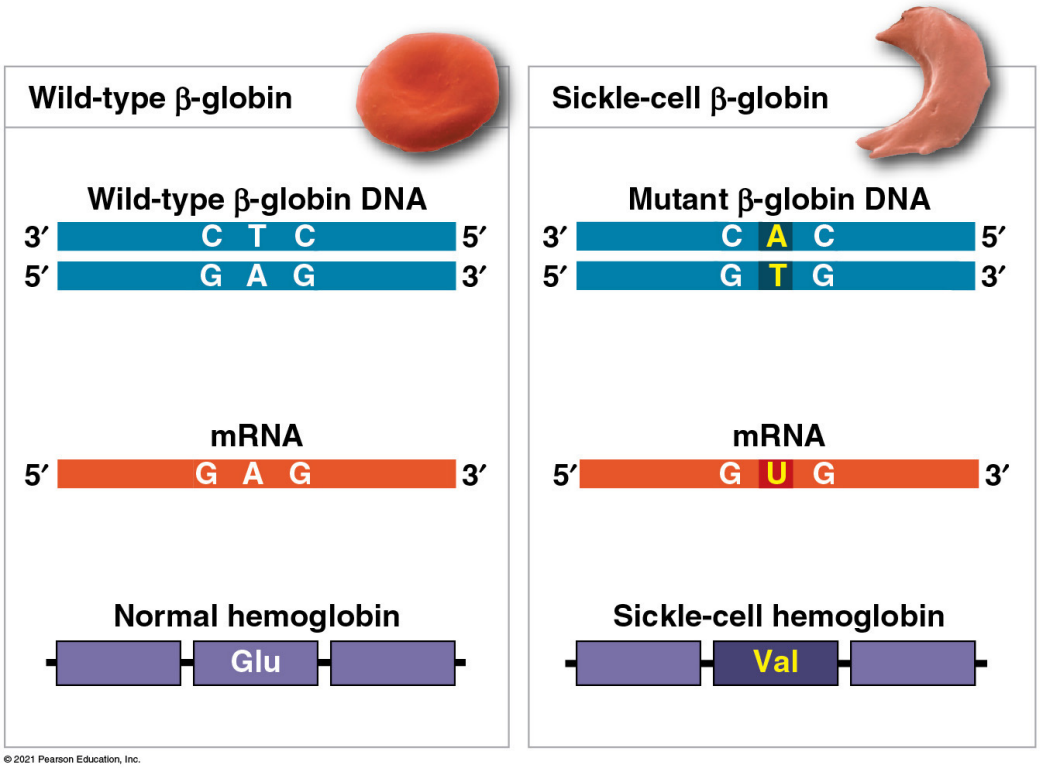
Delivering DNA with functional copy of CFTR gene to lung epithelial cells via nebulizer.

Extra copy makes good CFTR protein, restoring function to some cells.



Gene Editing with CRISPR Example: Sickle Cell Disease

- Sickle cell disease: defect in both copies of the HBB gene, Chromosome 11.
- Recessive, missense mutation.
- One copy of the allele protects against malaria.
- Two copies results in a tendency for the protein to clump resulting in sickle shaped red blood cells.



Gene Editing with CRISPR Example: Sickle Cell Disease

- The human foetus makes haemoglobin differently, using alpha and gamma chains (HbF).
- A gene called BCL11A shuts down the gamma genes at birth.
- CRISPR/Cas9 was used to break BCL11A in the patient's extracted bone marrow cells.
- The modified bone marrow cells were returned to the patient. Red blood cells now make HbF.

Victoria Gray: the first patient

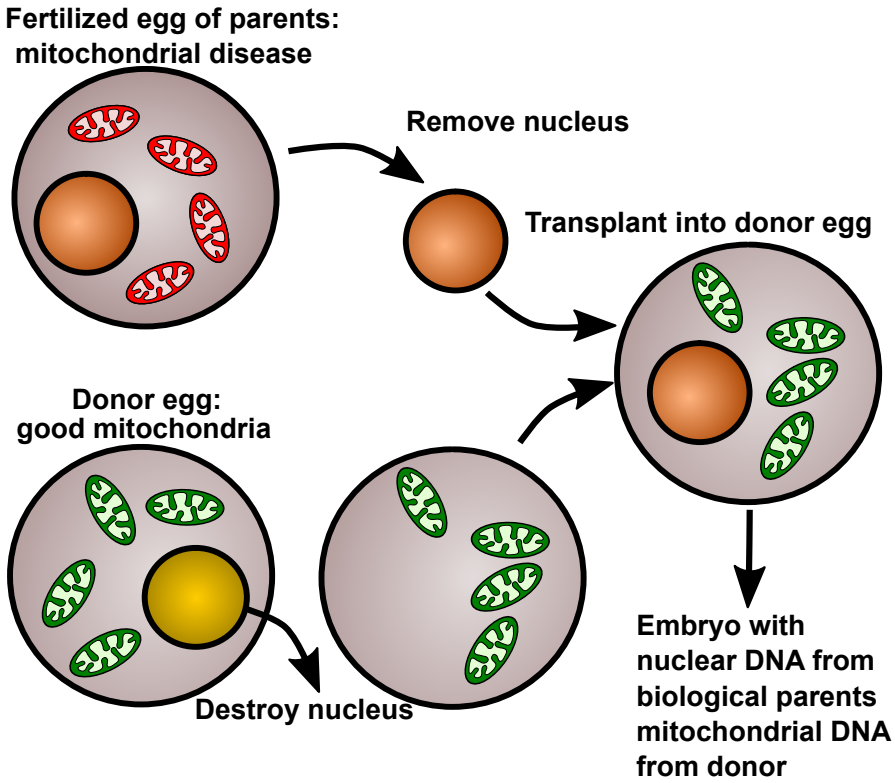
Image: Original C.Beck 2019 (3 Parent babies)

Genetic Disease: Can we fix it?

Yes (but only if we know what causes it, have a way to correct the defect, *and* have considered ethics).

GERMLINE

- **Pre-implantation genetic diagnosis:** in families with an identified risk, IVF can be used to make embryos from the parents' eggs and sperm. These embryos can be tested before implantation, and only healthy embryos implanted.
- **Three parent babies:** where the faulty gene is on the mitochondrial DNA, nuclear transfer to a donor egg can be used.
- **CRISPR gene "edited" babies** (Jiankui He, unpublished).



Lecture 25 Summary

- Mutations are a subset of variation, and do not always affect "fitness".
- By studying the phenotype of naturally occurring or engineered mutants, we can work out what a gene does.
- We can make use of the conservation of many genes in model organisms to study the effect of mutations.
- Transgenesis is where we add DNA to a genome to make a new protein or replace a defective gene.
- Knowledge of variation in the population or in parents, can be used to identify variants that may be disease causing (pathogenic) in an individual.
- CRISPR-Cas9 is one method that can be used to "break" or modify a gene to replicate a possible disease causing variant in a model organism, to see if the disease develops as a result.
- Gene editing and gene therapy could be used to correct some genetic disorders.
- These techniques can be targeted to somatic cells, to avoid modifying successive generations.

Objective-Based Questions

- Outline, in general terms, how you would get information about the function of a gene from its phenotype.
- Name three model organisms that can be used to make mutants. What is the most commonly used model organism to study human conditions? Give four reasons why this organism is the most commonly used model organism.
- Define the term transgenesis.
- CRISPR-Cas9 loaded with guide DNA must get into the cell.
  a. Describe what happens once the CRISPR-Cas9 is inside the cell.
  b. Outline the consequences of a cell trying to repair cut DNA in the absence of a template.
  c. How is gene editing achieved using CRISPR-Cas9?

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