Eye on the future

Medusa (Caravaggio) 1597

William Ayliffe
Gresham College
26/2/14

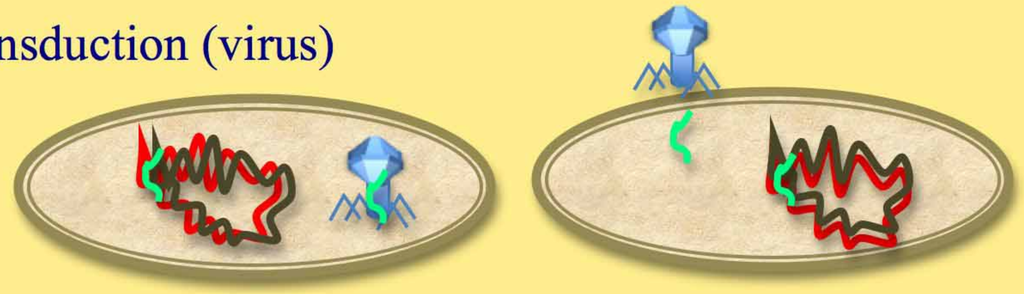
Infection
Genetic disease
Degenerative disease

Genes
Genetics
DNA to proteins

Genetic basis of antibiotic resistance
Antibiotics
Mobile genetic elements
Transfer of genetic information

Genetic Eye disease
Gene therapy
Prosthesis

Transduction (virus)



Jojhann (Gregor) Mendel (1822-84)

Studies mice discouraged by Bishop Schaffgotsch

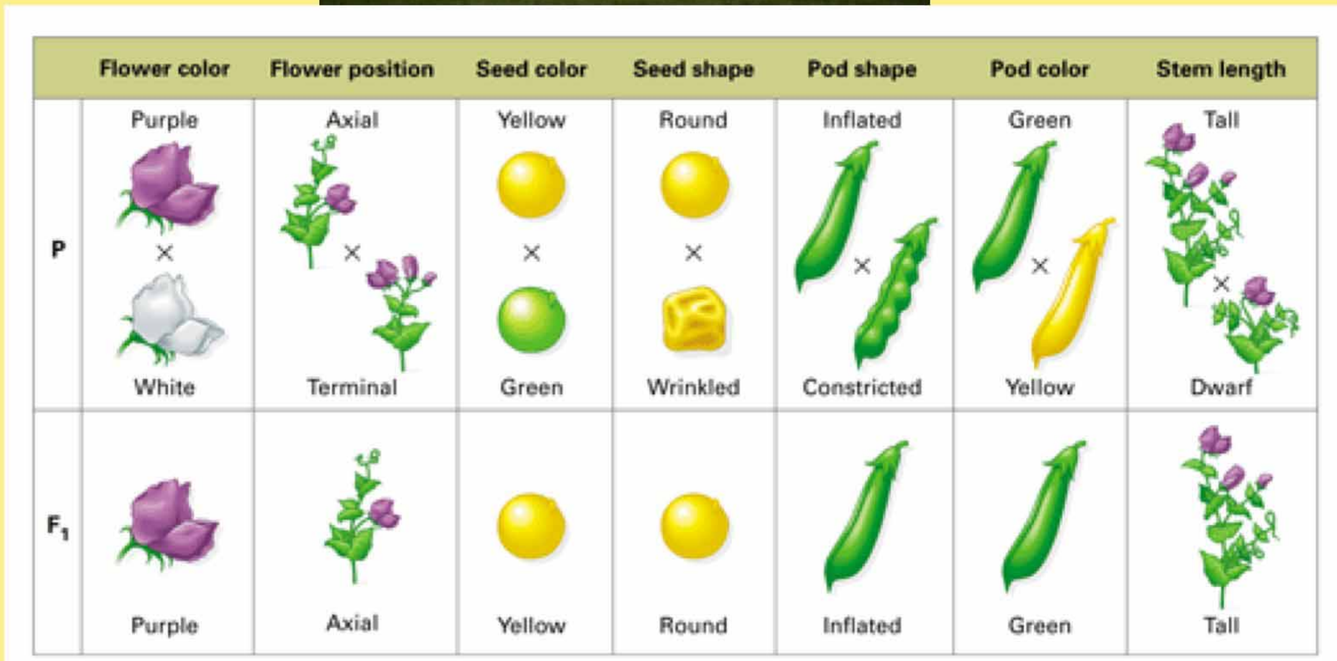
"bishop didn't understand that plants also have sex"

Mendel was studying the numbers and types of progeny produced by self-fertilized hybrids, not the inheritance of characters.

no concept of genes, work does not mention two (and only two) mutually exclusive factors or elements in heredity.

Merkmal refers to a feature that one can recognize; a "trait."

Elemente an unknown substances that might produce Merkmal.



7 traits: Inherited as discrete units, not blurred

flower color is purple or white

flower position is axial or terminal

seed color is yellow or green

seed shape is round or wrinkled

pod shape is inflated or constricted

pod color is yellow or green

stem length is long or short

"the behavior of each pair of differing traits in a hybrid association is independent of all other differences in the two parental plants".

pea hybrids form germinal and pollen cells that in their composition correspond in equal numbers to all the constant forms resulting from the combination of traits united through fertilization

Pollinated a white flower with a purple-flower

Seed produced all purple flowers

Self-pollinated F1. Obtained 929 seeds

Some of the resulting plants were white flowered;

The white phenotype had reappeared.

705 purple-flowered and 224 white-flowered plants.

705:224 almost 3:1 ratio

1865: monthly Bru□nn Natural Science Society

1866: 48-page paper in Proceedings of the Bru□nn Society for the Study of Natural Science.

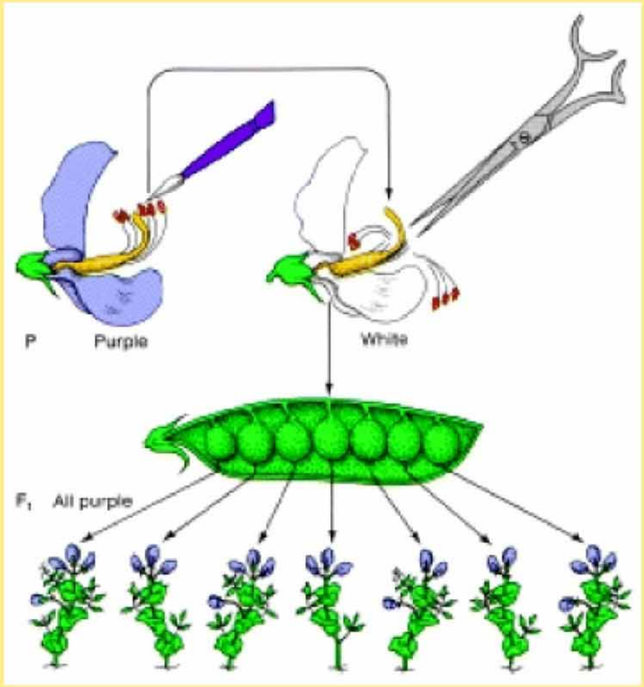
Trying to find "a generally applicable law governing the formation and development of hybrids."

$A/A + A/a + a/A + a/a = A + 2Aa + a$

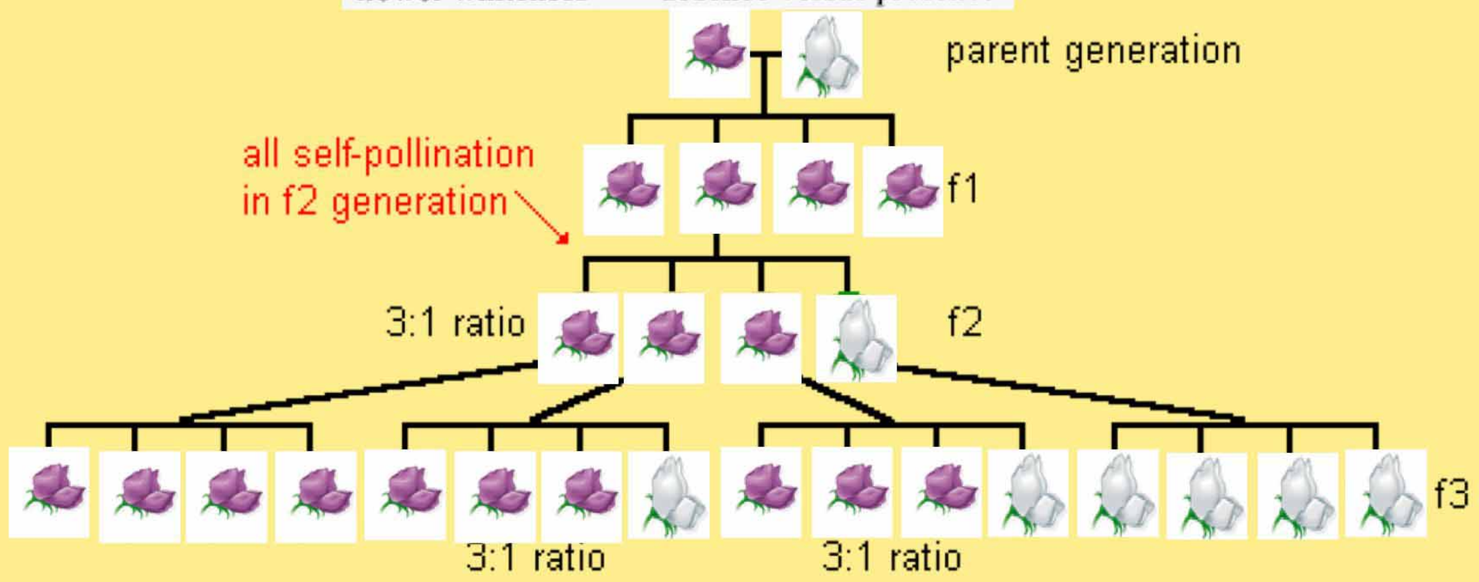
Some hybrids have characteristics of their parents

Studying the numbers and types of progeny produced by self-fertilized hybrids, not the inheritance of characters.

Mendel's paper one of many excellent studies

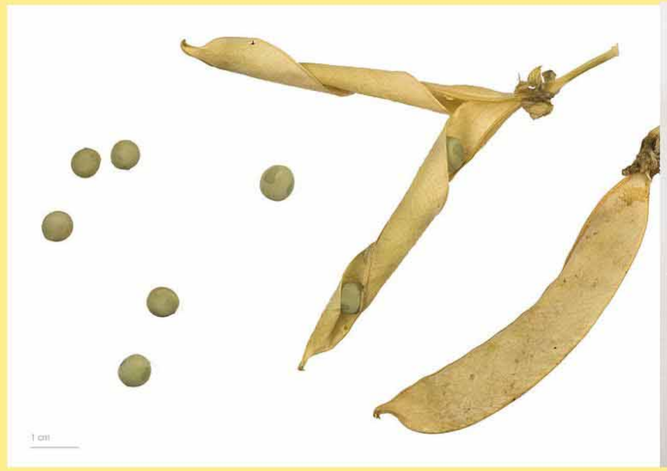


Character	Phenotype
flower color	purple versus white
flower purpleness	presence versus absence
flower whiteness	absence versus presence



first to describe hybrids with double letters (e.g., Aa), suggests he knew that hybrids carried two different character traits. However, he used only one letter for pure- breeding stocks. Believed that pure- breeding plants had only one such character, or may not have thought that his letters represented any sort of physical structure at all

Rediscovery of Mendel



1900: de Vries, Correns, and Tschermak each noted a 3:1 ratio in distribution of characteristics in hybrids

After seeing de Vries' paper, Correns quickly wrote a paper that gave Mendel credit for the findings

Three conclusions:

1. Inheritance of each trait is determined by "units" or passed on to decendents unchanged (genes)

2. Individual inherits one unit from each parent for each trait

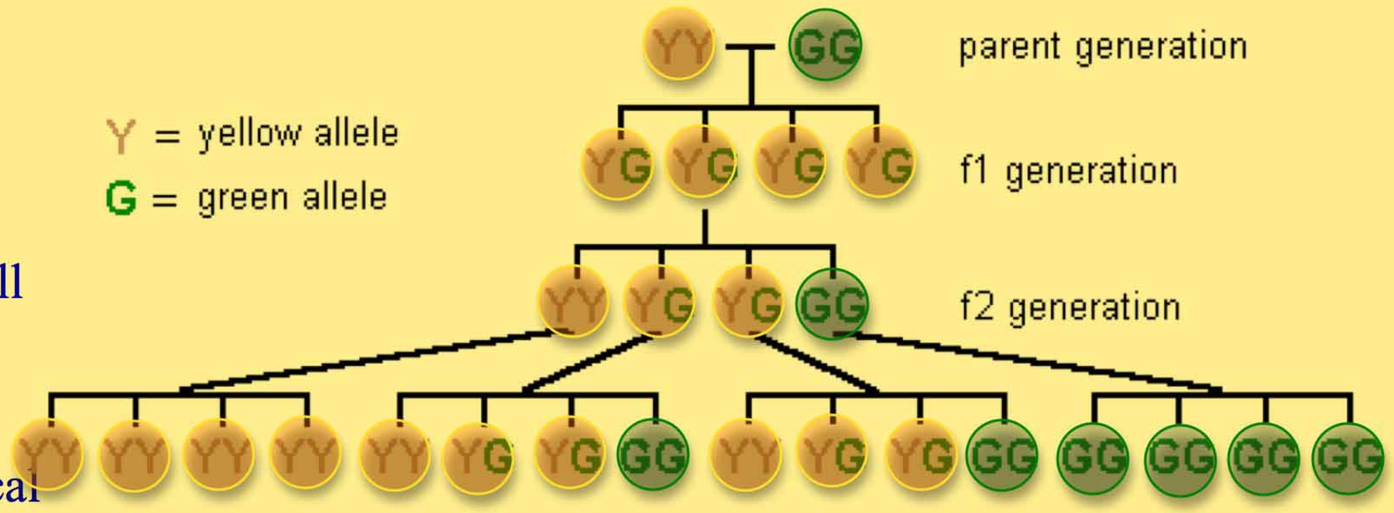
3. Trait may not show up in an individual but can still be passed on to the next generation.

Correns: Every trait based on an **anlage,** hypothetical nuclear unit that causes the trait to be expressed.

Hybrids express only one trait, one of their anlage suppresses expression of the another

Explains segregation, dominance, and recessiveness

1909: Wilhelm Johannsen: coins "gene" ("gen" in Danish) to describe the fundamental physical and functional units of heredity



Parent plants homozygous for pea seed color.
Each had two identical forms (alleles) of gene for this trait
f1 generation were all heterozygous .
Each inherited two different alleles--one from each parent
the genotype for pea seed color is YG (heterozygous)
the phenotype is yellow.
The yellow allele is dominant

Location of genes

1831: Robert Brown: Sailed with Flinders to Australia**:** Describes Nucleus in plant cells

1869 - Friedrich Miescher discovered nuclein in the nuclei of white blood cells.

1882: Walther Flemming: aniline dyes chromatin. threadlike structures in nucleus—chromosomes (*coloured body*)

1900: Theodor Boveri: Sea Urchins: Chromosomes needed to pass on information

1902: Sir Archibald Edward Garrod: Alkaptonuria recessive Mendelian trait.

1903 - Walter Sutton, American physician and geneticist, hypothesized that chromosomes are hereditary units.

1905 - William Bateson coined the term "*genetics*". *γεννώ*; "to give birth

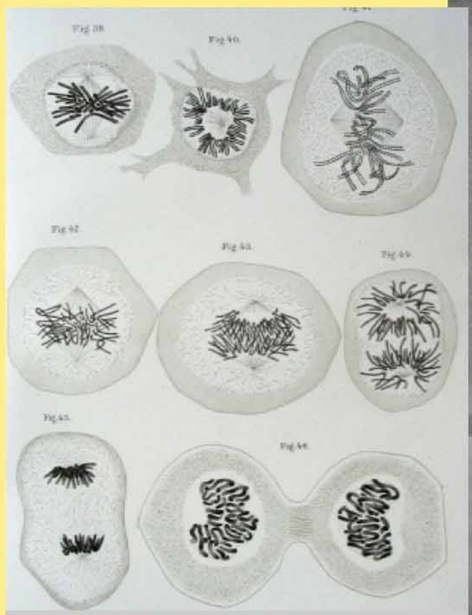
1910 - Thomas Hunt Morgan showed that genes are located on chromosomes.

1913 - Alfred Sturtevant genetic map of chromosome.

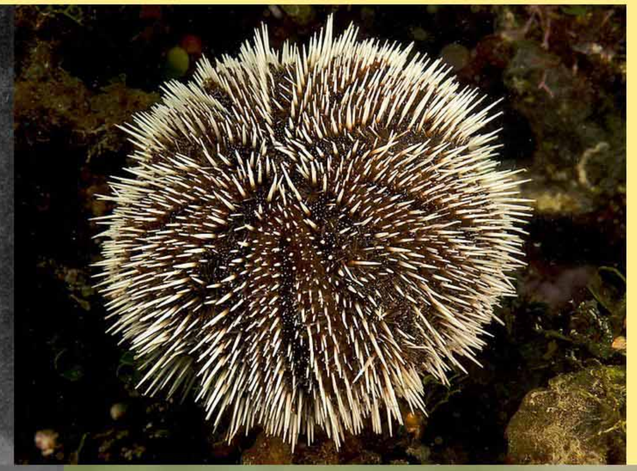
1933 - Jean Brachet: DNA was found in chromosomes and that RNA was found in the cytoplasm of cells.

1941 - George Wells Beadle and **Edward Lawrie Tatum**: genes code for proteins.

1952: Hershey–Chase experiments: Phages transmit DNA not their protein coat



Zellsubstanz, Kern und Zelltheilung (*cell substance, nucleus and cell division*; 1882) Walther Flemming (1843-1905)



Brachystola magna, grasshopper **Plains Lubber**

William Bateson
St John's Cambridge

Development of sea urchin eggs, male sperm nuclei and female egg nuclei each had a **half set** (haploid number) of chromosomes. As long as there was a set of both (diploid number of chromosomes), normal development of larvae. Any more or any less abnormal development.

Boveri–Sutton chromosome theory explains the mechanism underlying the laws of Mendelian inheritance by identifying chromosomes with the paired factors (particles) required by Mendel's laws. It also states that chromosomes are linear structures with genes located at specific sites called loci along them

DNA and genes

Deoxyribonucleic acid, DNA genetic instruction production of proteins and cell processes

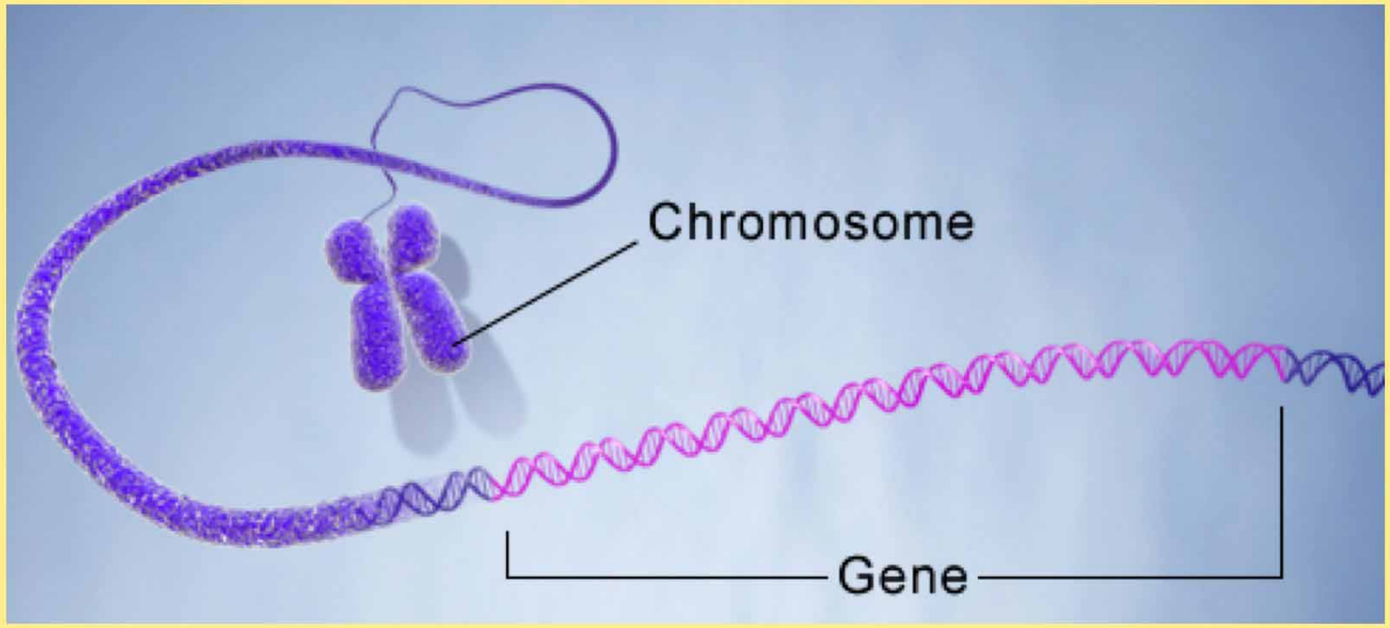
1953: Watson and Crick: 3D model of DNA based on Rosalind Franklins X-ray diffraction images

double helix: strands twisted around each other.

Nucleotides: deoxyribose sugar connect strands.

Adenine pairs with thymine:

guanine with cytosine, and vice versa.



human genome: 3000 megabases (Mb) 3 billion
More than 99% bases the same in all people.

20,000 - 25,000 genes.

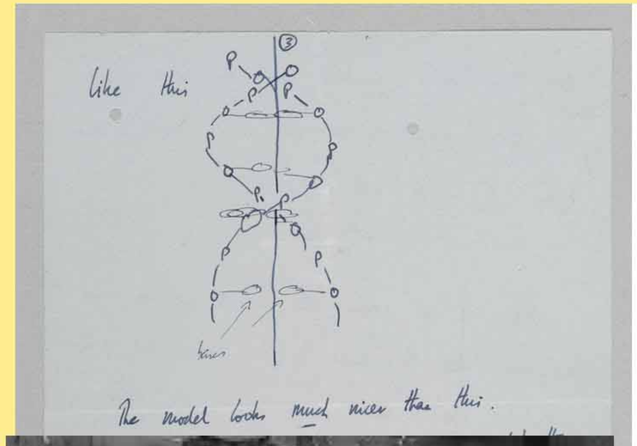
Packaged with proteins and RNA in 23 pairs of Chromosomes

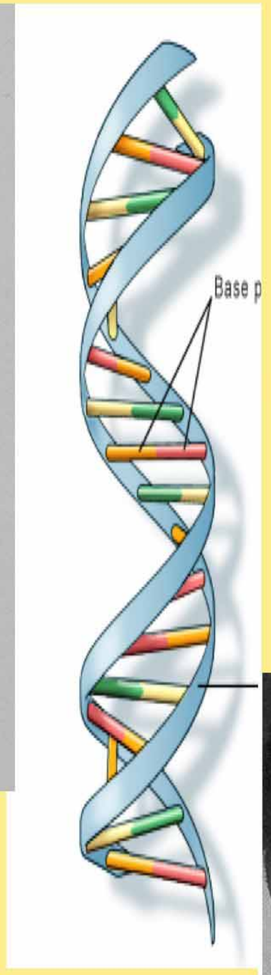
Genome *Escherichia coli* consists of a single 4.6 Mb circular chromosome.

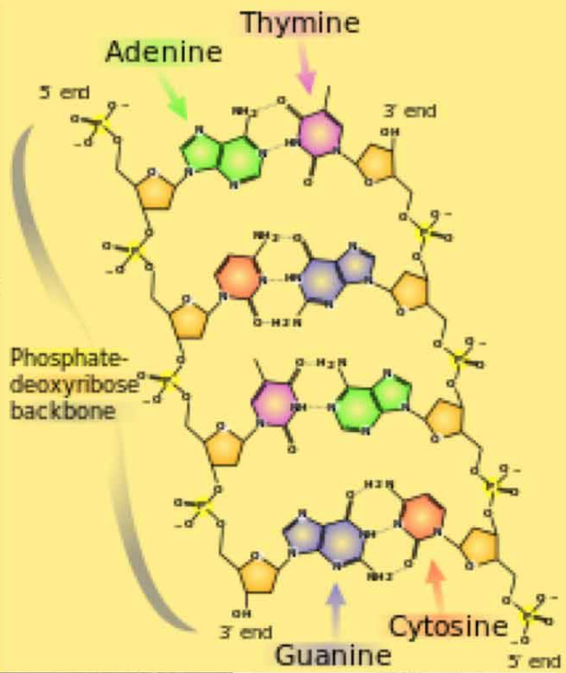
Gene: stretch of DNA that encodes information.

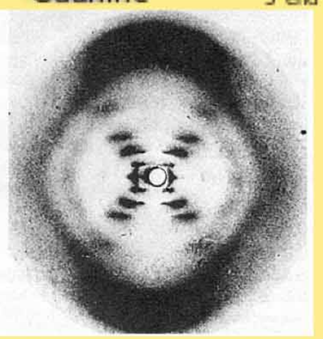
Basic unit of heredity.

Vary in size: hundreds to 2 million DNA bases.









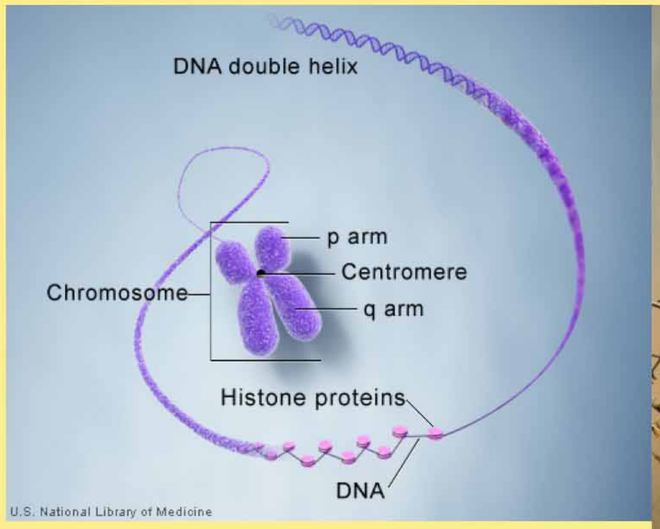
Every person has two copies of each gene

One inherited from each parent.

Most genes are the same in all people

Small number (less than 1%) are slightly different between people.

Alleles: Contribute to unique features (phenotype).



Mutations range in size from a single DNA base to a large segment of a chromosome.

Some genetic variants are very rare:

Others are common in the population.

Polymorphisms: Genetic changes that occur in more than 1% population, normal variation. responsible for many of the common differences between people; eye, hair colour, and blood type.

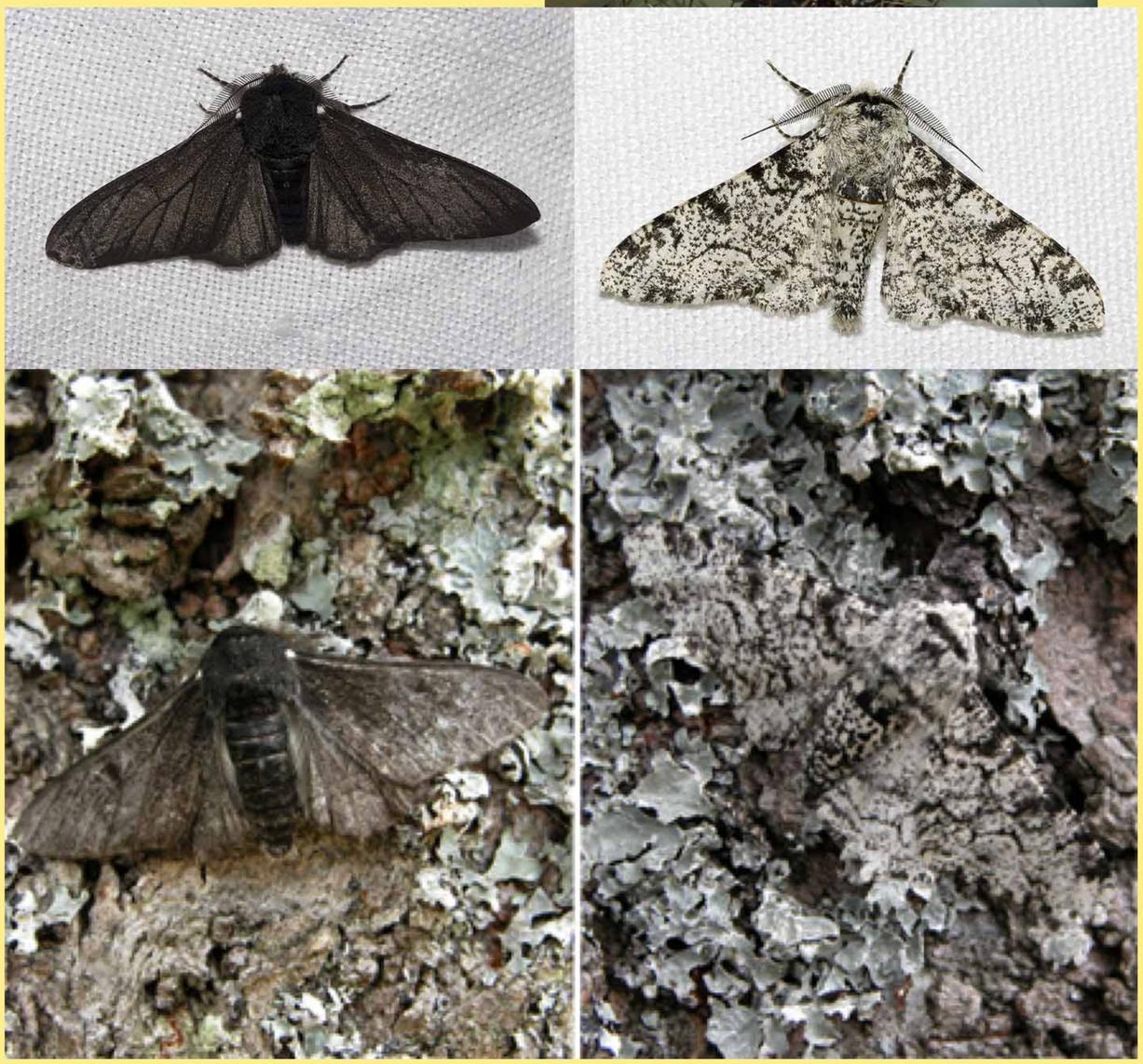
Before the Industrial Revolution: peppered moth light grey speckled.

Blends with lichens bark.

Black variant: more likely to be eaten by birds. Frequency of the dark allele was about 0.01%

1895, dark-coloured moths in the Manchester population was 98%, (increase 1000%) from the original frequency

Sexual selection of blue eyed partners



Reading the code

Most DNA is in chromosomes in cell nucleus

Small amount in the mitochondria

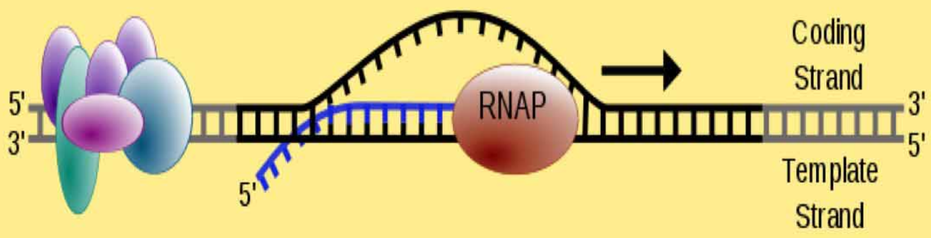
Transcription: production of RNA copies of genetic code by RNA polymerase

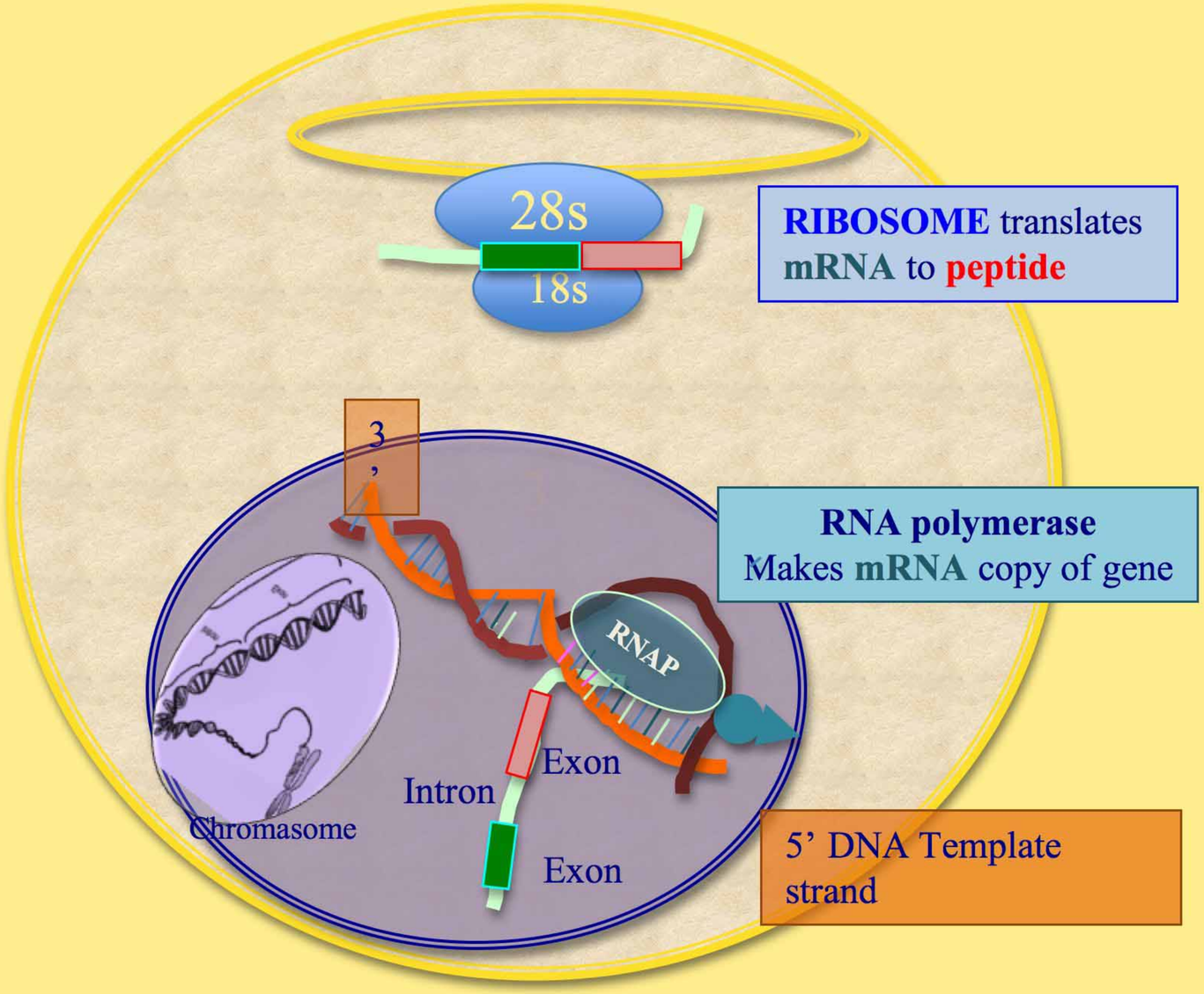
One strand runs **5' to 3'** other in opposite direction. **Template strand:** blueprint for **mRNA copies**

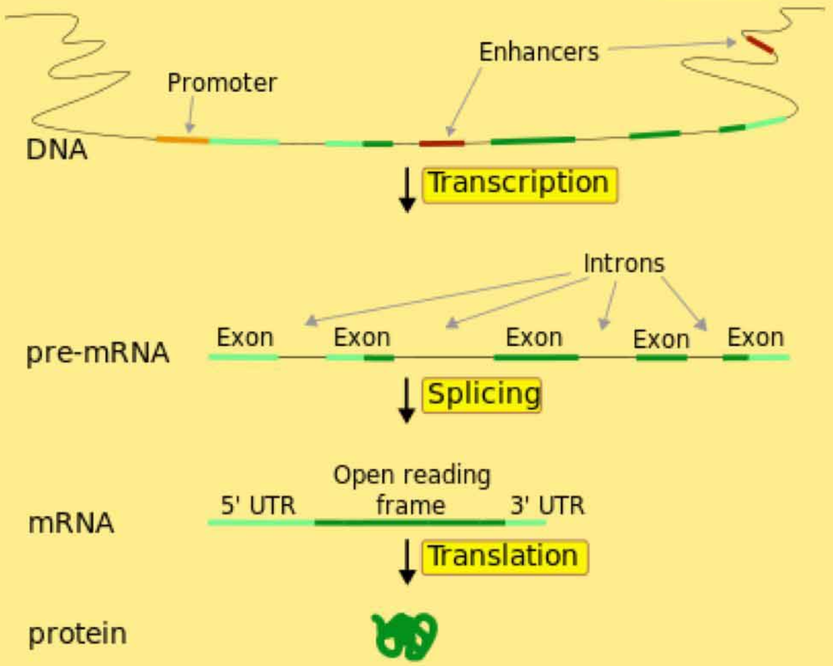
Not all the DNA codes for products

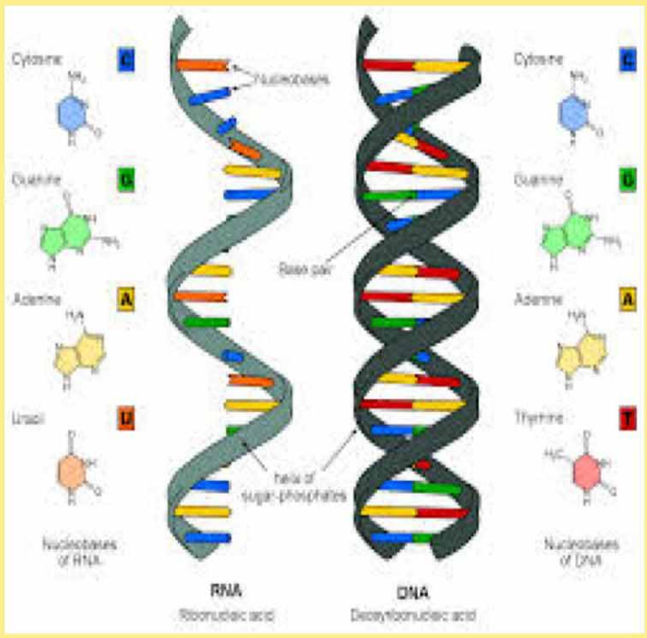
Gene contains some non-coding sequences introns

These need to be spliced out









Gene to Product

Spliced **mRNA** moved to **RIBOSOME** for translation

Alternative splicing gives different products

Ribosome: reads mRNA base sequence.

Each sequence of three bases, called a codon, usually codes for one particular amino acid

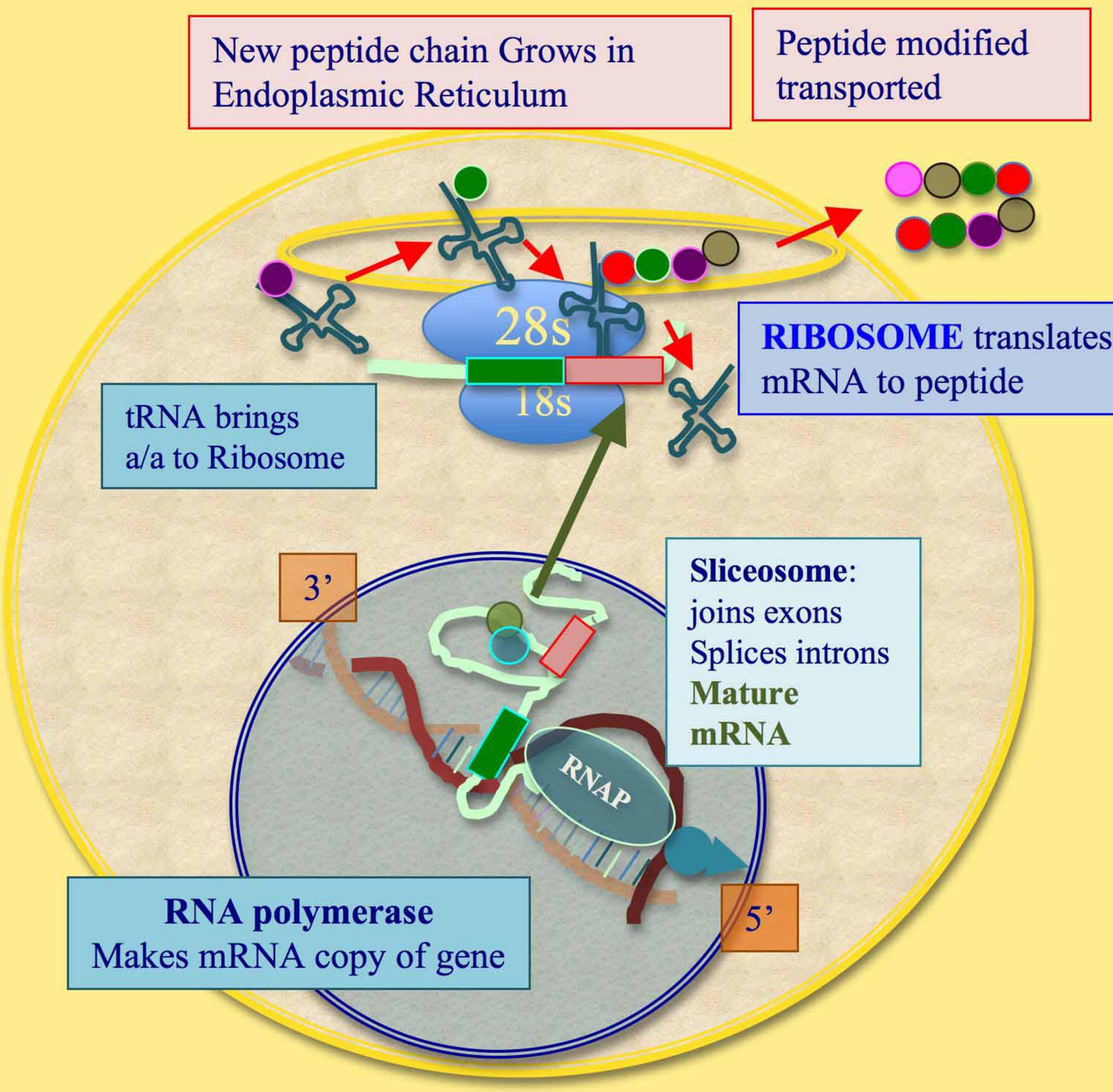
Genetic makeup is **genotype** of the organism.

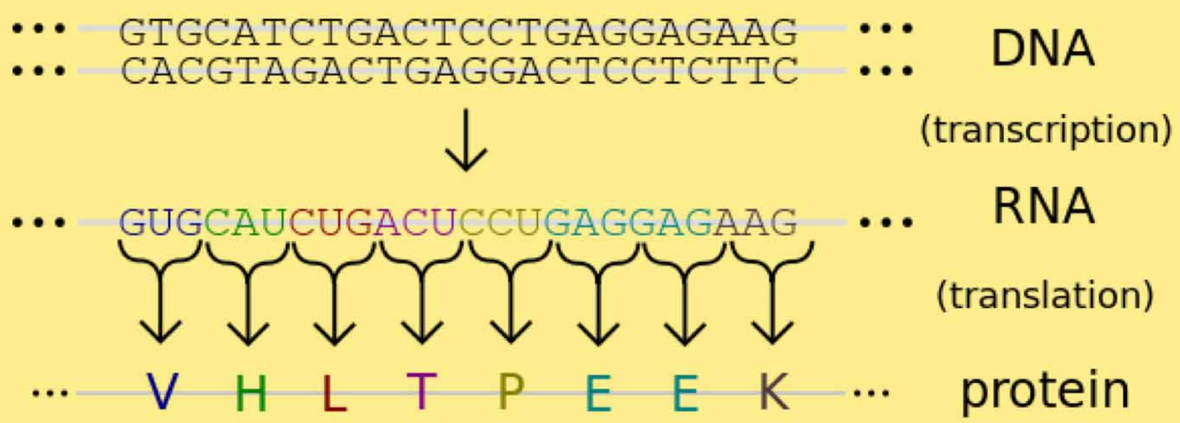
When the genotype is expressed, traits are created, called the **phenotype**

structural genes that code for proteins,

genes that code for RNA

regulatory genes that code for gene expression





Bacterial biochemistry

A primer

Cell wall Synthesis

Transpeptidase crosslinks the peptidoglycan net in the cell wall of Gram-positive bacteria.

Folic acid synthesis:

PABA used by bacteria to make DHF, needed for purine component of DNA,

Nucleic acid metabolism

Circular Double stranded DNA

No nuclear compartment

Unwinds for transcribing

RNA Polymerase binds to promotor region

makes a complementary copy

Protein synthesis: Translation

Messenger is **mRNA**

Binds machinery for **petide construction**

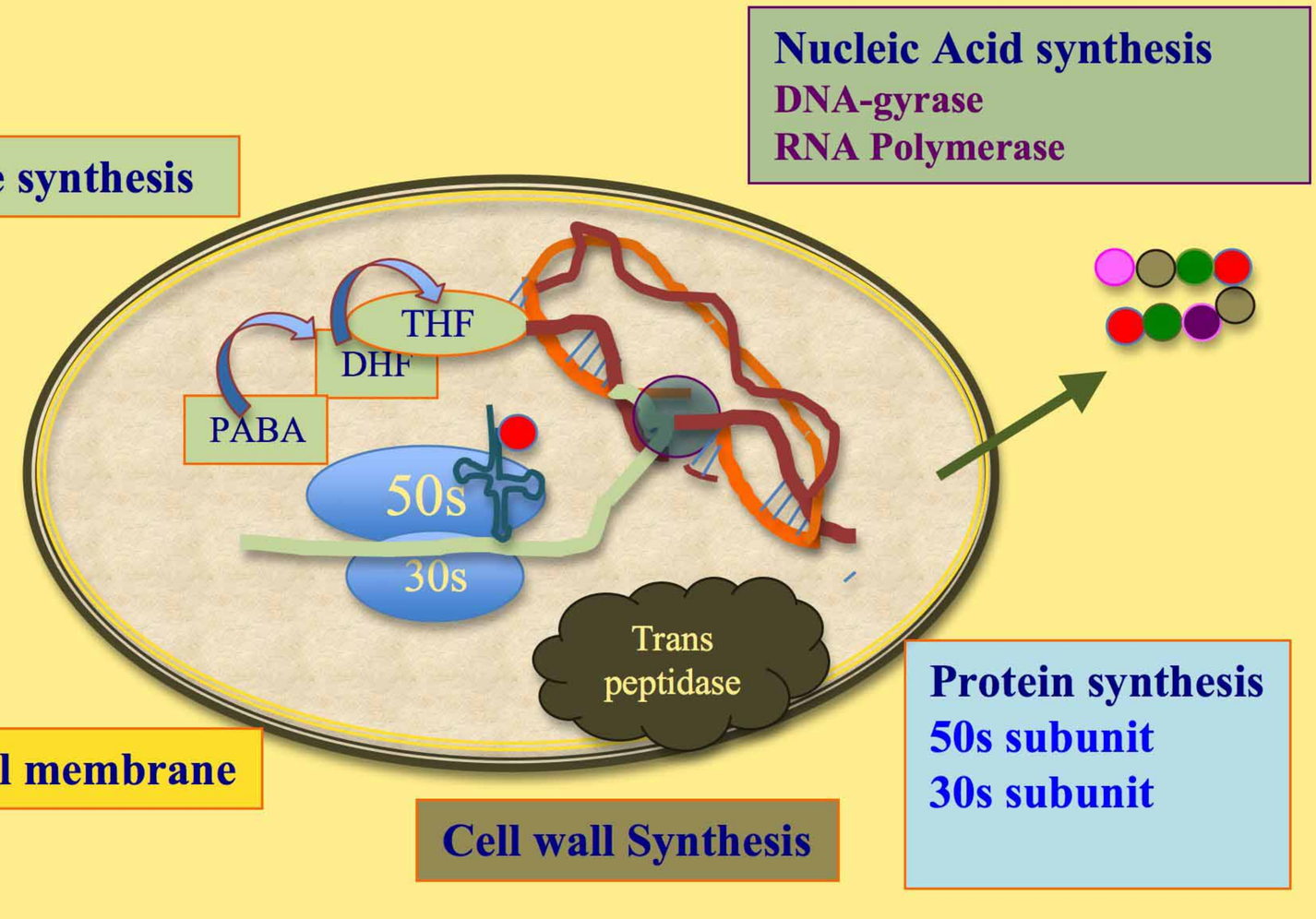
RIBOSOME

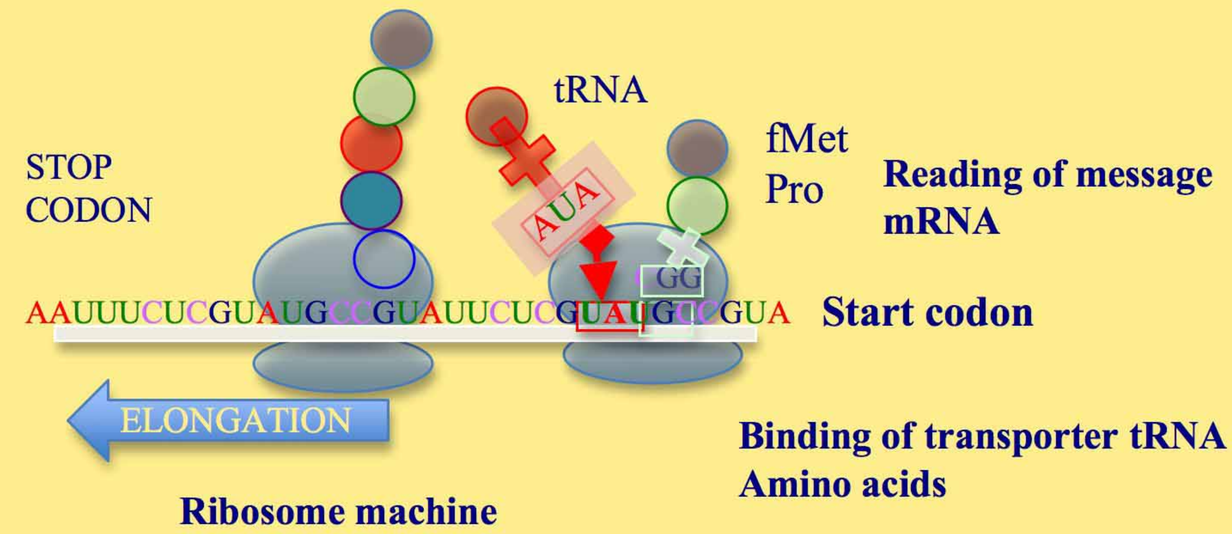
50s/30s subunits: Different than human

Amino acids brought to ribosome

Transporter tRNA has anticodon

Fits into slot





Pre-antibiotic world

1924: 16-year-old **Calvin Coolidge Jr.**, son of the President blister on the toe playing tennis. Septicaemia dead in a week.

November 1930, **W.W. ("Dodger") Whysall**, Nottinghamshire cricketer, Toured Australia: Scored 75 at Adelaide and 76 at Melbourne.

Wisden cricketer of the year 1925

slipped at a dance grazed elbow,

died of septicaemia a fortnight later

1:9 people with skin infection

3:10 pneumonia

5:1000 deliveries

Sore throats: kidney and chronic heart disease

After 90 years, antibiotics now less effective.

1945: Fleming's Nobel Address

"It is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to kill them… There is the danger that the ignorant man may easily underdose himself and by exposing his microbes to non-lethal quantities of the drug make them resistant."

Edvard Munch, *The Sick Child*, 1885–86
moment before the death of his older sister Johanne Sophie TB; aged 15.
Six completed oil paintings and many studies over 40 years.

Christian Krohg:
The Sick Girl

Before antibiotics

Arsenic and Mecury

VD rates of British x7 than the Germans, refusing to acknowledge any problem

1915: British medical officers Le Havre, counted 171,000 visits to brothels in one street

1916: Defence of the Realm Act, crime to approach men in uniform. No longer allowed to solicit openly

1918: 60,099 in France, more than trench foot and frost bite in entire war. Half contracted at home

VD caused **416,891** hospital admissions among British and Dominion troops: **1million** French

US Army lost use of 18,000 servicemen per day.

2nd commonest reason for absence from duty

7 million lost person-days and discharge of 10,000

Ettie Rout: NZ nurse in Egypt

VD a medical issue, not a moral.

1917 designed prophylactic kits on her own initiative. Letter ***NZ Times*** advocating condoms and clean brothels

Outrage her name forbidden in print £100 fine.

NZ authorities issue her kits carefully kept secret

Decorated by the French

Established hygienic brothel for NZ troops in Paris

H. Dewitt Welsh WWI

Ettie Rout

Sulphonamides

Synthetic antimicrobials contain the sulfonamide group

Bayer AG-IG Farben. Coal-tar dyes bind to bacteria and parasites might target harmful organisms

1932: Gerhard Domagk Fought at Ypres found a red dye synthesized by Josef Klarer inhibited mouse bacterial infections **Prontosil:** dyed skin red

1939: Accepts Nobel Prize arrested by Gestapo. (pacifist Carl von Ossietzky 1935 Peace prize, now in concentration camp)

1935 Leonard Colebrook Student of Almroth Wright at Mary's. Cure for puerperal fever. 38 pts

Isolation of burns patients reduced Strep contamination from 83% to 5%

Pro-drug of already known **sulfanilimide** off-patent rush of me-to drugs; **elixir sulfanilimide** disaster of 1937; 100 child deaths from ethylene glycol

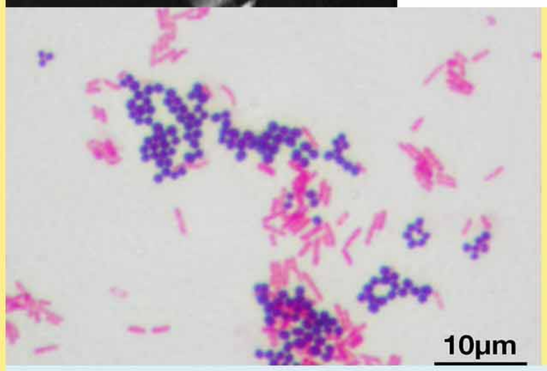
1936: Franklin D Roosevelt, Jr: strep throat life-threatening complications. Saved with Prontosil

Treatment prevents **rheumatic fever**

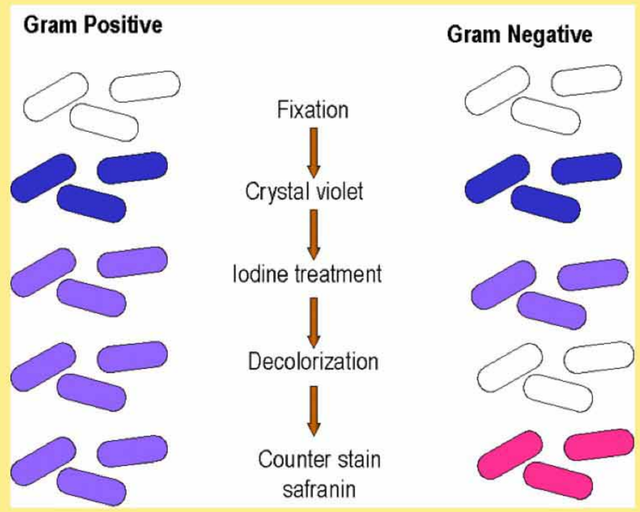
Occurring 20d later

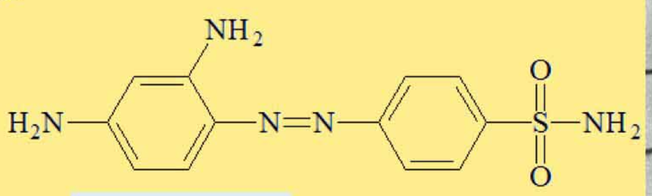
cardiac complications 5% mortality.

2010: 345,000 deaths, 1990/463,000

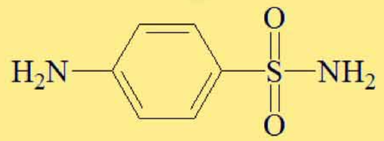


Hans Christian Gram
(1853 - 1938) Danish bacteriologist.





Prontosil



sulfanilimide

Carl von Ossietzky:
Editor of antifascist: *Die Weltbühne*
Guilty of treason for publishing about re-armament
1992: upheld by Federal Court of Justice.
1935: Nobel Peace Prize dies of TB under arrest

Sulphonamides: New versions

M&B 693 (**sulfapyridine**), made at the Dagenham lab of May and Baker

G.M. Evans: Birmingham, 100 patients lobar pneumonia mortality rate 78% to 8%.

Oct 1938, May and Baker licensed Merck USA. preferred treatment for pneumonia, saving 33,000 a year in the US.

1943: Winston Churchill: Pneumonia. **"This admirable 'M+B' (Sulphapyridine) from which I did not suffer any inconvenience, was used at the earliest moment and after a week's fever the intruders were repulsed."**

WWII, each G.I. carried a first-aid pouch containing 5g sulfa powder and a dressing bandage. Whenever anyone was wounded, the sulfa powder was sprinkled into the wound. Medics carried sulfa pills too.

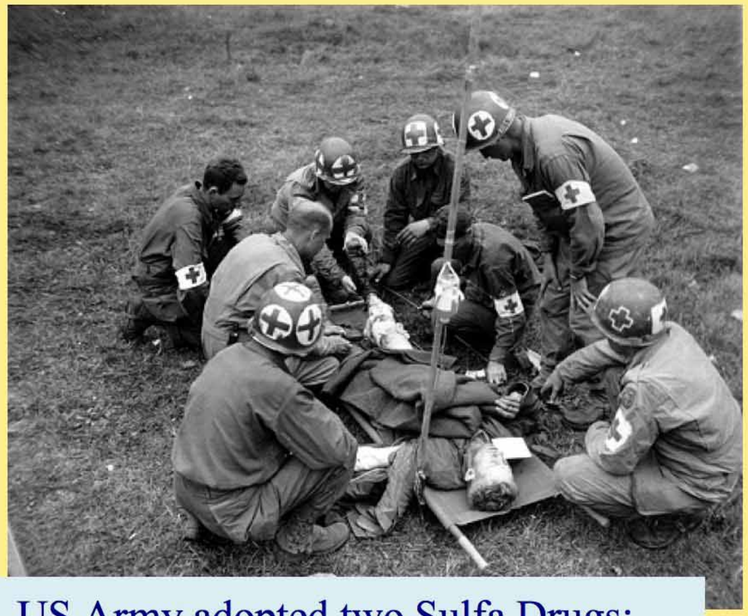
Use today limited: Resistance and side effects

Malaria and Toxoplasmosis: sulfadoxime and pyrimethamine (*Fansidar*)

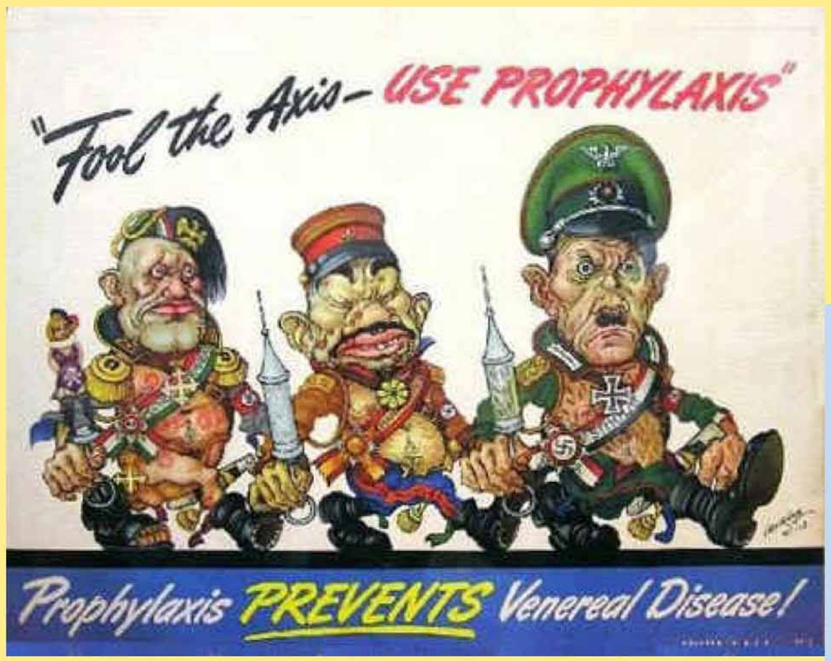
Co-trimoxazole UTI

NSAID, Diamox, Azopt,

allowed to drink only weak whisky and soda and not to smoke at all. No cigars. "*Dear Nurse, pray remember that man cannot live by M & B alone.*"



US Army adopted two Sulfa Drugs; **Crystalline Sulfanilamide** and **Sulfadiazine Tablets**.



Arthur Szyk caricature:

Every G.I. was issued with an **Individual Chemical Prophylactic Packet** 1 Tube containing 5 Grams of Ointment (30% Calomel + 15% Sulfathiazole)
Direction Sheet
Soap Impregnated Cloth
Cleansing Tissue

Penicillin

Sir Alexander Fleming, 1881- 1955 Q St Mary's WW1 battlefront

1923: discoveries enzyme lysozyme

1928: more than 650 penicillium moulds, few make penicillin. ***Penicillium notatum***

WW2 concerted research at Oxford to purify the mould juice and extract the penicillin from it.

1939: Ernst Boris Chain: discover penicillin's therapeutic action and its chemical composition. Theorized the structure of penicillin

Florey: first clinical trials in 1941

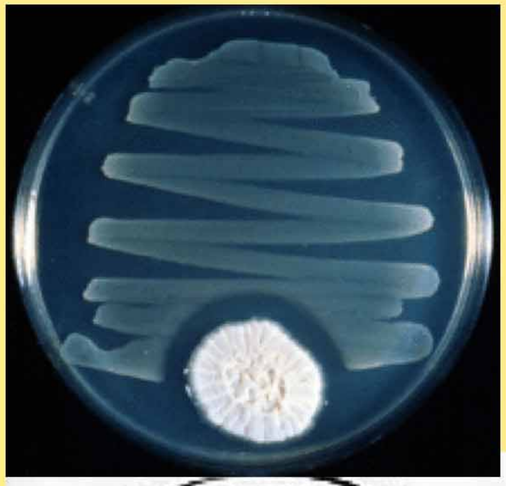
First patient, **Albert Alexander**, policeman scratched by a rose thorn severe facial infections. eye removed. Within a day started recovering. Not enough penicillin, died. 20 gallons of mould juice needed to treat infection

1941: **Heatley** & **Florey** visit Department of Agriculture's Northern Regional Research Lab Peoria, Illinois larger scale prod^{n}.

A. J. Moyer. addition of corn-steep liquor to the fermentation produced a ten-fold increase in yield.

Ironically, the most productive strain came from a moldy cantaloupe bought in Peoria fruit market

Outrage: Between 1947 and 1949, Moyer filled for four patents regarding his work on Penicillin.



Penicillin

1941 and 1945 STD's in US Army: 43/1,000/yr.
Continental US-based army personnel: 30/1,000/yr
(Vietnam War 1963 to 70: 262/1,000/yr)
90% gonorrhoea and 1% syphilis
1943: gonorrhea required a hospital treatment of 30d
Curing syphilis remained a 6m ordeal.

US War Production Board controlled the disposition of all penicillin produced. Aim to have adequate for D-Day

1943: **John Mahoney:** Public Health Service: Efficacy of Penicillin

Winston Churchill & General Poole:

'This valuable drug must on no account be wasted. It must be used to the best military advantage'.

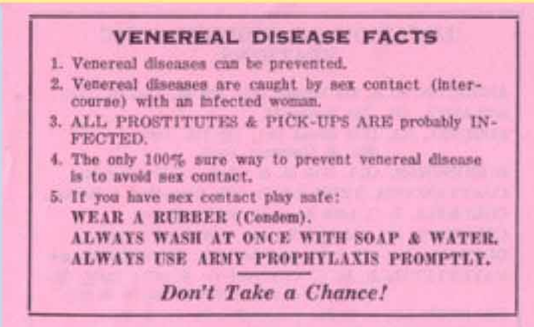
British medical officers in Sicily, treating 40,000 VD cases a month, x20 than number treated in England.

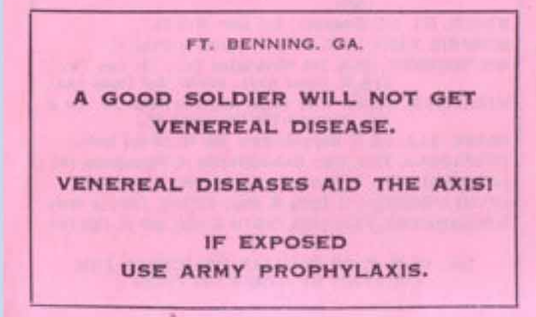
1944: VD reduced 30-fold, still 600 incapacitated/day.

Sickness reduced to 5days, patient remained on duty

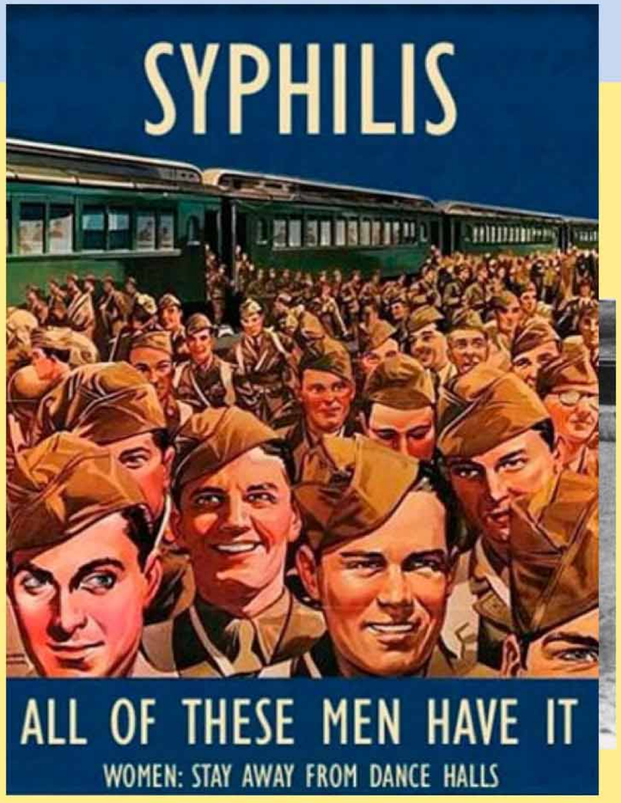
June 44: US Army adopted penicillin for wounds. Supply of penicillin had increased. British soon followed

Allocation of penicillin within the Military controversial:
1943: first shipment arrived North African Theatre, decision between using it for 'sulfa fast' gonorrhea or infected wounds.
Col Edward D. Churchill, Chief Surgical Consultant, opted for use in those wounded in battle.
Florey wanted to conduct trials on burns and gangrene
Military manpower shortage.
The Theatre Surgeon made the decision to use the available penicillin for those 'wounded' in brothels.





1943: Reginald Mount



Antibiotics in nature

50m years ago Amazon ants cultivate fungus for food

Leaf-cutter ants, dominant herbivore of the Neotropics. use fresh leaf substrate for their fungal partner

Symbiosis evolved to 230 spp of ants and diverse fungal strains.

Fungal crop is attacked by a parasitic fungus, *Escovopsis*.

To prevent infections, ants fungus grooming, run mouths over their crops removing parasite spores

Some ants have a second mutualism with (***Actinomycetes Pseudonocardia spp***) that produces many antibiotics

Queens carry fungal crop in their mouths and the bacteria on their exoskeletons to their new colony.

The ant–fungus–bacteria mutualism ancient system, special anatomical adaptations to house and nourish the *Actinomyces*

New Antibiotic discovery

1941: Selman Waksman Ukranian born American soil scientist Rutgers Agricultural College.

Antibiotic: molecule made by a microbe that antagonizes the growth of other microbes (not a good definition, includes CO_2 & O_2)

1943: Albert Schatz: Waksman's lab, search antibiotic effective against TB and Gram-negative bacteria – responsible for penicillin-resistant diseases.

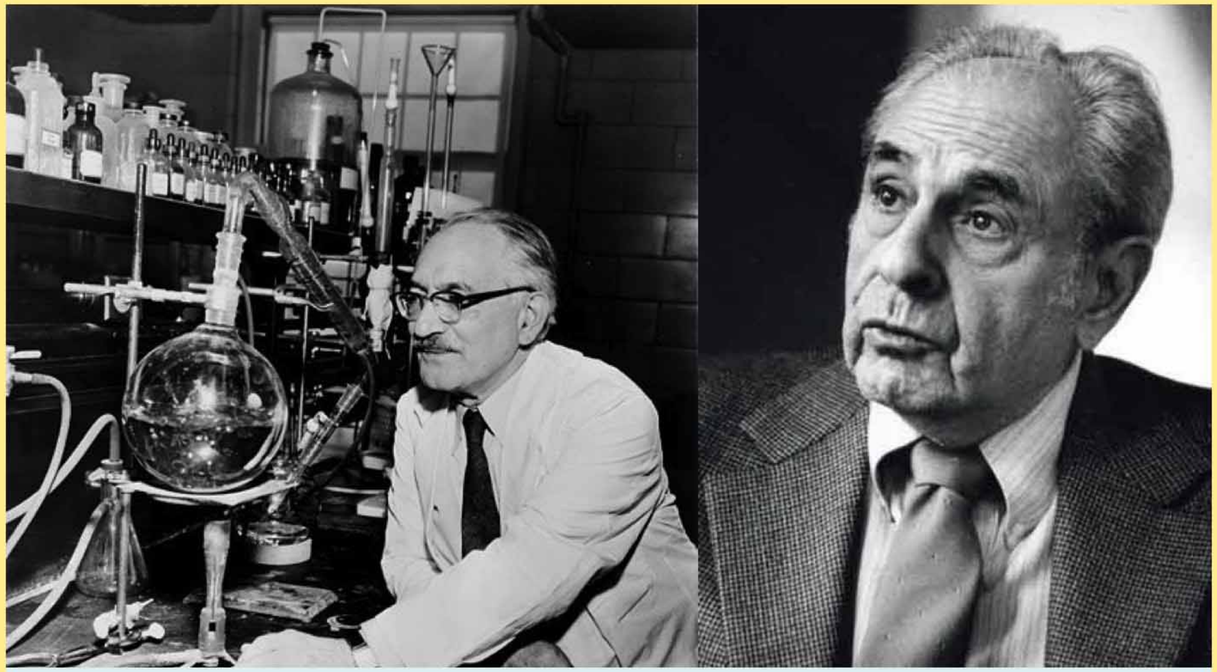
Two related strains of Actinomycetes inhibited tubercle bacillus and several Gram-negative bacteria. antibiotic named **STREPTOMYCIN**.

Controversy over Waksman's Nobel Prize and Royalties

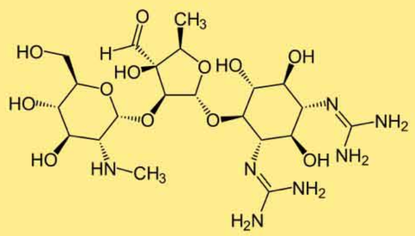
1945–1955: **penicillin**, produced by a fungus, **streptomycin, chloramphenicol**, and **tetracycline,** produced by soil bacteria,

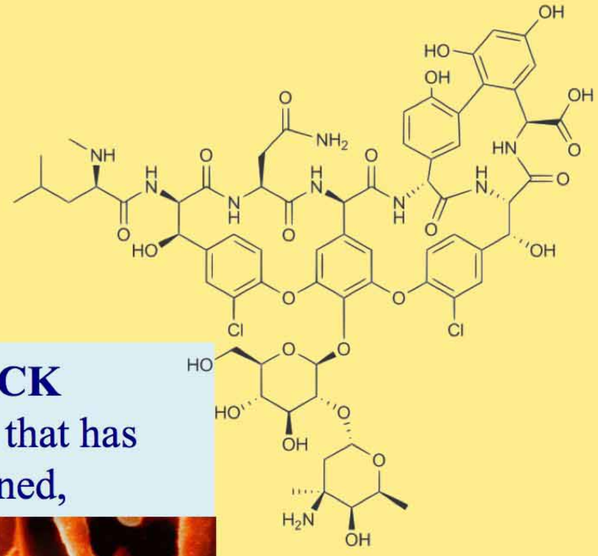
1953: Vancomycin Edmund Kornfeld (Eli Lilly); soil sample from jungles of Borneo by a missionary. *Amycolatopsis orientalis.*

Treatment of penicillin-resistant *Staph aureus*



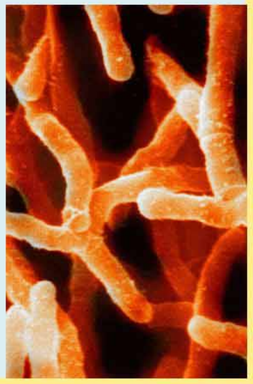
Albert Schatz (1920 –2005: two related strains Actinomycetes stopped the growth of tubercle bacillus and several Gram-negative bacteria. One strain mouth swab from a duck, the other from soil. Antibiotic derived from these bacteria "**streptomycin**".



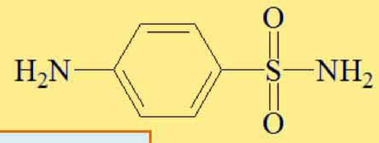


NEEDLE IN THE HAYSTACK

If 10,000 actinomycetes (soil bacteria that has produced most antibiotics) were screened,
2,500 would produce antibiotics.
2,250 would make streptothricins
125 streptomycin
40 tetracycline.
1:100,000 Vancomycin
1:1 million erythromycin
1: 10 million daptomycin



Mechanism of antibiotics

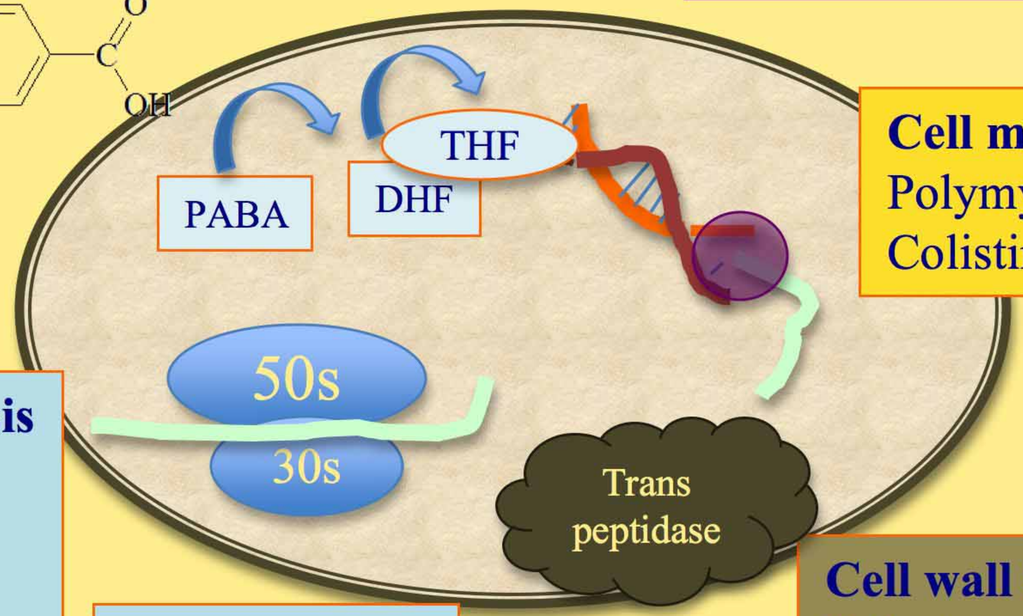


sulfanilimide

PABA

Folate synthesis
Sulfonamide
Trimethoprim

Nucleic Acid synthesis
DNA-gyrase Quinolones
RNA Polymerase Rifampicin



Cell membrane
Polymyxins
Colistin

Inhibitors of metabolism

Inhibit synthesis of purine and thymidylate precursors folic acid or tetrahydrofolate.

Sulfonomides inhibit bacteria-specific reaction.

Inhibitors of Cell wall

β-lactam ring mimics binding site of **transpeptidase**

Penicillin: (rupture) weak cell wall cannot contain growth.

Disrupters of nucleic acid synthesis

Rifampin inhibits prokaryotic RNA

Fluoroquinolones inhibit DNA gyrase, a bacterial enzyme that unwinds DNA in preparation for replication and transcription.

Protein synthesis
50s subunit
Macrolides
Clindamycin
Linezolid
Chloramphenicol

30s subunit
Tetracyclines
Aminglycoside

Cell wall Synthesis
B-lactams (penicillin)
Vancomycin
Bacitracin

Disrupters of protein synthesis

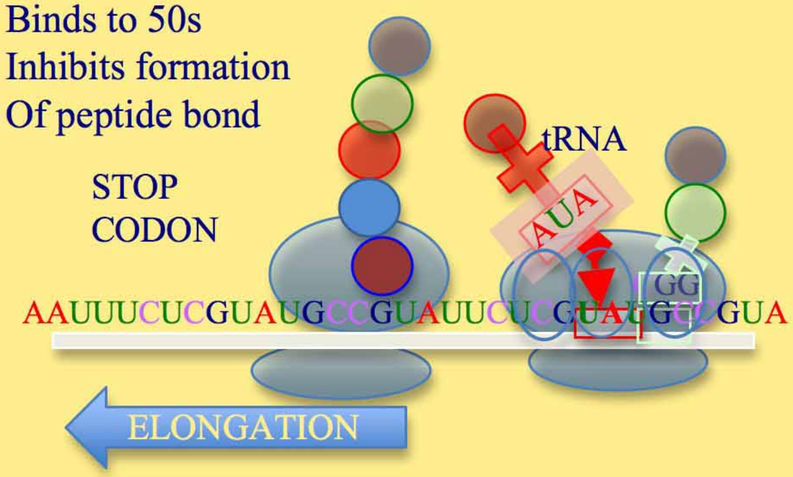
Aminoglycosides inhibit nucleic acid or protein synthesis

shaped molecules fit in pockets of bacterial ribosomal RNA.

disrupt ribosomal structure.

specific to bacteria. No effect on human L-shaped pocket

Chloramphenicol
Binds to 50s
Inhibits formation
Of peptide bond



Erythromycin
Binds 50s rRNA
Blocks exit of peptide

Streptomycin
Changes shape of 30s
mRNA read incorrectly

Tetracyclines
Interfere with t-RNA
Anti-codon reading

Antibiotic resistance

1950s, bacterial diseases no longer public health threat!

1943: Abraham & Chain reported strains that could hydrolyse and inactivate B-lactam before use as an antibiotic.

Organismsthat make toxic products either produce resistance factors to stop themselves being killed by their own products or do not use that metabolic pathway

Many microbes also carry resistance genes for antibiotics that they themselves cannot produce,

B-lactamases found in remote Alaskan soils

Pathogens with multiple mutations and combinations of r genes evolve and survive successfully.

Naturally occurring antibiotic resistance is common

Environmental resistome.

Comprises all of the antibiotic resistance genes.

Includes cryptic resistance genes (not necessarily expressed) present in bacterial chromosomes.

20,000 potential resistance genes (r genes) of 400 types, predicted from bacterial genome sequences

CDC estimates that in the United States, more than two million people are sickened every year with antibiotic-resistant infections, with at least 23,000 dying as a result .

URGENT THREATS
Clostridium difficile
Carbapenem-resistant Enterobacteriaceae (CRE)
Drug-resistant Neisseria gonorrhoeae

SERIOUS THREATS
Multidrug-resistant Acinetobacter
Drug-resistant Campylobacter
Fluconazole-resistant Candida (a fungus)
Extended spectrum β-lactamase producing Enterobacteriaceae (ESBLs)
Vancomycin-resistant Enterococcus (VRE)
Multidrug-resistant Pseudomonas aeruginosa
Drug-resistant Non-typhoidal Salmonella
Drug-resistant Salmonella Typhi
Drug-resistant Shigella
Methicillin-resistant Staphylococcus aureus (MRSA)
Drug-resistant Streptococcus pneumoniae
Drug-resistant tuberculosis

CONCERNING THREATS
Vancomycin-resistant Staphylococcus aureus (VRSA)
Erythromycin-resistant Group A Streptococcus
Clindamycin-resistant Group B Streptococcus

Antibiotic resistance

Mediated by acquisition of genetic elements containing resistance genes.

Horizontal gene transfer: common in bacteria, even distantly related ones.

a) Transformation: naked DNA is released on lysis of an organism and is taken up by another organism. Genes integrated into the recipient cell.

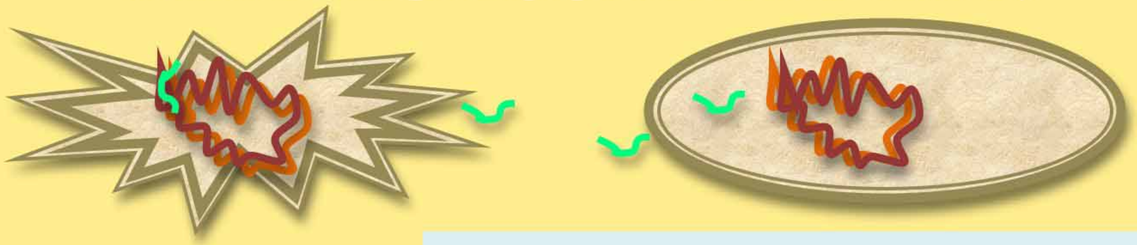
b) Transduction: antibiotic-resistance genes are transferred by bacteriophages integrated into the chromosome of the recipient (lysogeny).

c) Conjugation form a mating bridge DNA is exchanged, can result in acquisition of antibiotic-resistance genes.

Plasmids, transposable genetic elements, and genomic islands, which are transferred between bacteria via horizontal gene transfer

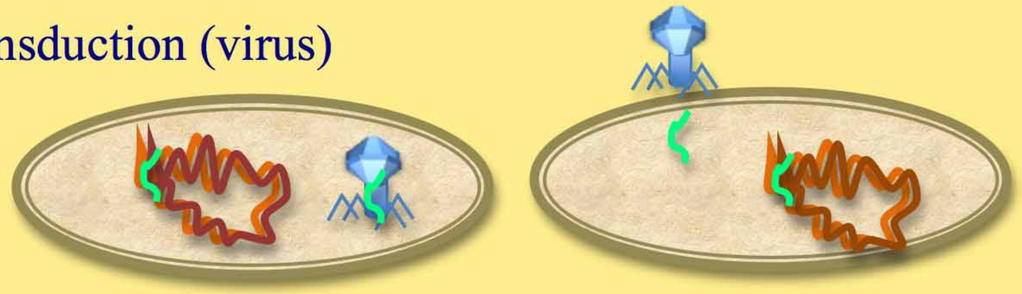
Resistance gene, ***mecA***, stops β-lactam antibiotics from inactivating the enzymes (transpeptidases) that are critical for cell wall synthesis.

Transformation (picking up DNA from environment)



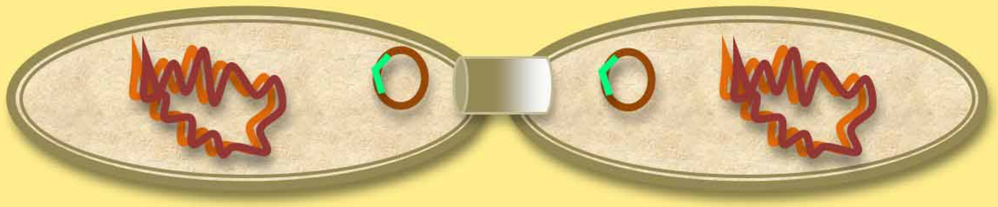
Pneumococci: *rough* (R) and virulent *smooth* (S).
slippery polysaccharide coat: evasion of Φ
1927: Griffiths Liverpool showed R could transform to S by factor from killed S.
1943: Avery as an old man showed this factor was DNA

Transduction (virus)



vast majority of bacteria contain prophages, either integrated into chromosome or extra-chromosomal elements, accounting for substantial genetic variability and vehicles for HGT

Conjugation (bacterial sex)



Mechanisms of antibiotic Resistance

Drug inactivation or modification:

β-lactamases: enzymatic deactivation

chloramphenicol acetyl transferase: modified antibiotic no longer binds to ribosomes.

Alteration of target site:

Alternative binding proteins: **PBP_{2a}** binds penicillin less well. Still functional as alternative transpeptidase

The ribosome can be methylated so that an antibiotic cannot bind to it. (erythromycin)

Random mutations alter **gyrase** make it unrecognizable to antibiotics but functional.

Alteration of metabolic pathway:

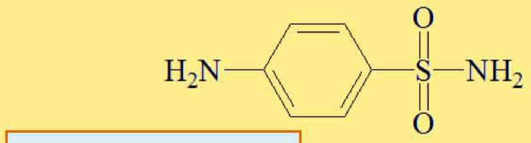
some sulfonamide-resistant bacteria do not require (PABA), use preformed folic acid.

Reduced drug accumulation:

Decreasing drug permeability

Increasing active pumping out

Carbapenem resistance in *E.coli*



sulfanilimide

PABA

Folate synthesis
Sulfonamide
Trimethoprim

Nucleic Acid synthesis
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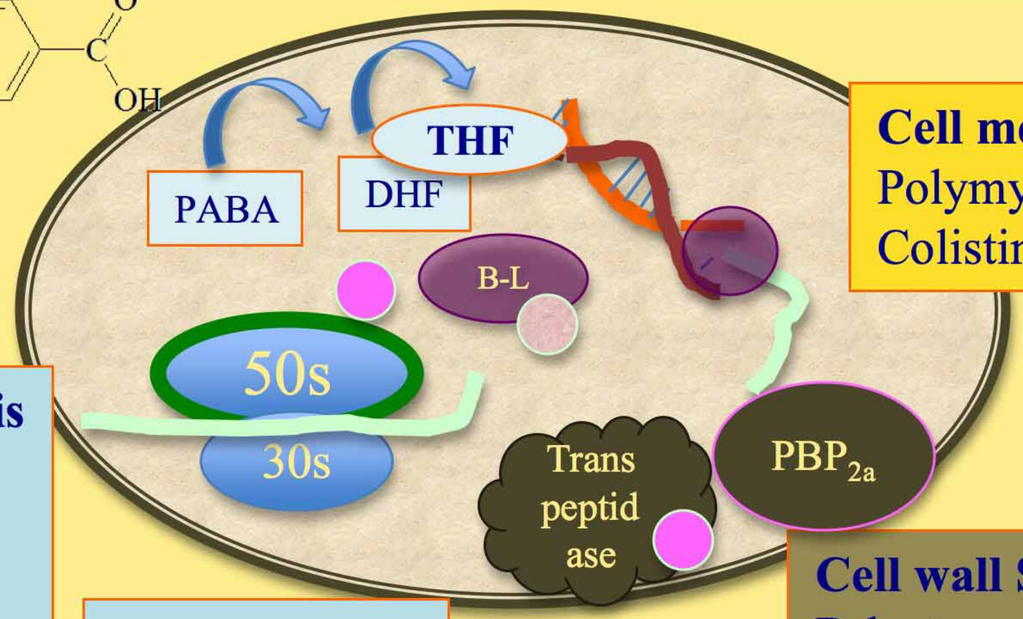
RNA Polymerase Rifampicin



Cell membrane
Polymyxins
Colistin

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50s subunit
Macrolides
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Linezolid
Chloramphenicol

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Cell wall Synthesis
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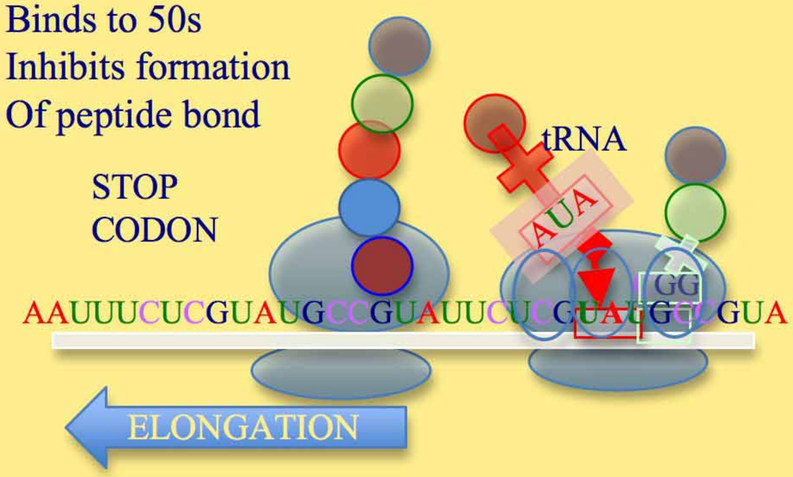

Erythromycin
Binds 50s rRNA
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Changes shape of 30s
mRNA read incorrectly

Tetracyclines
Interfere with t-RNA
Anti-codon reading

Methicillin Resistant StaphA

S. Aureus 1880: Sir Alexander Ogston's coccus

Nasal commensal in 30% of the population

Skin infections such as boils: Deadly if enters body

Many virulence factors

Quorum sensing: α-pyrone signals to LuxR solo receptor on other bacterial cells. bacteria to recognize and clump together

Bind to host/prosthesis: eg S. aureus surface protein G adheres to epithelium; heart valve:

Tissue Penetration: Proteases, lipases etc

Evade defence: Protein A binds AB, avoids Φ: Leucocidins: antiΦ microcapsule: Biofilm

Toxins: Enterotoxin, Toxic Shock

S. aureus genomes are circular and contain approximately 2.8 million bp 2700 coding sequences, structural and regulatory RNAs.

Core genome, 80% DNA conserved: contains mostly housekeeping genes

Accessory genome mobile DNA contains varying virulence factors.

Mobile genetic element:

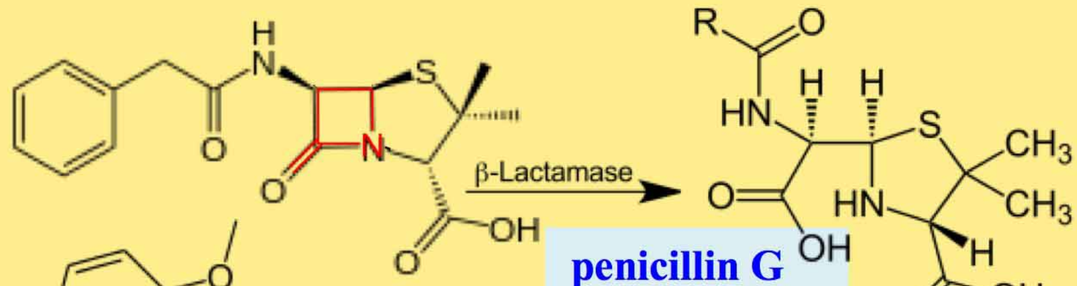
Plasmids: auto-replicating DNA molecules. Varied genes Transposons (Tn),

Insertion sequences (IS),

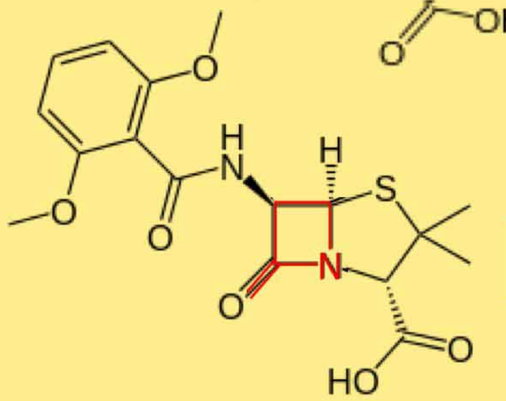
Bacteriophages,

Pathogenicity islands: Discrete DNA sequences acquired by HGT

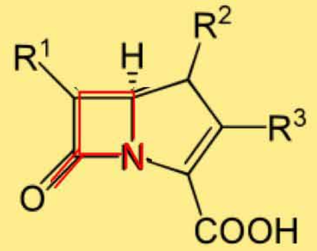
Staphylococcal cassette chromosome



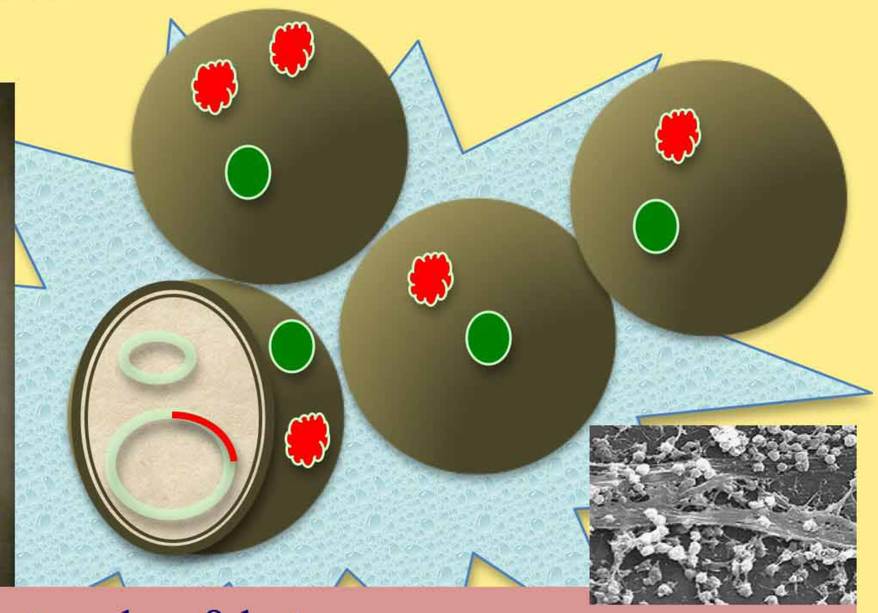
penicillin G
β-lactam ring



Methicillin.



Carbapenems broad spectrum β-lactam antibiotics resistant to most β-lactamases



1944 penicillin: Resistant produce β-lactamase
1950's: Erythromycin Boston City Hospital, withdrawn <year because 70% of S. aureus became resistant.
1959: **Meticillin**: First designed anti-resistance antibiotic:
1961: The first MRSA strain (NCTC 10442) in UK, archaic clone spread around the world
1997: Vancomycin resistance

MRSA

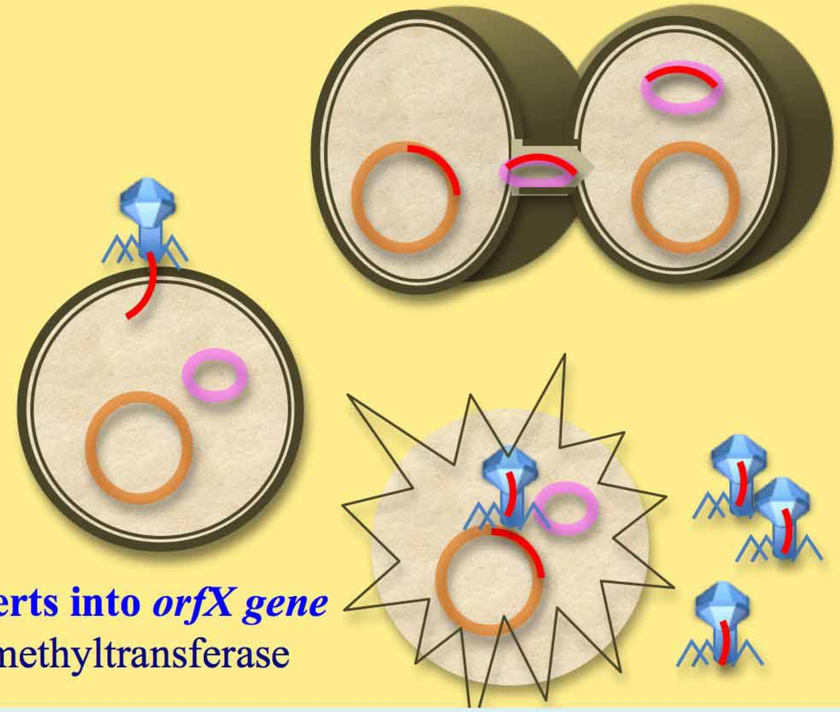
Unlike E.coli Staph not very good at picking up DNA from environment; acquire Plasmids by transduction (virus) or conjugation (bacterial sex)

Antibiotic resistance propagated by conjugative plasmids like pLW1043, the first vancomycin-resistant S. aureus vector

1: small plasmids carry a single resistance determinant;

2: larger (15–30 kb) low copy (4–6/cell) plasmids carry several resistance determinants;

3: conjugative multi-resistance plasmids



mecA: Inserts into *orfX gene*
ribosomal methyltransferase

BlaZ gene: β-lactamase: Plamids Human/Chr bovine isolates

SCCmec low affinity binding pr for penicillin **PBP_{2a}** flanked by recombinase genes (ccrA/ccrB or ccrC) permit horizontal transfer

6 types SCCmec MRSA clones.

Types I, II, III health care–associated clones: large 35-60kb; contain multiple resistance determinants.

Types IV, V, VI; community-associated clones. 15kb Less resistance genes but more toxins

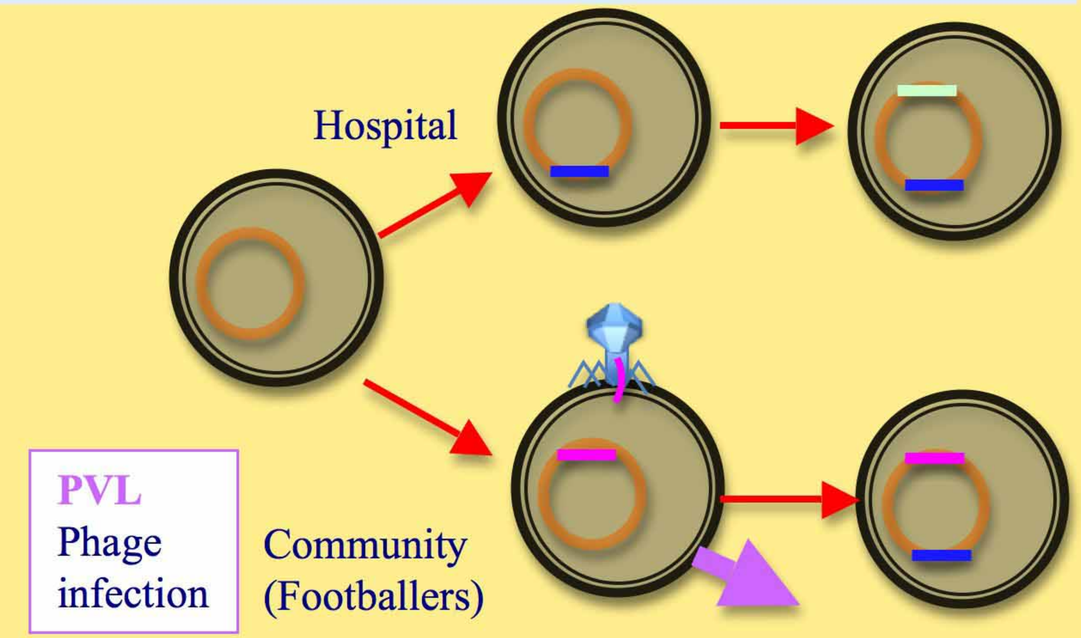
1997: new community MRSA, with enhanced virulence.

USA_{400} clone: Killed healthy children.

CA-MRSA has most of the properties of MRSA, but different mec gene clusters, and has acquired new pathogenicity genes, cytotoxic **Panton-Valentine leukocidin** for abscess formation in skin and lungs

Hospital MRSA has become Multi-resistant

Phages encode most virulence factors and variety of toxins,
immune modulator staphylokinase (*sak*) host tissue destruction
chemotaxis inhibitory protein CHIP (*chp*)
staphylococcal inhibitor of complement SCIN (*scn*)
superantigens (*sea*, *seg*, *sek*, *sek2*, *sep*, *seq*). cause food poisoning, toxic shock syndrome and necrotizing fasciitis.
Panton-Valentine leukocidin (*lukF-PV*, *lukS-PV*)
leukocidins (*lukM*, *lukF*)) form pores in leukocytes necrosis
exfoliative toxin A (*eta*) severe skin infections.



Reducing antibiotic resistance

Who's Hogging the pills?

(i) growth promotion/prophylactic use in animals;

(ii) therapeutic/prophylactic use in humans;

(iii) therapeutic/prophylactic use in aquaculture;

(iv) therapeutic/prophylactic use in household pets;

(v) pest control/cloning for plants and agriculture;

(vi) use as biocides in toiletries and in hand care and household cleaning products; and

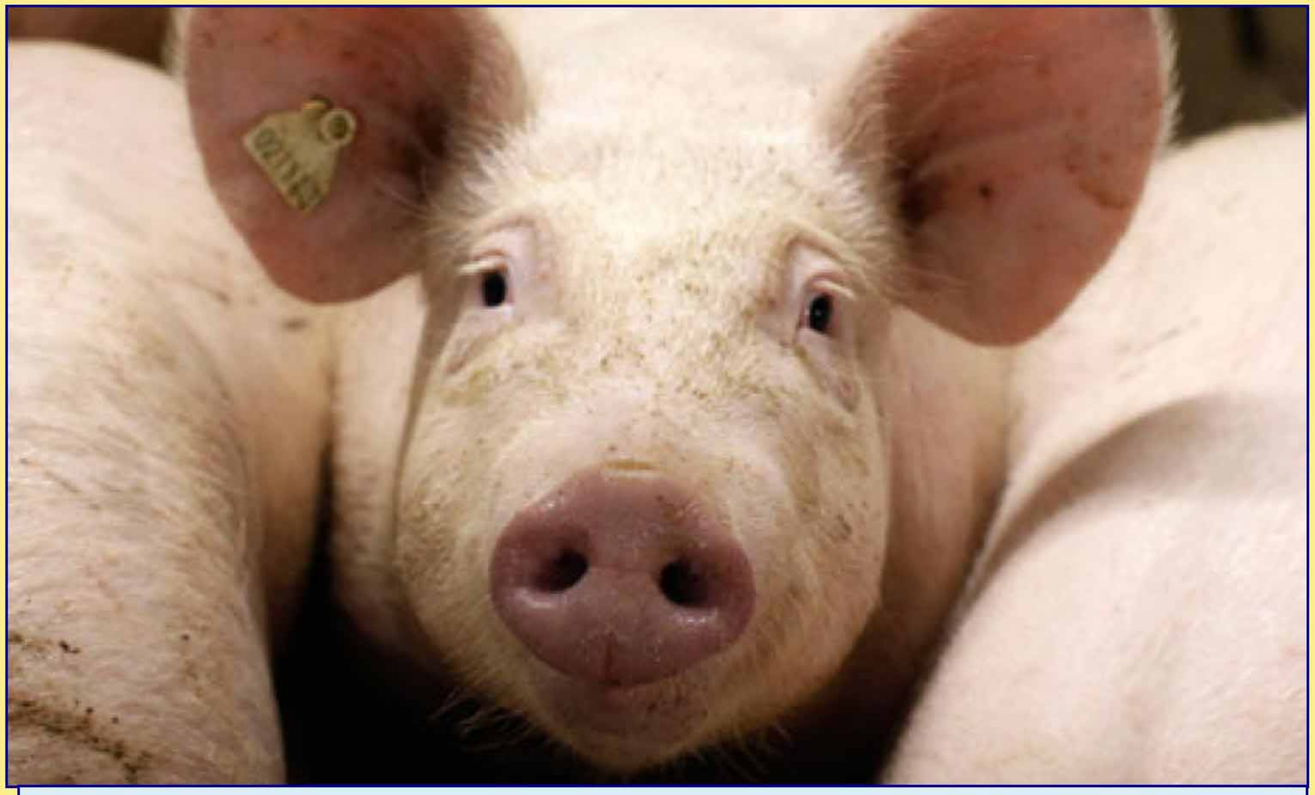
(vii) culture sterility, cloning, and selection in research and industry.

Therapeutic use in humans: less than half of all antibiotics produced commercially.

Dumping of ciprofloxacin into rivers 50 kg a day by manufacturers in Hyderabad

Millions of tons of antibiotic compounds dumped into biosphere over the last half-century

Antibiotic resistance is a multifaceted global issue, and a coordinated **international effort will be needed**



Farms use double amount of antibiotics than humans
Small doses in feed curb low-grade infections
Antibiotics increase pigs' growth rate by 2.5%, the difference for farmers between profit and loss.
US$1 per pound for a pig that costs about $0.94 per pound to produce.
300mg of antibiotics to produce 1Kg meat and eggs
2009: 13,600 tonnes 80% of the antibiotics sold in US used on on animals that aren't sick. fatten up faster and prevent health problems in animals kept in extreme confinement (often filthy) conditions

Antibiotics in Farming

which antibiotic with your bacon sir?

2004: Holland, girl awaiting heart op screened +ve for MRSA. So did her family and their pigs!

Swine have their own varieties of staph, and shouldn't get *S. aureus*, the human strain.

Sequence Type 398 *S.Aureus*: strain of resistant staph different from hospital and community variety; also resistant to tetracycline, routinely used in factory farming

Spread widely found on retail meat across world

2012: Price et al: genetic analysis of **ST398** strains Originated humans; crossed into livestock, acquired the **SCCmec** cassette resistance genes then back to humans

Germany: 24% of farmers poultry and pigs were colonized with the animal MRSA strain **ST398**

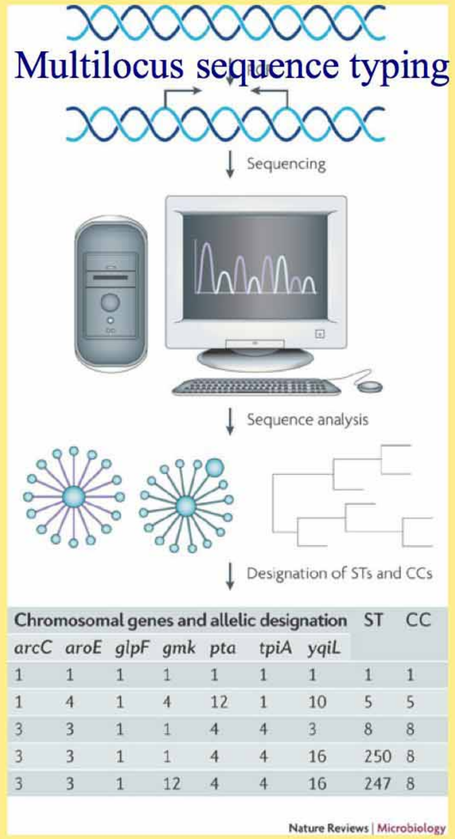
Meat in 5 US cities, 47% contained *S.aureus*, 96% resistant to at least one antibiotic. 52% resistant to at least three antibiotics **ST1** in pigs, **ST5** in chickens and **ST398** in turkey.

2013: livestock-associated MRSA, drug-resistant staph, in UK poultry (Xmas Turkey's) for the first time

Risk is not food poisoning but transfer to skin

Resistant bacteria from farms are escaping

E.coli, new highly resistant type found on a large number of dairy, pig and poultry farms in England and Wales.
2006: EU banned antimicrobials to promote growth
Still able to be prescribed by a vet.
Tetracyclines, previously used as a growth promoter 50% of antimicrobial sales to farmers in the UK
2009: 350 tonnes of antibiotics used on UK farm animals
Dec 2013, FDA's new guidelines simply ask that industry voluntarily eliminate the routine use of antibiotics



Chromosomal genes and allelic designation							ST	CC
arcC	*aroE*	*glpF*	*gmk*	*pta*	*tpiA*	*yqiL*		
1	1	1	1	1	1	1	1	1
1	4	1	4	12	1	10	5	5
3	3	1	1	4	4	3	8	8
3	3	1	1	4	4	16	250	8
3	3	1	12	4	4	16	247	8

Nature Reviews | Microbiology

Antibiotic-resistance

2013 CDC: antibiotic-resistant infections cost $20bn US: 2m infected with resistant organisms a year

untreatable superbugs kill 23,000 of them

TB drug resistance. Cocktails of anti-TB drugs essential multidrug resistance

2005: HIV in KwaZulu-Natal TB: lethal descendant: extensively drug-resistant **(XDR-TB)** resistant to both the first-line antibiotics and at least 3 of the 6 remaining second-line drugs.

Between HIV and TB : Africa India and China so many deaths that it will change societies,

TDR strains, which are totally drug resistant

carbapenem-resistant Enterobacteriaceae (CREs).

antibiotics of last resort for *(E. coli*) and *Klebsiella*.

N. Carolina ***Klebsiella pneumoniae*** gene that encoded **Carbapenemase** enzyme on a **plasmid**

Jan 2008: 59yr-old Swede man ***K. pneumoniae*** resistant to carbapenems. dismantled the antibiotics with a different enzyme, a **metallo-β-lactamase**

Link to clinics in India, through medical tourism new enzyme **New Delhi metallo-β-lactamase** (NDM),

Dec 2013: NDM-1 in wastewater treatment plants in China.

Dame Sally Davies, the UK chief medical officer, **CREs as a risk as serious as terrorism**

CONSEQUENCES OF RESISTANCE DIVERSE

Nov 2013: **BP:** operated on in Vietnam, returns to NZ dies of an infection totally resistant to everything!

A world where infection is so dangerous that even minor symptoms locked in confinement until they recover or die.

No routine surgery, no prosthetics (1:6 hip ops will die)

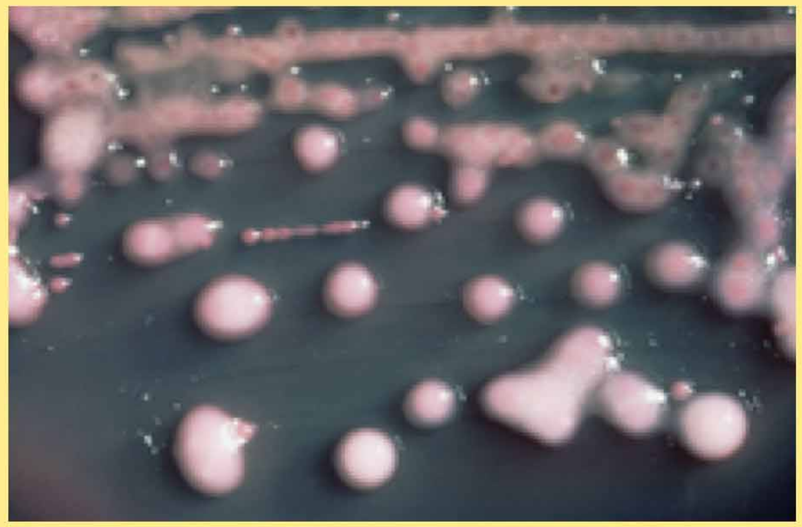
Childbirth becomes dangerous

Animal and fish protein prohibitively expensive

Unprotected sex resistant Gonorrhea and Syphilis

Streptomycin-resistant fire blight, destroyed Michigan's orchards, now in upstate New York

Louise Slaughter: Democratic Representative for New York. "Every year, more than 100,000 Americans die from bacterial infections acquired in hospitals, 70% of these infections are resistant to drugs. This abuse and overuse must stop."



Transferring genes therapeutically

Can Mobile genetic elements be used to transfer healthy genes to sick humans?

Suicide genes to cancer cells?

Inhibit blood vessels in AMD?

Replace faulty genes?

Absent gene: Failure of function

Replace the gene with healthy copy

Identification of the defect at the molecular level

A correcting gene

Introduce the gene into host cells (i.e., a vector)

Hostile environment for nucleic acids

Get across nuclear membrane

Integrate into a safe place (mutagenesis)

Get the gene to work

Vectors

Naked DNA plasmid

viruses

bacterial plasmids

nanoparticles

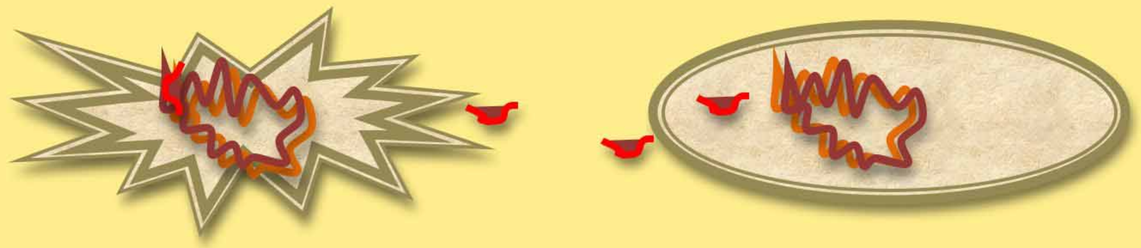
Variant gene: Abnormal protein.

Non functional

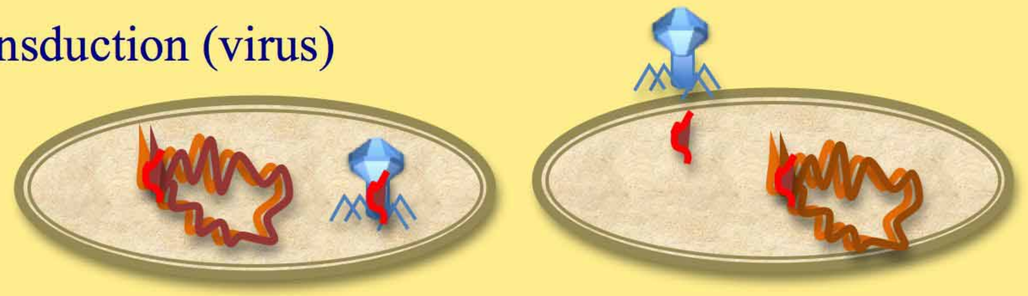
Malfunction

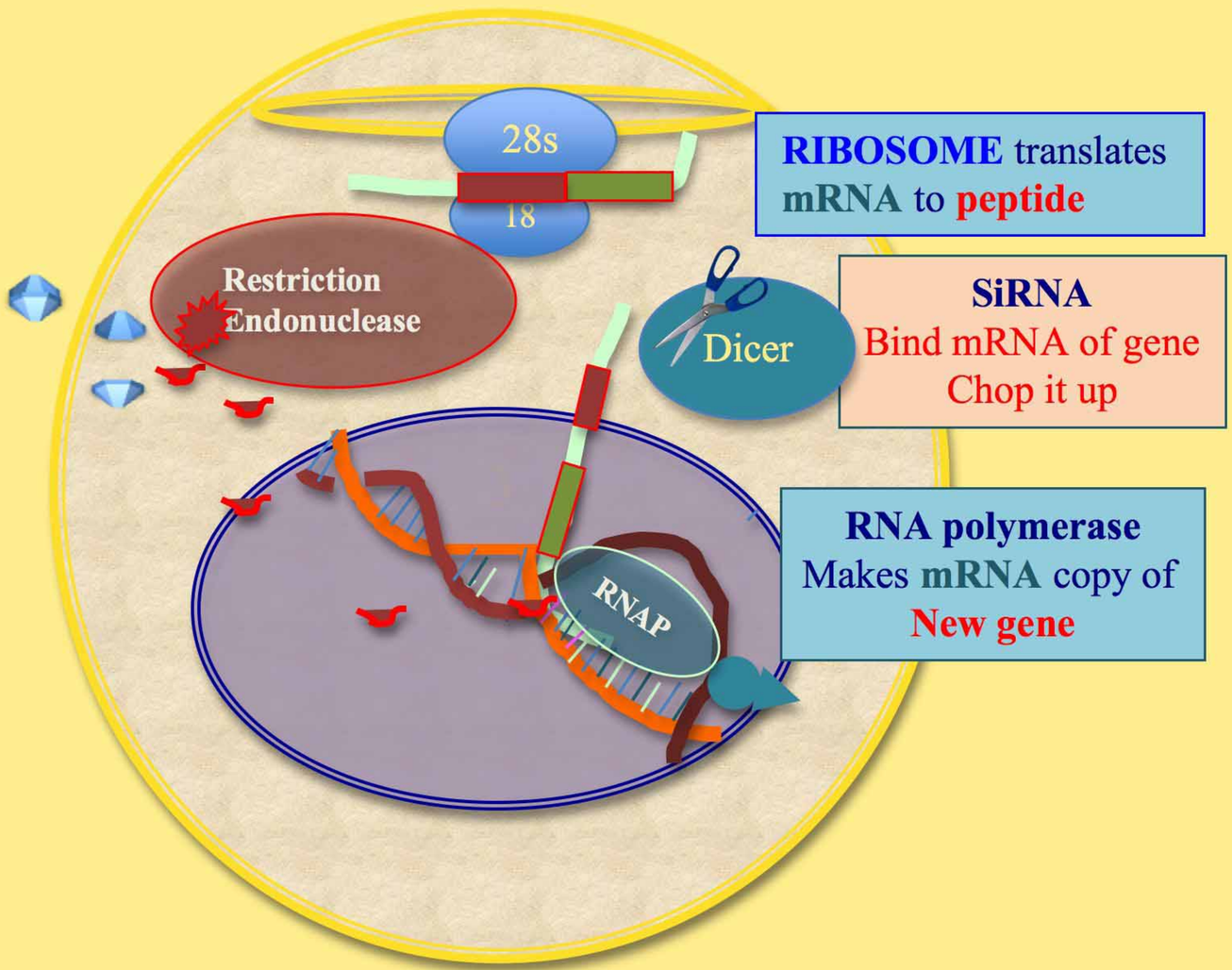
Gene deletion/silencing

Transformation (picking up DNA from environment)



Transduction (virus)





Gene editing

Restriction endonuclease: natural defense to viruses
Cutting invading DNA into pieces. discriminate self from viral DNA
Cuts DNA at specific recognition sequences: Molecular scissors
Allows specific gene knock-out: using nucleases to snip it out

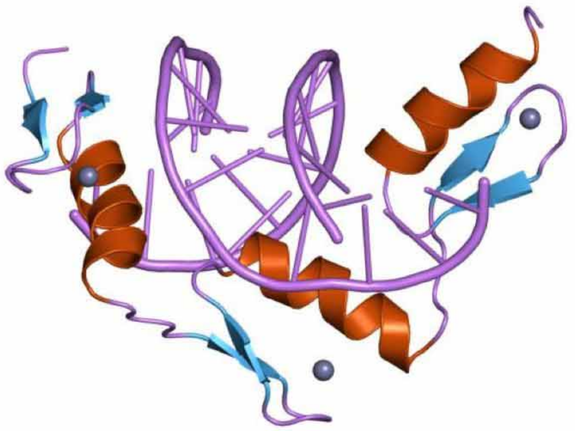
Zn finger nucleases: Artificial proteins (Fok1 restriction enzymes)
Wispy amino acid strands on a zinc ion.
The fingers bind to miscoded strands of DNA: Ds cut
Innate repair mechanism inserts the correct gene sequence.
Genome of billions of nucleotides several sites recognised by chance, $10^9/4^6$

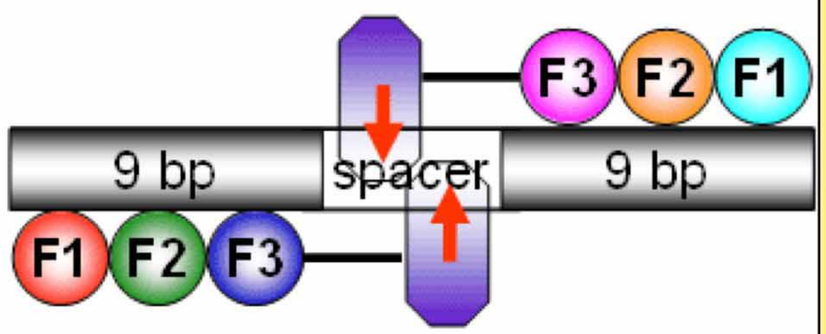
I-CreI homing endonucleases
a 22-nucleotide sequence might occur only once ($10^9/4^{22}$).
makes I-*Cre*I a promising tool
Necessary to engineer it to recognize/cut sequences of DNA different from its native homing site

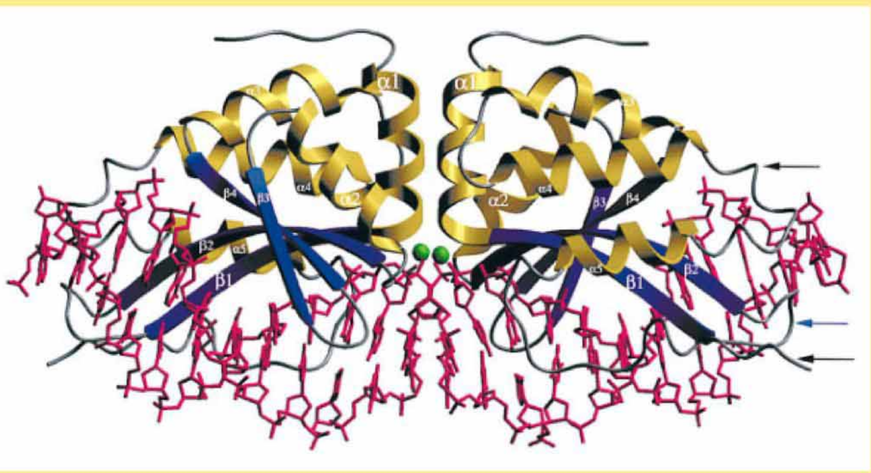
CRISPR: defense mechanism against bacteriophages. "clustered, regularly interspersed short palindromic repeats,"
A peculiar 20-50 **non-coding bp sequence** in bacterial DNA:
palindromic – reading the same forward and backward –
followed by a "spacer" sequence of 30 base pairs,
followed by the same **non-coding palindrome** again,
followed by a different spacer, repeatedly.

Spacers short sequences of bacteriophage DNA, transcribed to sRNA
A protein; **Cas9** uses these **sRNA** to find the same sequences in invading viruses and cut the viral DNA at the targeted site.
Bacteria add new spacers when infected with new viruses, gaining immunity from those viral strains.

Artificial guiding RNA to target and snip out any gene you like







Silence the gene

RNA interference (RNAi)

Block specific genes.

siRNA or **miRNA**,. Both processed by **Dicer** enzyme and incorporated **RISC complex**.

Small interfering RNA,

21bp: exogenous dsRNA taken up or enters via viruses

Destroys mRNA "interferes" with the translation of proteins

Natural mechanism: cytoplasmic cleavage of long dsRNA by **Dicer enzyme** forms short RNA duplexes. incorporated into the RNA-induced silencing complex (**RISC**).

miRNA: ssRNA endogenous (made by cell) non-coding ssRNA, introns of larger RNA molecules.

Expressed by genes whose purpose is to make miRNAs,

Regulate other genes, by Inhibiting translation of mRNA

Occur naturally in plants and animals

Imperfect base pairing between the small RNA and the target. , a single miRNA may target up to hundreds of mRNAs

The mRNA is either destroyed or stored.

Molecular Scissors

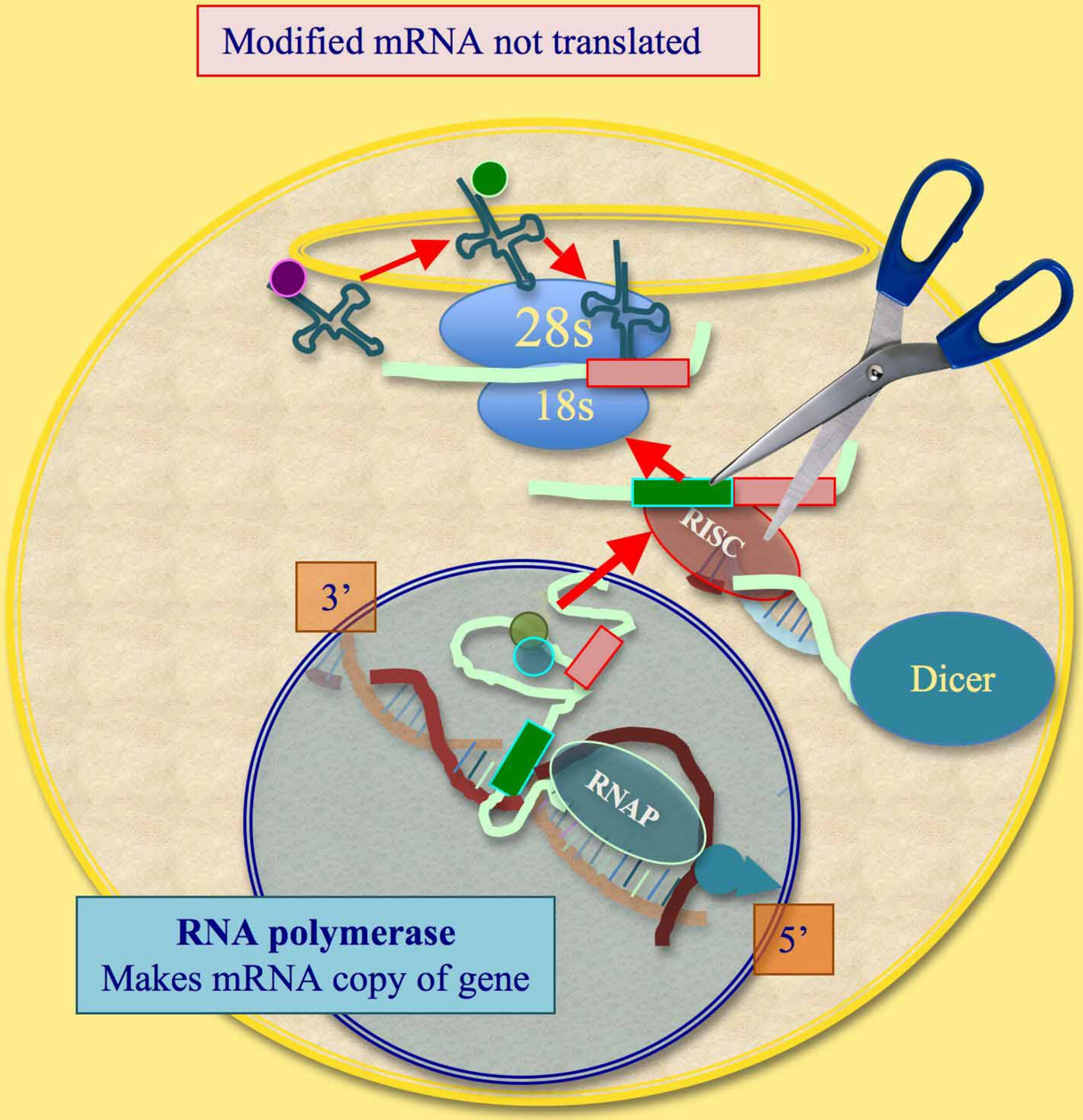
Binds and promotes cutting of mRNA at specific sequences.

Prevent the production of specific proteins.

dsRNAs can be designed to target essentially any protein-coding mRNA

Act as gene-silencing guardians in plants and animals that do not have antibody-or cell-mediated immunity

siRNAs delivered by inhalation with a nebulizer or intraocular injection investigated for treating respiratory syncytial viral & AMD



Huntington's. Short pieces of dsRNA (short, interfering RNAs or si RNAs) are used by cells to degrade RNA of a particular sequence. si RNA is designed to match the RNA copied from a faulty gene, abnormal protein product of that gene will not be produced

Gene therapy

Germ line Gene Therapy - altering the genetic makeup of egg or a sperm or blastomere

Advantages - cure passed to future generations. Avoids immunity.

Disadvantages - very controversial. numerous risks, error during transfer

Somatic Gene Therapy altering genes/chromosomes of body cells.

Advantages – effective treatments and cures, have been developed using gene therapy.

Disadvantages - not be passed on to offspring. Methodology of somatic gene therapy, such as the use of viral vectors, is difficult.

Ex-vivo Gene Therapy – cells cultured

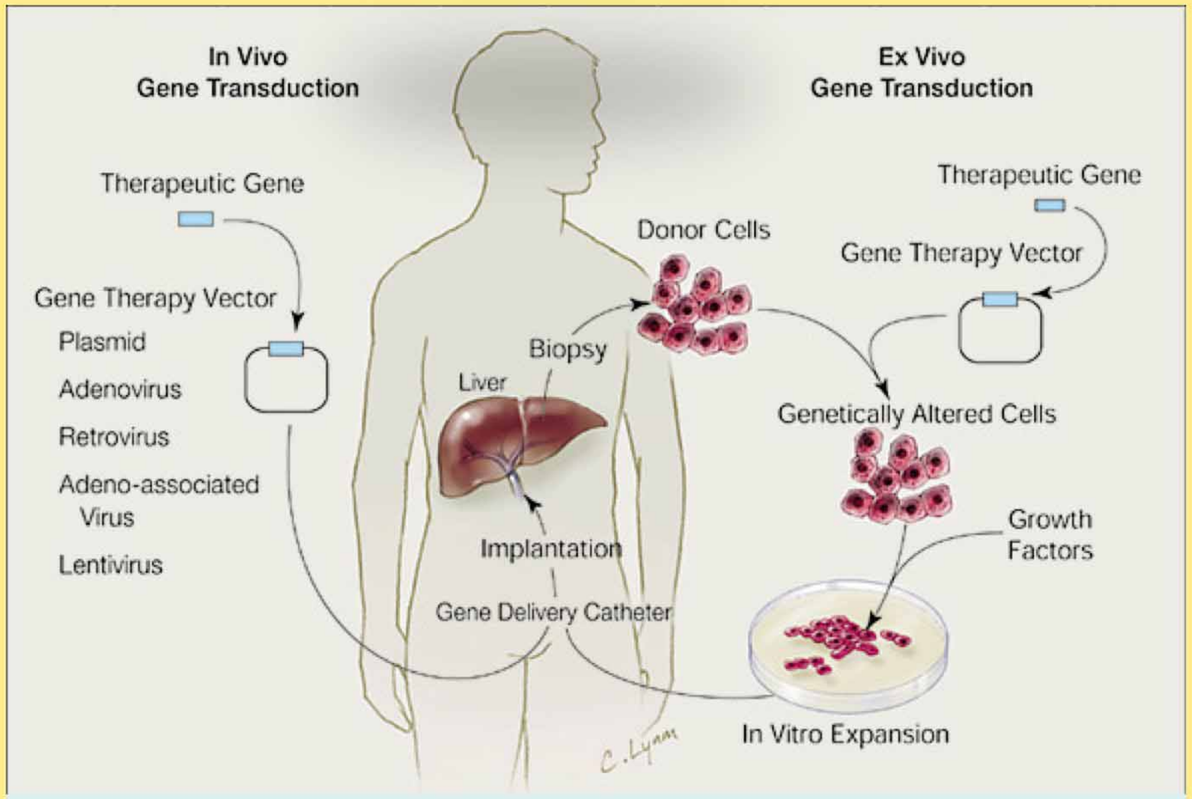
limitations - introducing engineered cells may trigger immune

Cells may malfunction

In-vivo Gene Therapy – gene is directly delivered to the recipient

More risky

possible immune reaction from the organism.



Transfer Genes: Vectors

Naked DNA,
plasmid,
viruses,
bacterial plasmids,
nanoparticles.

Ways to Deliver the Altered Gene

NON-VIRAL VECTORS

Non-infectious capacity to transfer large genes and low production costs. relatively low gene delivery and transgene expression efficiencies.

Physical Methods to Enhance Delivery

· Electroporation- short pulses of high voltage carry DNA across the cell membrane. High rate of cell death limited use

· Gene Gun- DNA is coated with gold particles forced penetration into the cell

· Sonoporation- ultrasonic acoustic cavitation disrupt the cell membrane

· Magnetofection- DNA is complexed to a magnetic particles, and a magnet is placed underneath the tissue culture dish

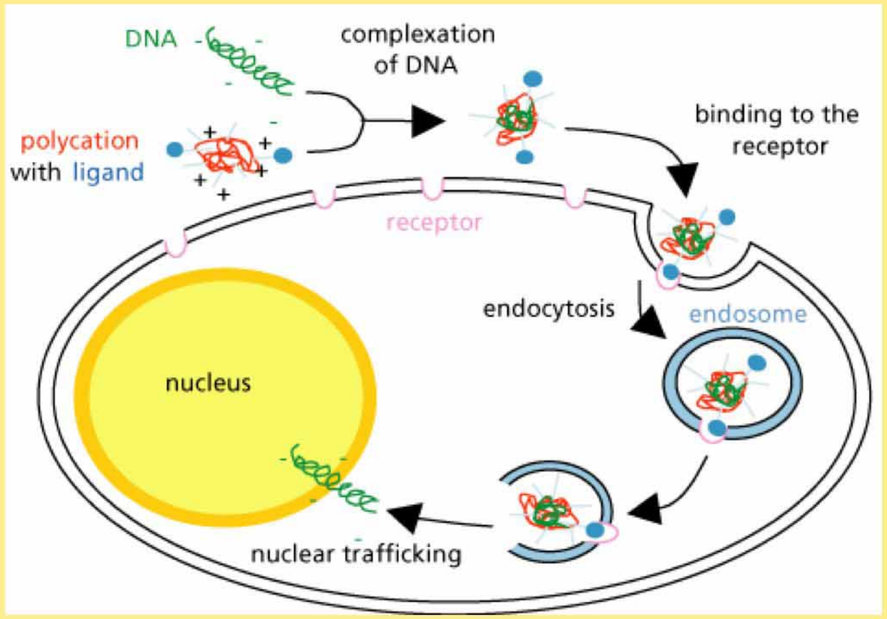
Chemical Methods to Enhance Delivery

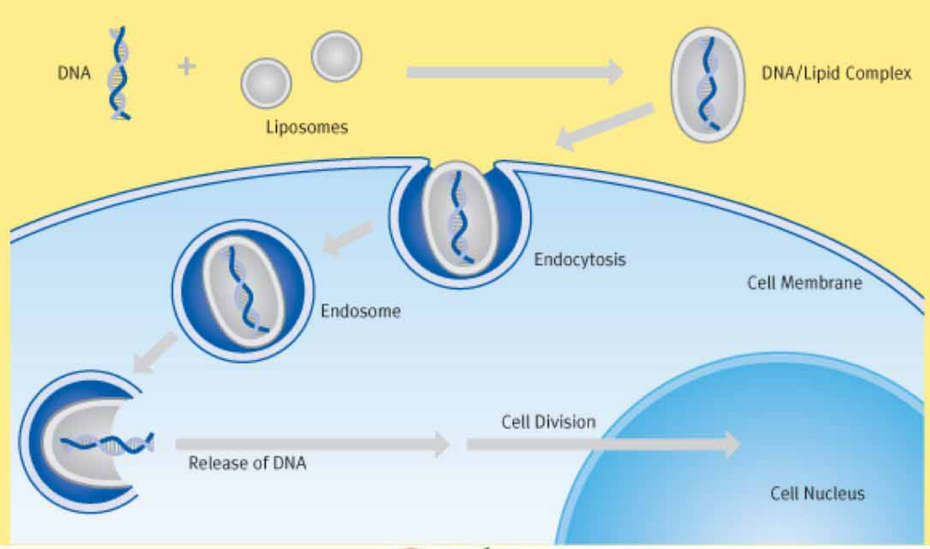
· Lipoplexes and polyplexes- DNA must be protected from damage and (positively charged). Initially, anionic and neutral lipids were used for the construction of lipoplexes for synthetic vectors.

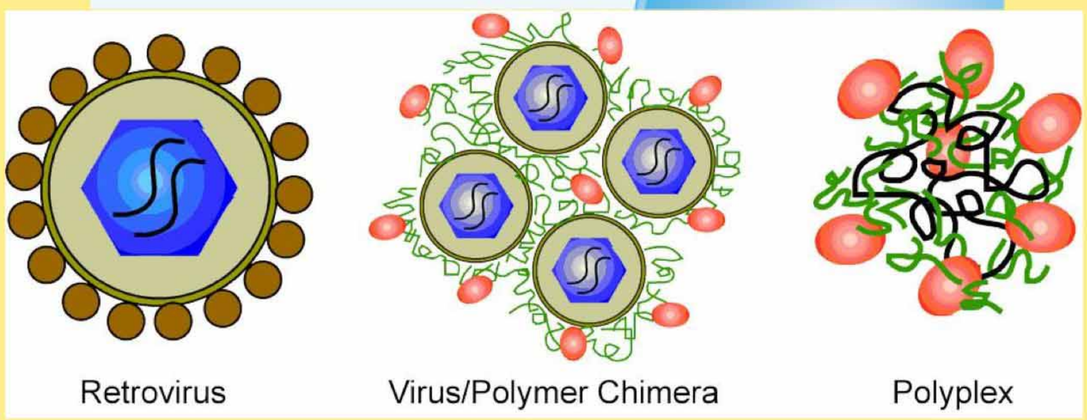
· Dendrimers- highly branched macromolecule with a spherical shape. dendrimer-nucleic acid complex is then taken into the cell via endocytosis.

· Hybrid methods- Virosomes combine liposomes with an inactivated HIV or influenza virus.

More efficient gene transfer in respiratory epithelial cells than either viral or liposomal methods alone.







Viral Vectors in gene therapy

Adenoviruses- 36kb dsDNA genomes cause respiratory, intestinal, and eye infections in humans.

causes URTI (common cold)

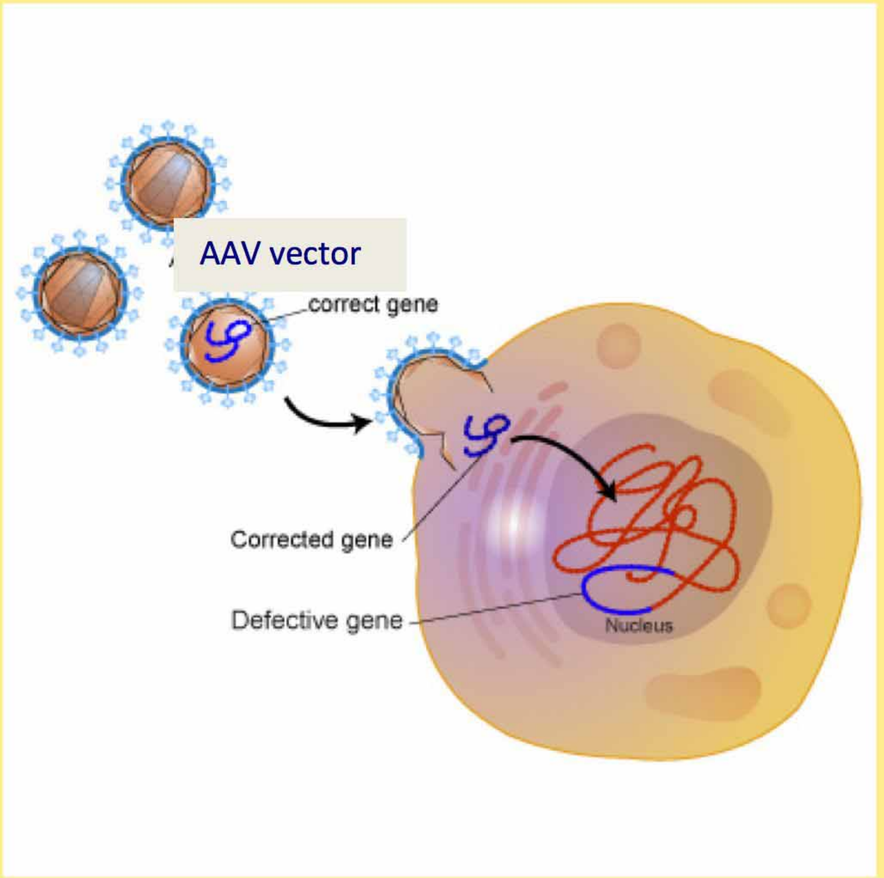
adenoviral DNA **does not** integrate into the genome and is not replicated during cell division

Adeno -associated viruses-AAV

small (25-nm), nonenveloped virus 4.7kb linear ssDNA genome.

infects humans and some other primate species. AAV is not currently known to cause disease.

Inserts DNA at specific site on chr19. However vectors miss the targeting sequence and insert at random low frequency (10^{-7})



Herpes simplex viruses- 150kb dsDNA infect neurons. HSV1 causes cold sores

Oncolytic for brain tumours melanomas

Can carry large payload

Persists in neural tissue: prolonged effect

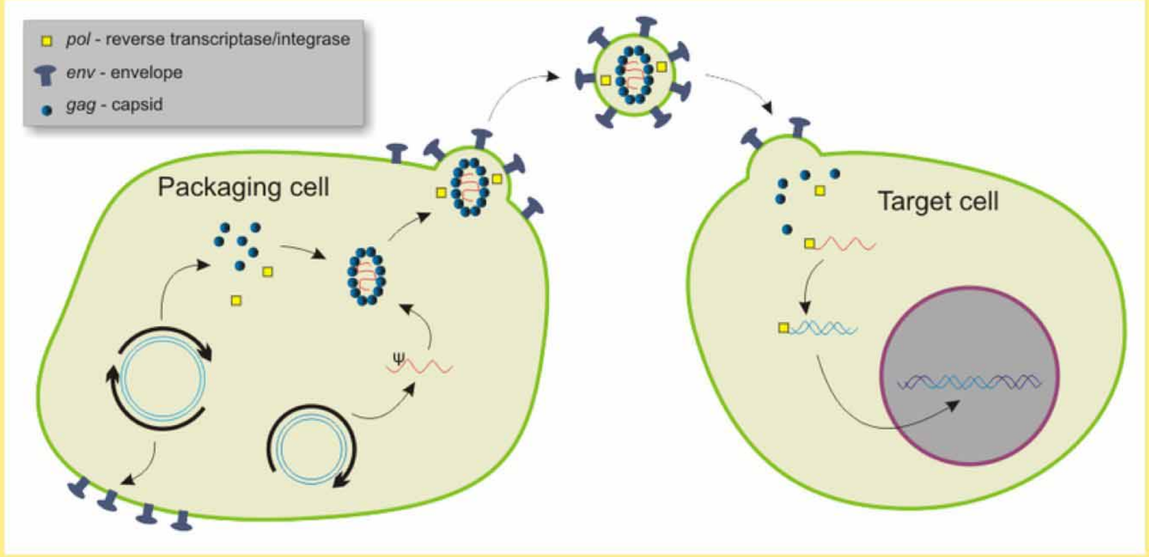
Retroviruses- 7-10kb RNA genomes. create dsDNA copies integrated into DNA of host cells. (HIV) is a retrovirus.

Problems is integrase enzyme randomly inserts viral gene

If inserted within a gene (insertional mutagenesis).

If regulating gene, uncontrolled cell division (cancer) can occur.

Addressed by utilizing zinc finger nucleases to direct the site of integration to specific sites.



Adenoviral and Adenoviral-Associated vectors

Adenovirus:

Large payload

1999: A **September 13, 1999**, Gelsinger ornithine transcarboxylase deficiency injected with adenoviral vector carrying corrected gene. He died four days later, September 17, at 2:30 pm, violent innate immune response to adenoviral vector leading to multiple organ failure

AAV2: vector for in vivo gene therapy stable and didn't cause much immune reaction.

Avigen hemophilia, muscule or intravenous administration.

little expression, and some immune toxicity.

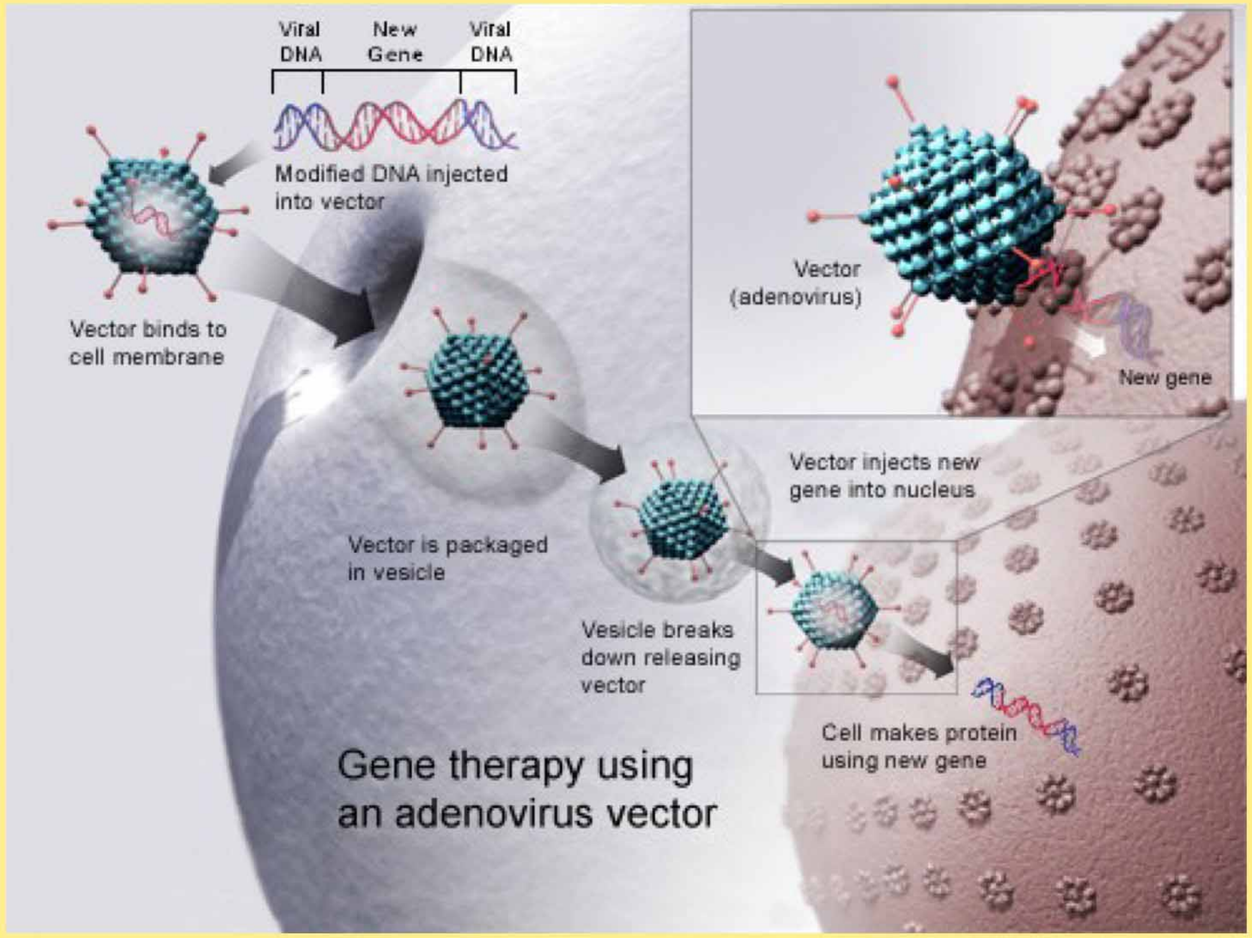
Targeted Genetics: cystic fibrosis, failed because there was no evidence for gene transfer.

Injected into arthritis joints: didn't work.

AAV2 Lebers

Don't integrate well: Effect decays with time

Useful for organ targeted therapies (Eye)



CLINICAL TRIALS INVOLVING AAV VECTORS

Condition	Gene product(s)	Phase
Cystic Fibrosis	CFTR	I/II
Canavan's disease	Aspartoacylase	I
Parkinson's disease	GAD65, GAD65, AADC, neurturin	I
Alzheimer's disease	Beta nerve growth factor	I
Alpha-1-antitrypsin deficiency	AAT	I
Arthritis	TNFR:Fc	I
Leber congenital amaurosis	**RPE65**	**I**
Hemophilia B	Factor IX	I
Late infantile neuronal lipofuscinosis	CLN2	I
Muscular dystrophy	Minidystrophin, sarcoglycan	I
Heart failure	SERCA-2a	I
Prostate cancer	Gran-mφ CSF	I/II/III
Epilepsy	Neuropeptide Y	I

Retro virus vectors

Mutations in at least 9 different genes cause inherited SCID

X-linked: IL-2Rγ gene defect: Maturation of T-lymphocytes, die within 1 year due to severe, recurrent infections

Modified retroviruses: Moloney murine leukemia virus

cells need to be dividing, nuclear membrane broken down

Integrate into DNA on cell division.

2000: first successful gene therapy

Ex vivo retroviral gene transfer of the γc-chain (common to several cytokine receptors) to stem cells from bone marrow

Cured boys with X-linked SCID

However, 4/10 French and 1/10 children in UK developed leukemia

T cells abnormally expressing *LMO-2*, powerful promoter sequence in the vector, needed to boost expression of the corrective gene, activated *LMO-2*, triggered cancer.

Adenosine deaminase deficiency

15% cases SCID caused by Defective ADA

Accumulation deoxyadenosine formed during DNA breakdown

Toxic to immune system

30 children treated with gene therapy

None developed leukaemia,

21 discontinued the enzyme replacement therapy

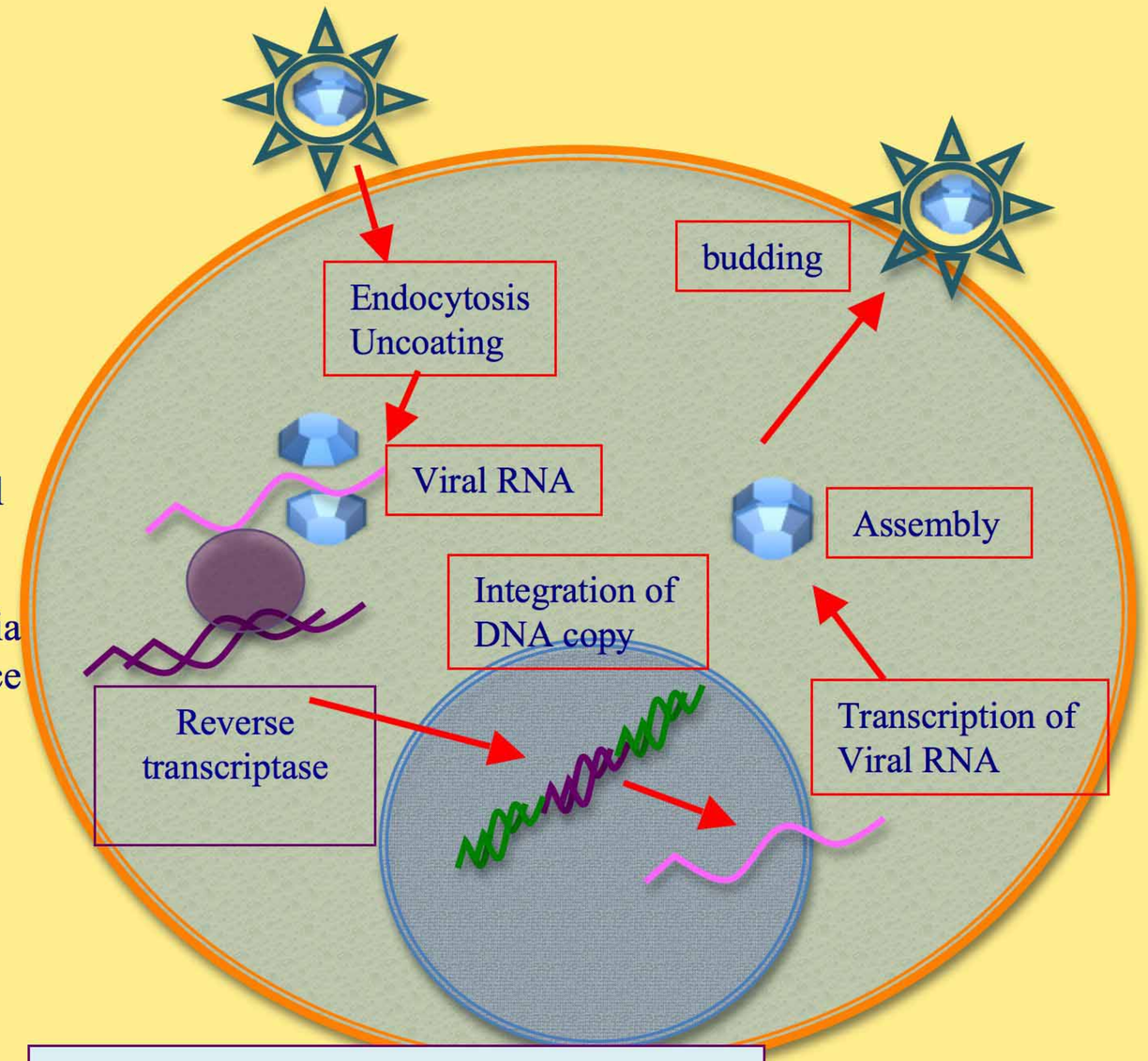
1990, 4-yr-old Ashanti DeSilva, ex vivo gene therapy trial.

Subset of T cells treated with a γ-retrovirus expressing ADA gene. cells reintroduced. Repeated monthly for 2 years. Now a healthy adult.

JAK3 gene, signaling protein in cytokine receptor path.

Cause of SCID

Cannot control where retroviral vectors insert themselves.



Severe combined immunodeficiency

γ- chain, JAK 3 kinase, purine nucleoside phosphorylase (PNP), adenosine deaminase (ADA), MHC class II or recombinase activating gene (RAG) deficiency.

Boy in the bubble: David Vetter

Sister not a match. Kept in a bubble. Eventually has a BM transplant but dies of EBV Burkitt's Lymphoma aged 12 not detectable in donor (sister Katherine)

Lentiviral vectors

Lentiviral vectors (LV)

Type of retrovirus (HIV) dividing and nondividing cells

Expands cell types able to be targeted

Useful for muscle, heartneurons, RPE and photoreceptors.

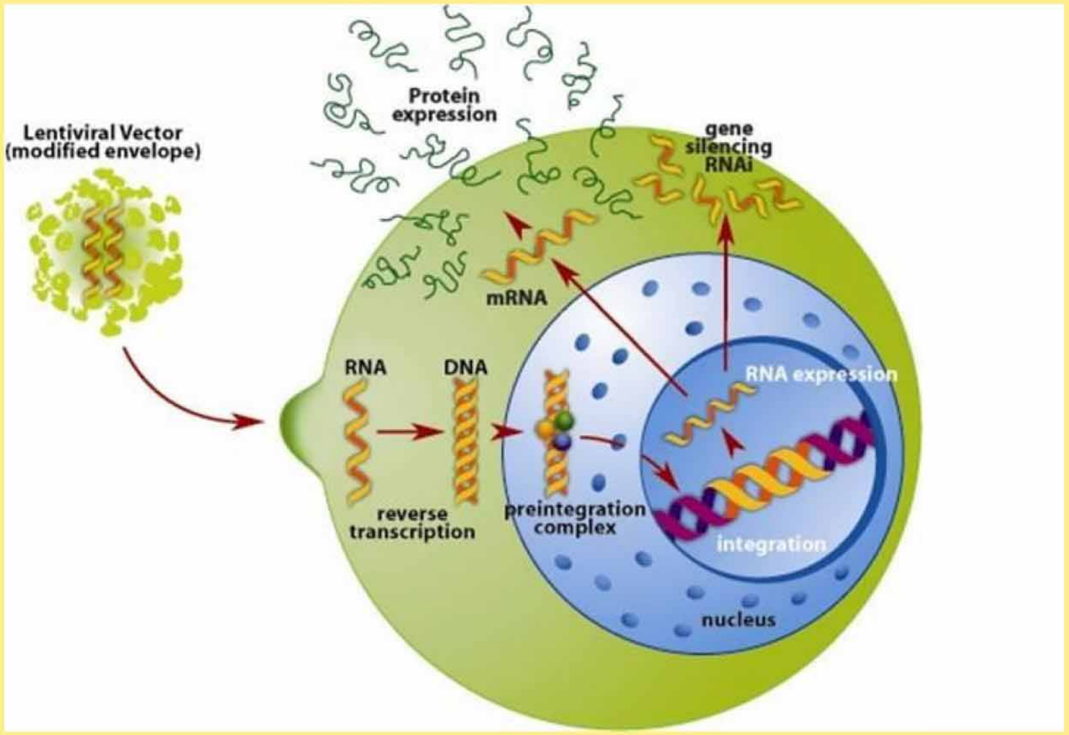
Deliver genes or RNAi into cells with 100% efficiency.

Bind to target cells using envelope protein which allows for release of the LV RNA containing the gene or gene silencing sequence into the cell.

Reverse transcriptase converts RNA to DNA which enters the nucleus and integrates into chromosomal DNA.

Gene delivery is stable because target gene integrated

Copied every time the cell divides.



long-term stable expression of transgene, low immunogenicity.

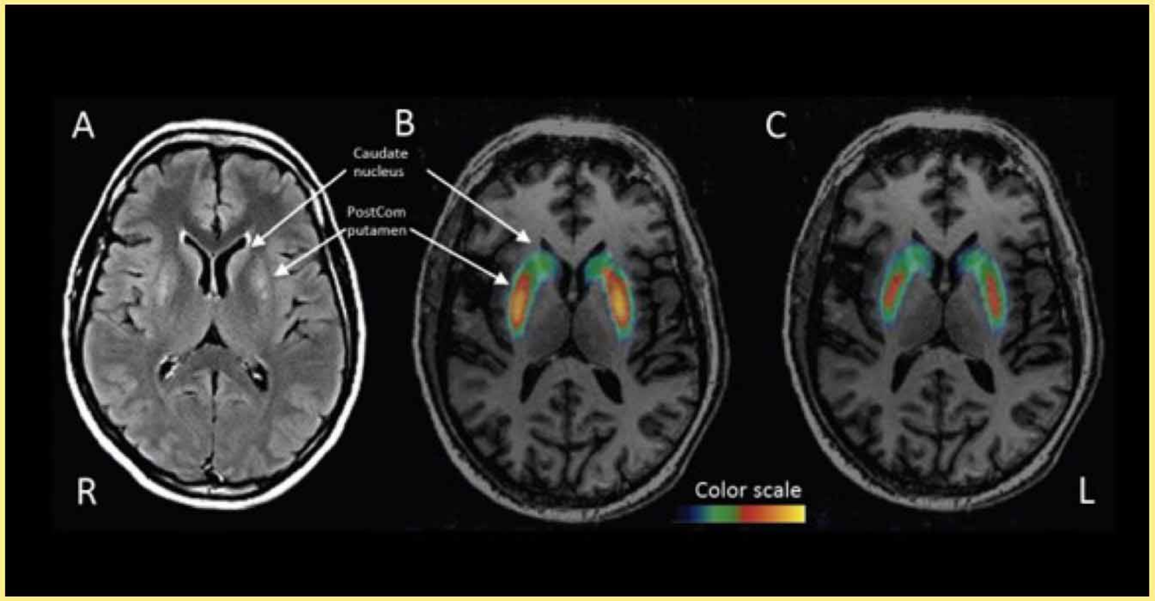
mouse haemophilia corrected by expressing wild-type platelet-factor VIII,

Transfection of diabetic mice with the PDGF gene

Parkinson's: genes that drive dopamine production in a lentivirus, injected into the region of the brain that controls movement.

15 patients improvements of movement-related symptoms. PET scans showed more dopamine production, and none of the patients experienced major adverse reactions.

Oxford BioMedica ProSavin.



FAMILIAL LIPOPROTEIN LIPASE DEFICIENCY

very high levels of triglycerides in their blood, cause severe pancreatitis.

2012, the European Medicines Agency approval, for gene therapy

healthy LPL gene is packaged in an adeno-associated virus which targets muscle cells (serotype 1).

The virus invades and inserts its healthy DNA payload, allowing the altered cells to produce LPL.

Muscles most important tissue contributing towards healthy LPL production

designed to restore the LPL enzyme activity required to enable the processing, of fat-carrying chylomicron particles formed in the intestine after a fat-containing meal.

Alipogene tiparvovec (Glybera)

$1.6 Million dollars for treatment most expensive drug in the world

engineered copy of the human LPL gene packaged with a tissue-specific promoter in a non-replicating AAV1 vector, with affinity for muscle cells

Mystery of Amaurosis solved

Blindness in a normal looking eye was called Amaurosis

Neither patient nor the ophthalmologist saw anything!

Cause mystery until the back of the eye visualised.

Many causes of previously enigmatic condition discovered

Various patterns of hereditary retinal diseases, retinal dystrophies

1853: Coccius retina detachment and retinitis pigmentosa.

1847 **Charles Babbage** (1792-1871), gave a device to ophthalmologist Thomas Wharton Jones,
1850: **Hermann von Helmholtz** (1821-1894)
Augenspiegel' "eye-mirror"

Eye useful organ for study of genetic disease

Wide-variety of phenotypes be directly visualized

Effects on the organ can be quantified by psychophysical measures (acuity, field, colour contrast) electrophysiology

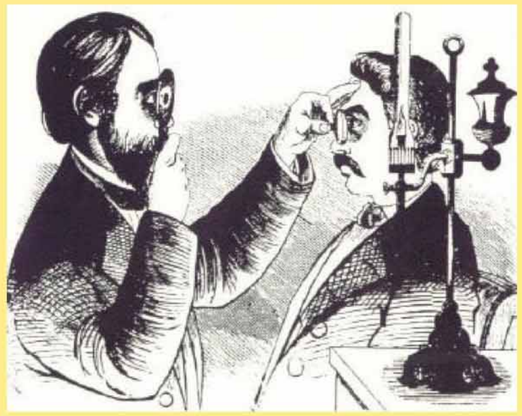
Retinal dystrophies slowly progressive

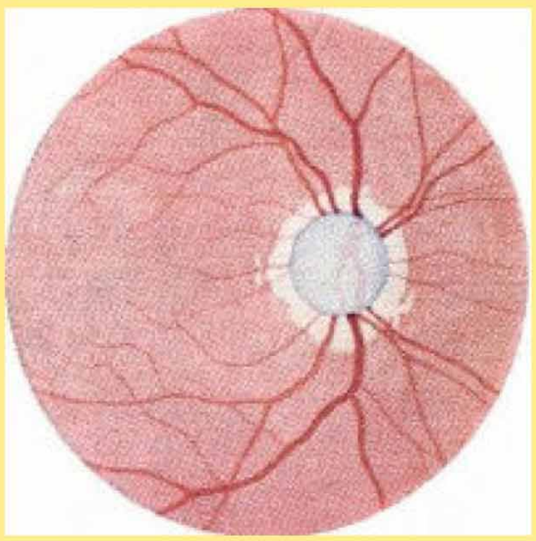
families large enough for genetic linkage studies.

large number of animal models

RP: 1:4000 people

150+ diseases





Phototransduction

Biological conversion of a photon into an electrical signal in the retina.

via G-protein coupled receptors: opsins bound to chromophore 11-cis retinal.

Photon changes shape of chromophore to all-trans

creates bleached opsin that **activates transducin**.

activates **phosphodiesterase**.

breaks down cGMP.

Decreased cGMP closes **Na^+ channels**

K^+ continues to leak out **hyperpolarizing** the membrane.

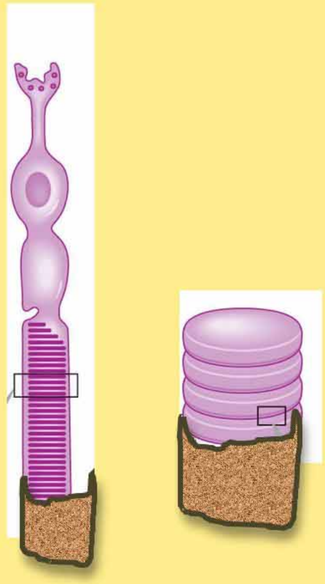
Hyperpolarization **spreads** to the inner segment and closes Ca^{++} channels.

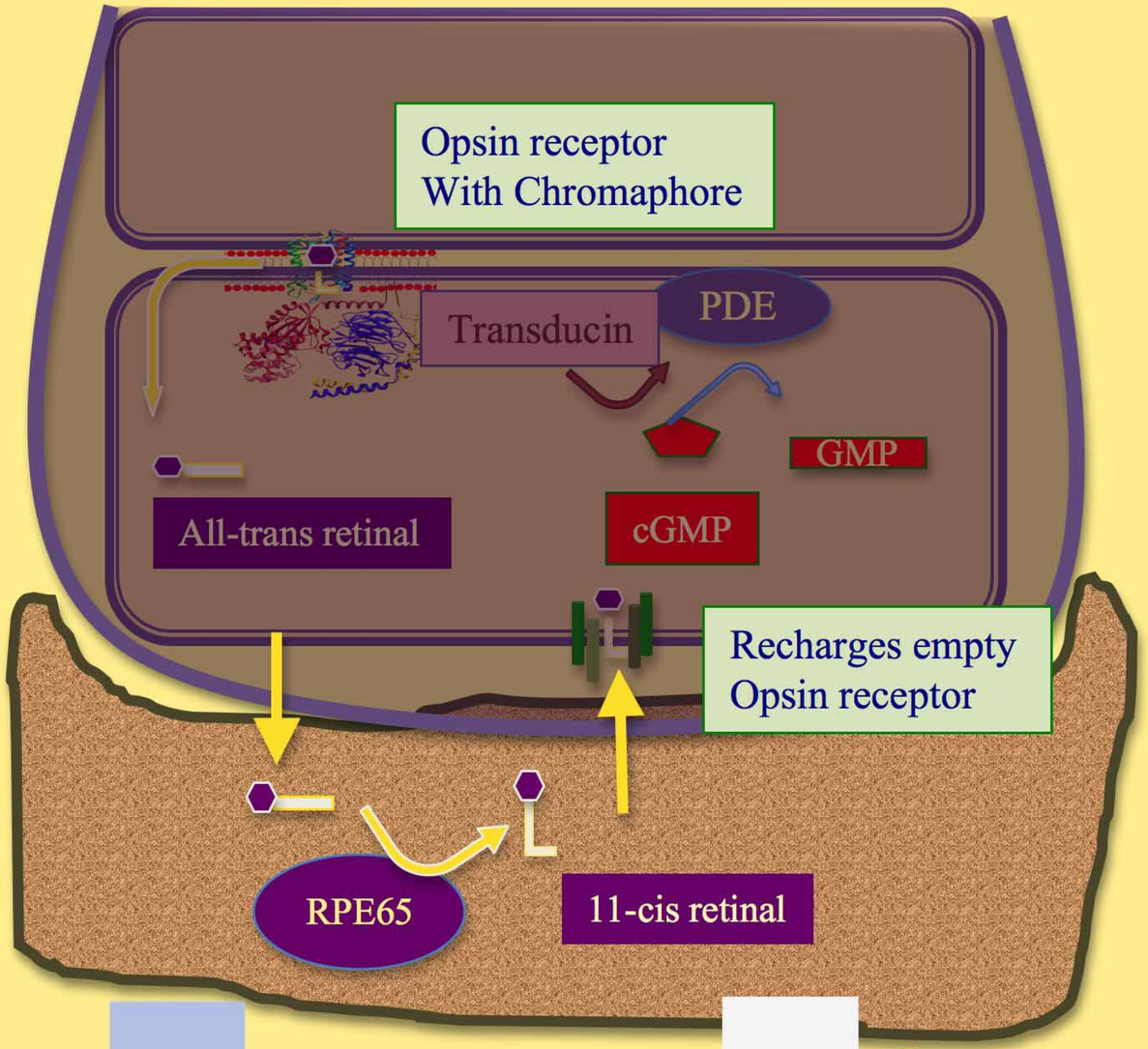
Less Ca^{++} Less transmitter is released.

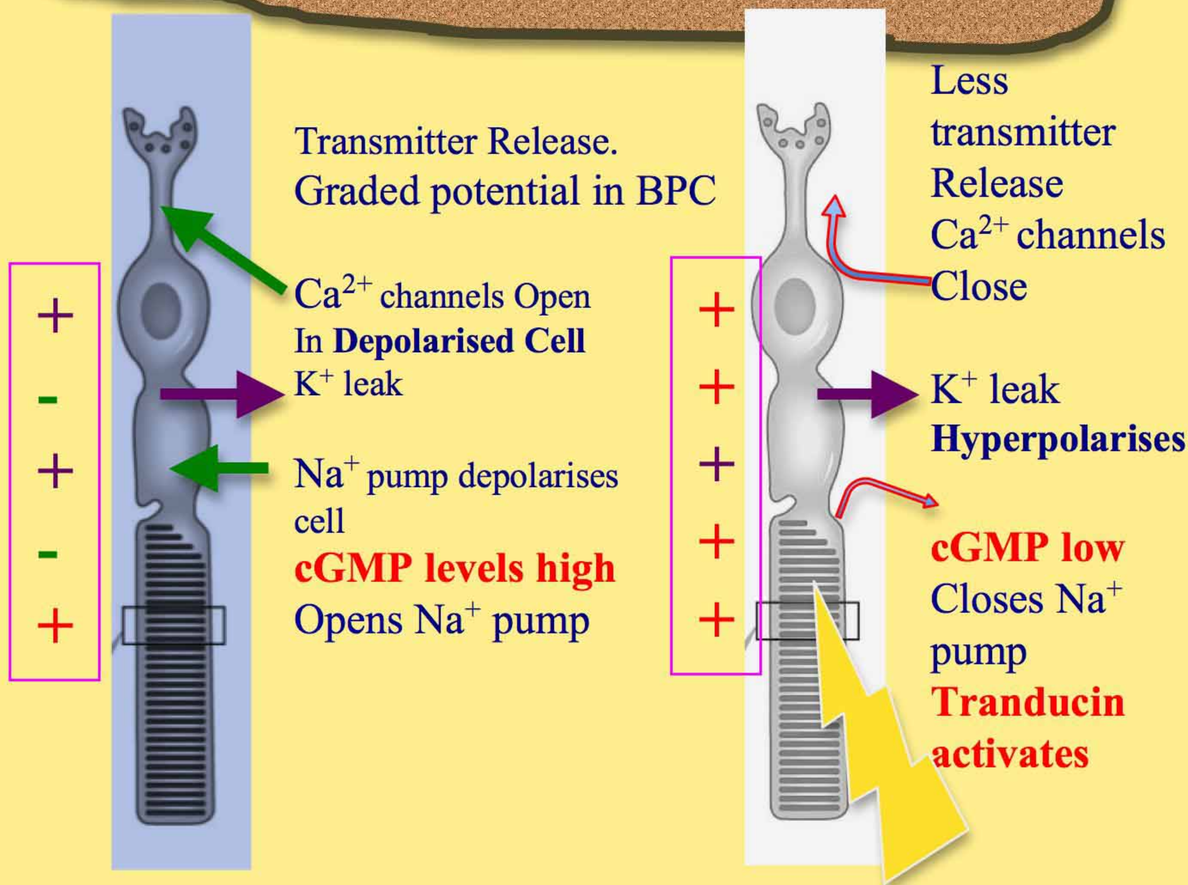
Graded potential in bipolar cells **decreases**.

Following release from the opsin protein, all-trans retinal is reduced to all-trans retinol and travels back to the retinal pigment epithelium to be recycled

converted to 11-cis retinol by the enzyme **RPE65**.







Genetics of eye disease

Receptors developed to detect radiation.

Contain chromophore (Vit A derivative) linked to membrane protein-enzyme (opsin)

Eye sensitive to a small fraction of the energy the spectrum between 400-700nm.

Captured photons cause a chemical reaction; The altered chromophore leads to a change in shape of the opsin, activation and eventually to electrical signals which travel to the brain

Retinitis pigmentosa common inherited diseases of the retina.

1 in 4,000 people

Mutations in 60+ genes cause retinitis pigmentosa.

1989: mutation of rhodopsin gene identified.

150 mutations have been found in this gene,
the most common cause of autosomal dominant RP,
25% Most are missense mutations (code for alternative amino acids)

Dominant inheritance.

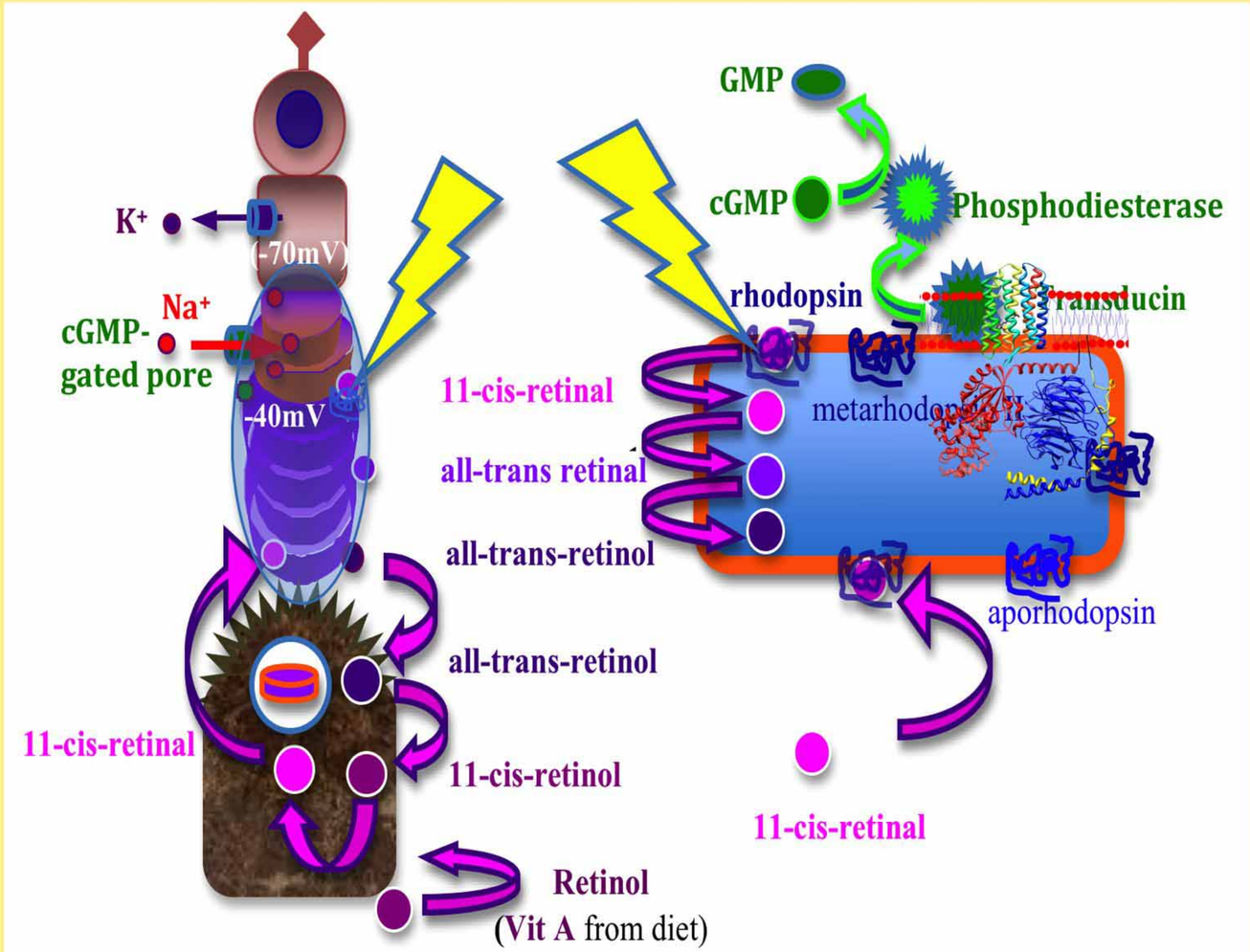
35 other genes autosomal recessive RP
mutations in *USH2 15%* AR RP

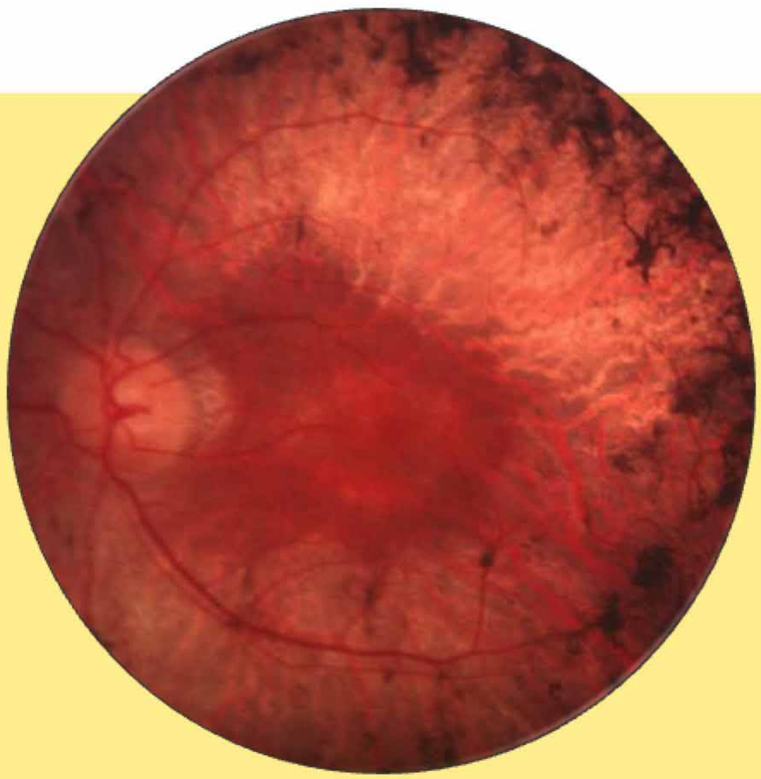
6 genes X-linked RP: *RPGR* and *RP2* most cases

These genes structure and function of photoreceptors

Mutations in any of the genes of RP lead to a gradual loss of rods and cones in the retina.

Rods degenerate before cones,
night vision impairment first sign of the disorder.





Colour blindness corrected by gene therapy

Opsin is the primary photopigment a/a sequence determines the spectral sensitivity of its cone

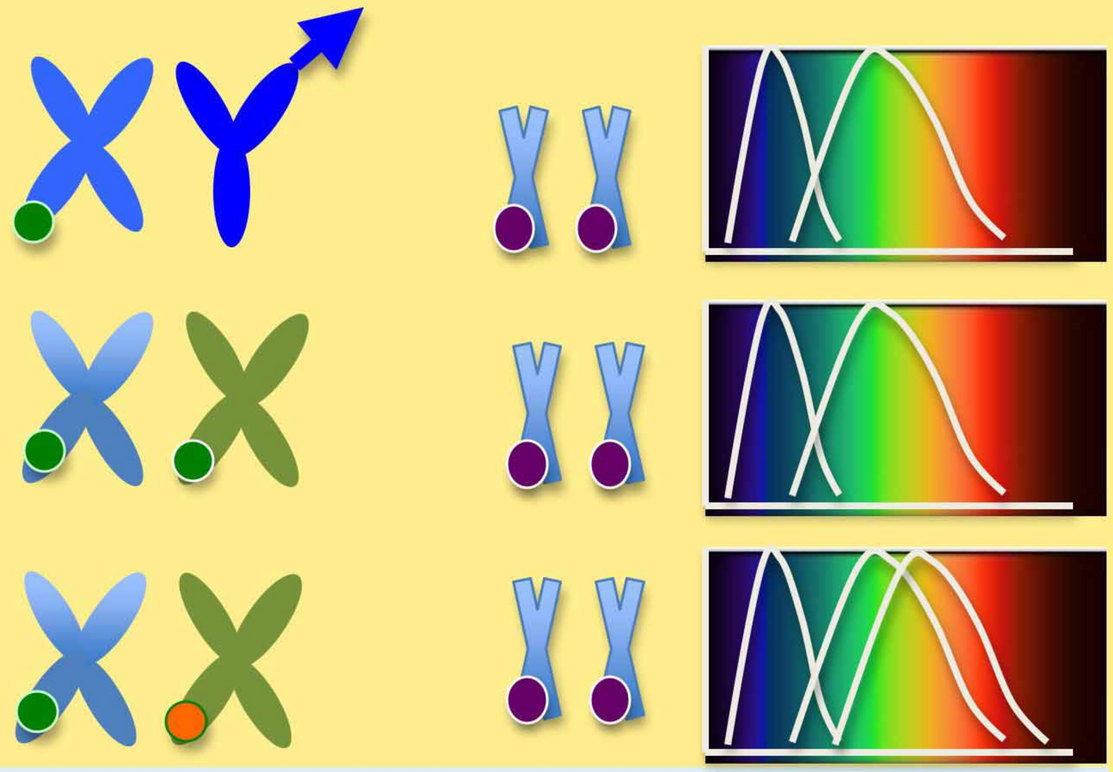
Mutations in gene change spectral sensitivity

Protanomaly (1% males) "red-weakness" Red, orange, and yellow-green appear shifted in hue towards green, and all appear paler

Deuteranomaly (5% males): "green weak". poor at discriminating small differences in hues in the red, orange, yellow, green region

hues appear somewhat shifted towards red

Absence of function –opia in either variant 1% males severe colour deficiency



Primate trichromacy 3 λ-sensitive cone cells, spectral peaks in the short λ (430nm violet), mid (530 green) long (560 yellow-green) λ

New world monkeys: Did not evolve the second colour gene on X chromosome Male squirrel monkeys (*Saimiri sciureus*) dichromats

Have *SWS* opsin on autosome

Only one X chromosome, only one copy of the MWS gene

Naturally Red–green colour blind

The MWS opsin gene has many variations; alleles in the population

Opsin proteins coded by the different variants sensitive to different λ

individual males see the world differently

Females can be trichromats if the two alleles on their two X chromos code different opsin variants sensitive to different λ.

If the females have identical alleles on both X chromosomes, the are dichromats like the males,

Viral vector containing human red-detecting opsin gene injected subretina in male squirrel monkeys —Dalton & Sam

Assessed the monkeys' ability to find coloured patches of dots on a background of grey dots rewarding with grape juice

Adding the missing gene enabled full colour vision

Brain able to code for colour despite colour blind from birth

Leber's Congenital Amaurosis

1869: **Theodor Karl Gustav von Leber** (February 29, 1840 - April 17, 1917) student of Hermann von Helmholtz in Heidelberg, doctorate in 1862

1867-70: assistant to Albrecht von Graefe in Berlin

Congenital amaurosis: the most severe form of early-onset inherited retinal dystrophy congenital blindness

Patients present in infancy with pendular nystagmus, unusual roving eye movements and absent ocular pursuit upon ophthalmic examination.

habitually rub their eyes

Similar disease in Briard Dogs, puppies eventually become blind

Le Livre de la Chasse (Book of the Hunt) of Gaston III Phoebus (1331-91), Comte de Foix.

Leber congenital amaurosis

A 65 kDa protein located in RPE is involved in the Defective RPE65 genes produce a **mutant form of the RPE65 protein**, early vision loss, degeneration of the retinas and near-total blindness later in life.

The mutated enzyme fails to convert vitamin A into a form needed for the photosensitive cells of the retina to detect light.

all-trans retinol to 11-cis retinal

The unconverted form of vitamin A builds up and kills cells in the retinal pigment epithelium.

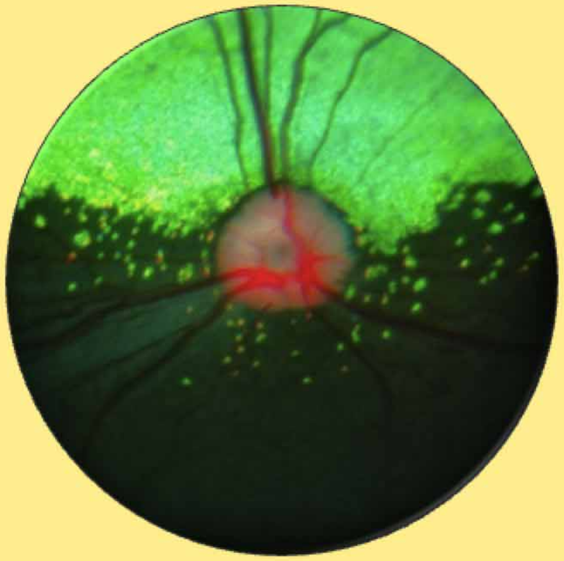
Vision loss occurs early, usually within the first six months of a child's life. And it's severe.

Early infancy, the optic discs and fundus are normal

Progressive abnormalities with time, thinning of vessels, nerve pallor, pigmentation, atrophy of RPE

Leber mutations severest consequence.

Other mutations in these genes cause RP & retinal dystrophies.



2001, gene therapy. Congenital Stationary Night Blindness in Briards analogous to Leber congenital amaurosis in humans, both defects in the RPE65 gene.

Healthy dog RPE65 genes cloned

recombinant adeno-associated virus vector injected subretinal space briard-beagle mix pups with defective RPE65 gene blind since birth.

treated eyes produced normal RPE65 protein.

Restoring visual function over 3m.

Chewbacca based on Briard

1 of 80,000 births.

~ 3,000 people in the US have LCA and will become blind in their lifetimes

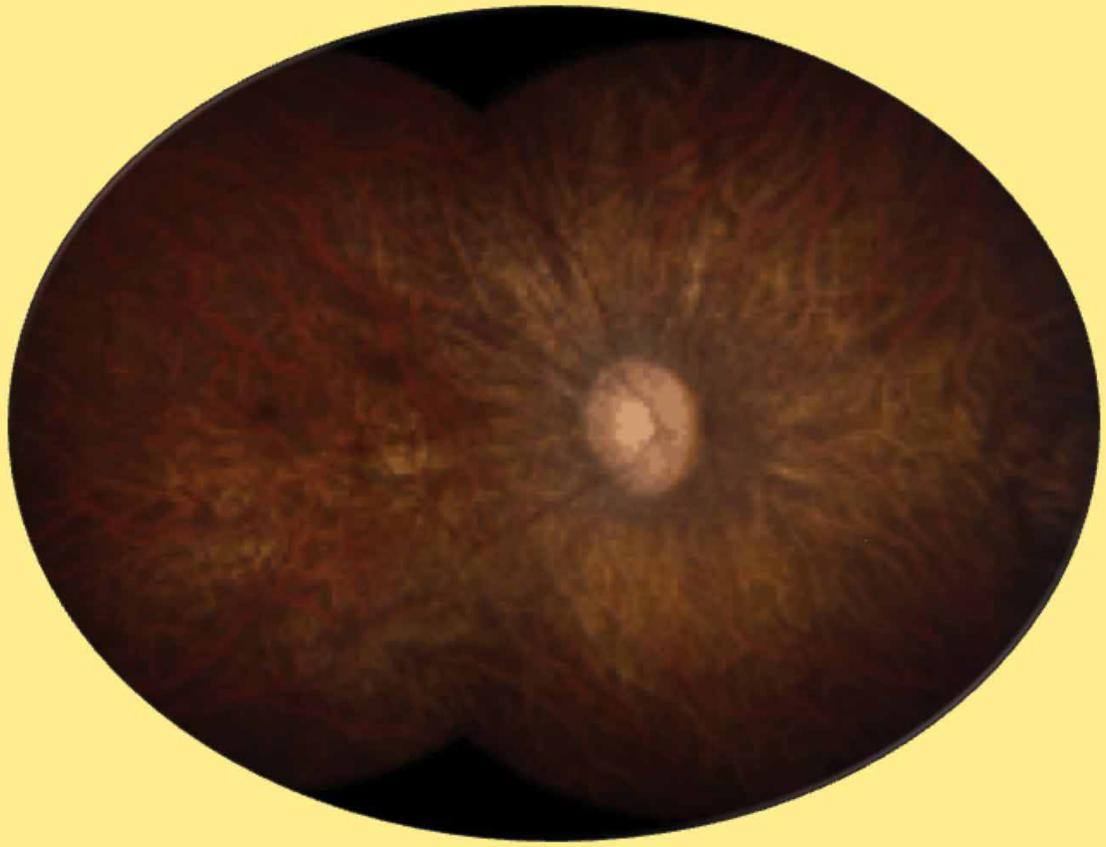
LCA and related early-onset retinal degenerations are caused by mutations in at least 15 genes.

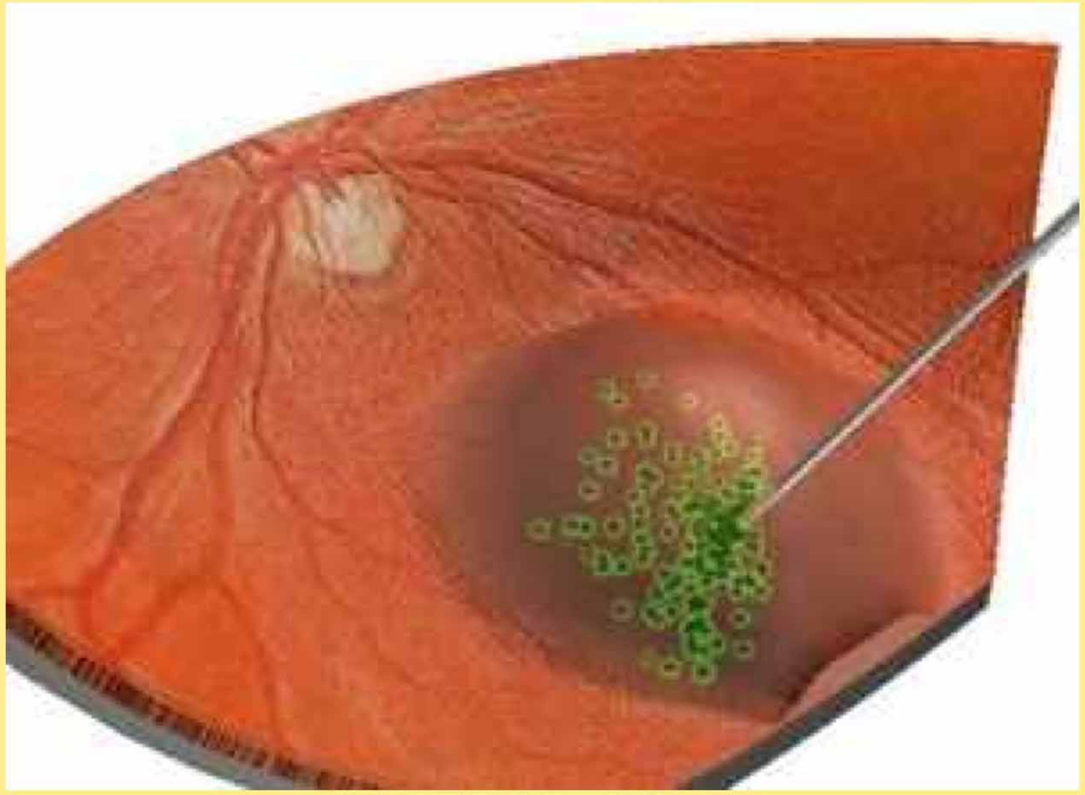
LCE caused by mutation in **RPE65 gene** treatable with normal copy of the gene.

Gene testing complex as benign variants also exist in this gene that do not cause disease

2008: two teams in US and one in UK reported success in patients using the AAV2 vector (Bainbridge et al., 2008; Cideciyan et al., 2008; Maguire et al., 2008).

Local (subretinal, an immune privileged site) administration of a vector expressing R**PE65** led to gain of light sensitivity and, in some cases, of vision





Retinitis pigmentosa

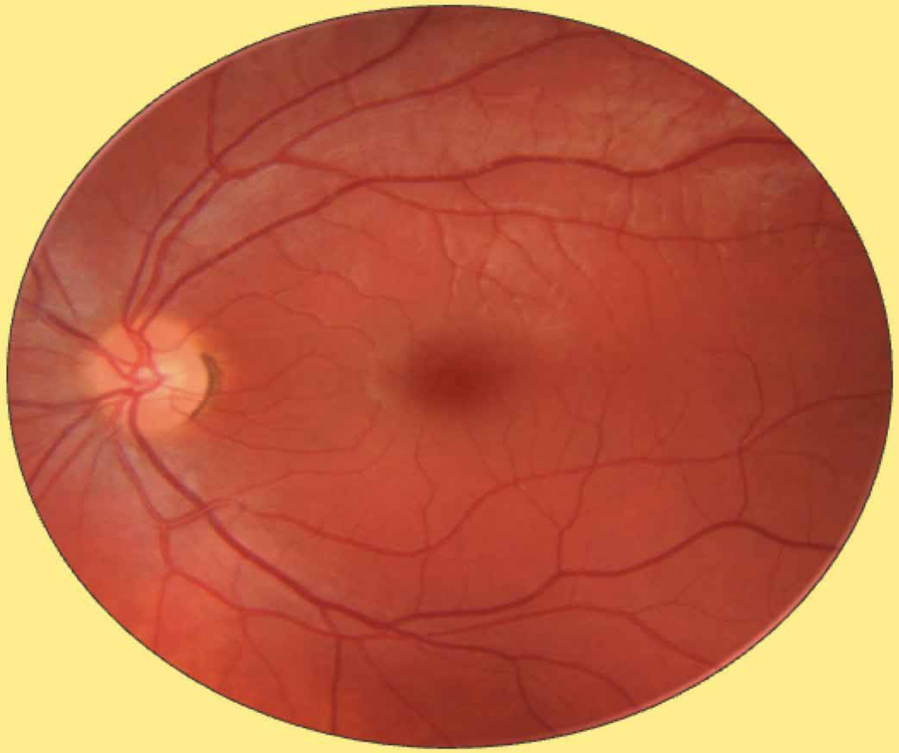
Most RP mutations affect rods selectively.
Many genetic defects lead to this clinical picture

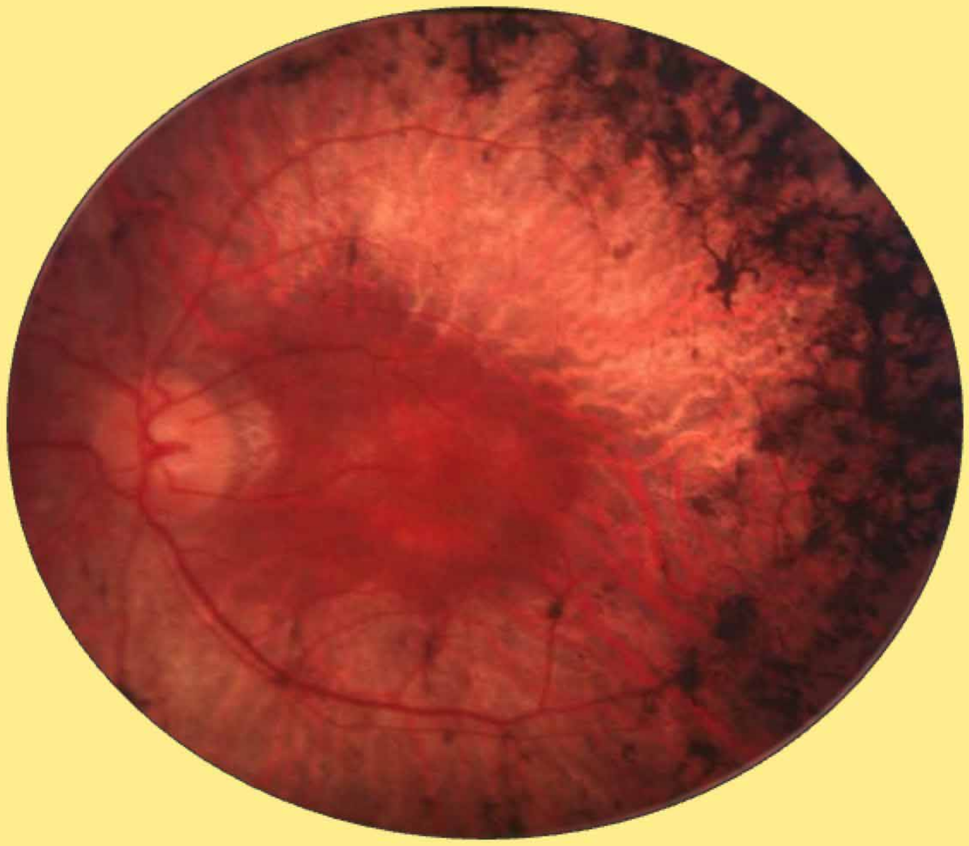
Encode proteins for phototransduction
Structural
Transmembrane proteins
Transcription factors.

Cones are seldom directly affected by the mutations
degenerate secondarily to rods, accounting for the loss of central vision and complete blindness sometimes seen at the end-stage of the disease

Gene therapy to prevent and reverse X-linked RP mutation in the RPGR gene in rodents.

One of the most common inherited forms of retinal degeneration in man.





Choroideremia

progressive vision loss that mainly affects males. The first symptom night blindness in early childhood.

A progressive (tunnel vision) follows, as well as a decrease in (visual acuity).

1:100,000

4% cases of blindness

CHM gene on X-chromosome Rab escort protein-1 (REP-1).

attaches hydrophobic groups (prenylation) to Rab proteins directing them to lipid membranes of cell compartments.

Rab proteins are involved in the movement of proteins and organelles within cells (intracellular trafficking).

Mutations absence of REP-1 protein or the production of a REP-1 protein that cannot carry out escort function.

Without the aid of Rab proteins in intracellular trafficking, cells die prematurely.

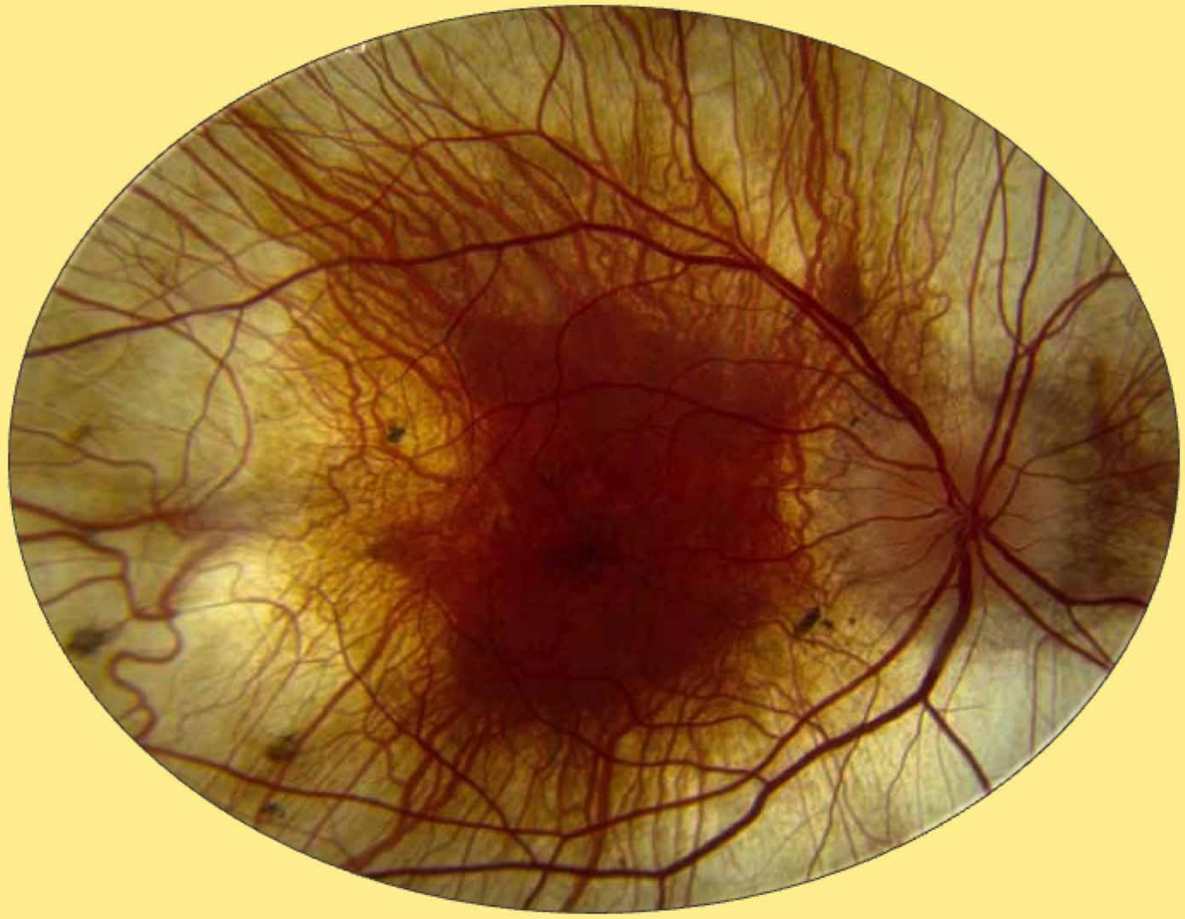
The REP-1 protein is active (expressed) throughout the body, as is a similar protein, REP-2.

when REP-1 is absent or nonfunctional, REP-2 can perform the protein escort duties of REP-1

Very little REP-2 protein is present in the retina, however, so it cannot compensate for the loss of REP-1

subsequent misplacement of Rab proteins within the cells of the retina causes the progressive vision loss

2014: six male patients 35—63 yrs AAV.REP1 ($0{\cdot}6$—$1{\cdot}0\times10^{10}$ genome particles, subfoveal injection
Professor MacLaren: 'It is still too early to know if the gene therapy treatment will last indefinitely, but vision improvements have been maintained for as long as we have been following up the patients.
The results showing improvement in vision in the first six patients confirm that the virus can deliver its DNA payload without causing significant damage to the retina.



Gene Therapy Trials

Disease	Gene	Vector	Mode	Phase	Locations
Leber's congenital amaurosis type II	CBSB-RPE65	AAV2	Subretinal	I	University of Pennsylvania Children's Hospital of Philadelphia; University of Florida
Leber's congenital amaurosis type II	RPE65	AAV2	Subretinal	I/II	Nantes University Hospital
Leber's congenital amaurosis type II	CB-RPE65	AAV2	Subretinal	I/II	Applied Genetics Technologies Corp Casey Eye Institute, Oregon University of Massachusetts
Leber's congenital amaurosis type II	RPE65	AAV2	Subretinal	I/II	Children's Hospital of Philadelphia
Leber's congenital amaurosis type II	RPE65	AAV2	Subretinal	III	Children's Hospital of Philadelphia University of Iowa
MERTK-associated retinitis pigmentosa	VMD2-MERTK	AAV2	Subretinal	I	King Khaled Eye Specialist Hospital, Riyadh, Saudi Arabia
Neovascular ARMD	Endostatin & Angiostatin	LV	Subretinal	I	Oxford Biomedica Wilmer Eye Insitute, Johns Hopkins Hospital;
Neovascular age-related macular degeneration	SFLT01	AAV2	Intravitreal	I	Genzyme; Johns Hopkins Hospital; Ophthalmic Consultants of Boston;
Neovascular age-related macular degeneration	SFLT-1	AAV2	Intravitreal	I/II	Lions Eye Institute, Perth Avalanche Biotechnologies
Choroideremia	REP1	AAV2	Subretinal	I/II	University of Oxford St Mary's Hospital, Central Manchester University
Stargardt's disease	ABCR	LV	Subretinal	I/II	Oxford Biomedica Casey Eye Institute, Oregon Health & Science University;
Usher type IB	MYO7A	LV	Subretinal	I/II	Oxford Biomedica NCT01505062 Casey Eye Institute, Oregon

bioengineering of lentiviral vectors safe enough for clinical trials properties that make them particularly suitable for gene delivery in ophthalmic diseases, including high expression, consistent targeting of various post-mitotic ocular cells *in vivo* no inflammation mediate efficient and stable intraocular gene transfer.

Emerging treatments

Optogenetics:

Artificial photoreceptors constructed by gene delivery of light-activated channels to surviving cell types in the retinal circuit

Restore photosensitivity in animal models of RP

Silicone Prosthesis

Argus II: two decades of R&D and US$200 million investment, clinical trials 2007 FDA approval for sale in Europe in 2011.

tiny video camera mounted in glasses wirelessly linked to a receiver and microelectrode array implanted onto retina.

20^0 of visual field, stimulates remaining cells electrical pulses.

patients learn to interpret these signals

The Artificial Silicon Retina (ASR) is a subretinal implant

Stem cells

Already in use for cornea.

Promising studies to generate monolayers of RPE for transplant under retina in AMD.

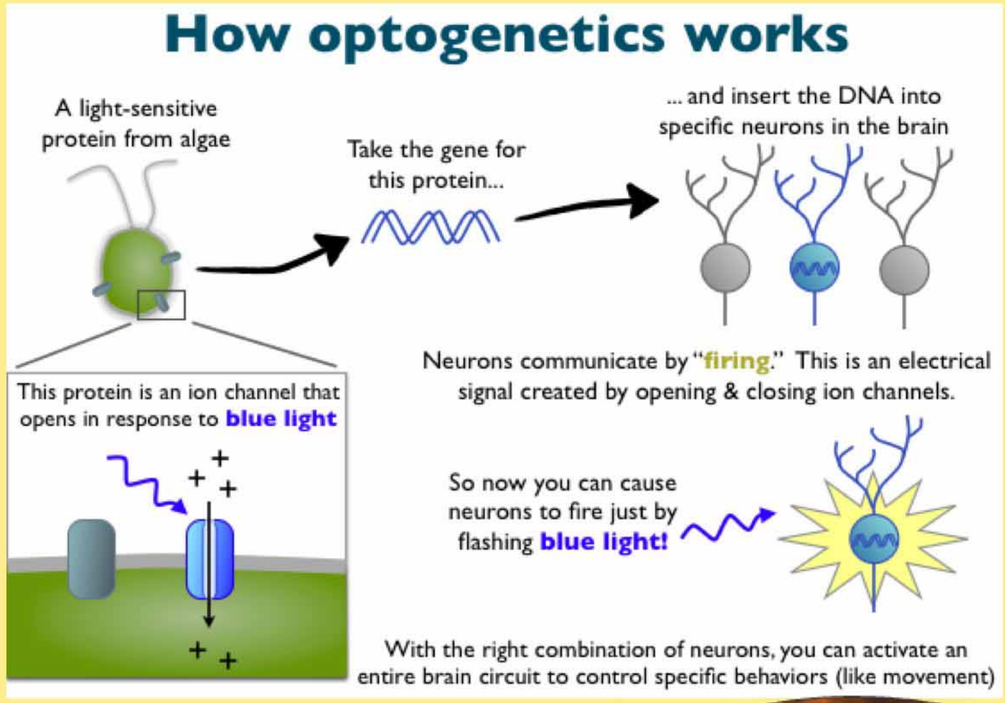
Trials use human embryonic, foetal and umbilical cord tissue-derived stem cells and bone marrow-derived stem cells to treat AMD, Stargardt's disease and RP

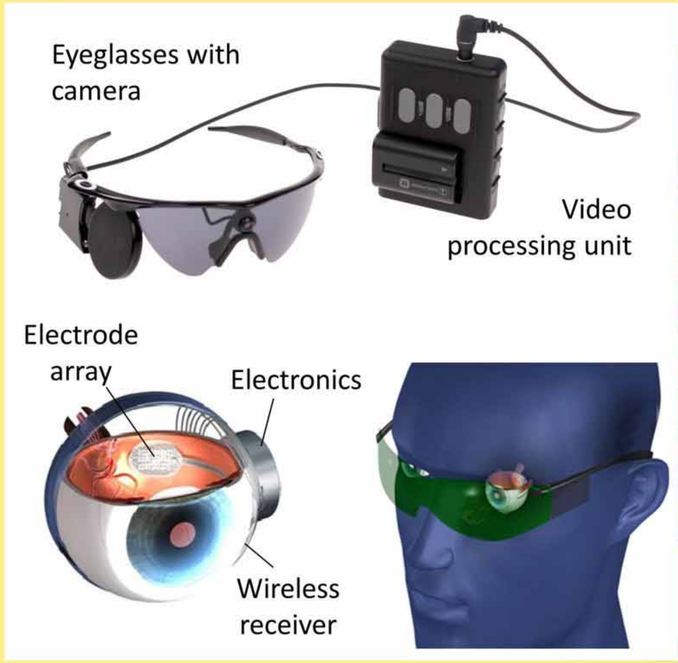
Pluripotent Stem cells iPS cells "reprogramming" genes into adult cells. Revert to embryonic state. injecting proteins that instruct embryonic stem cells to become liver, retina or any other type of cell.

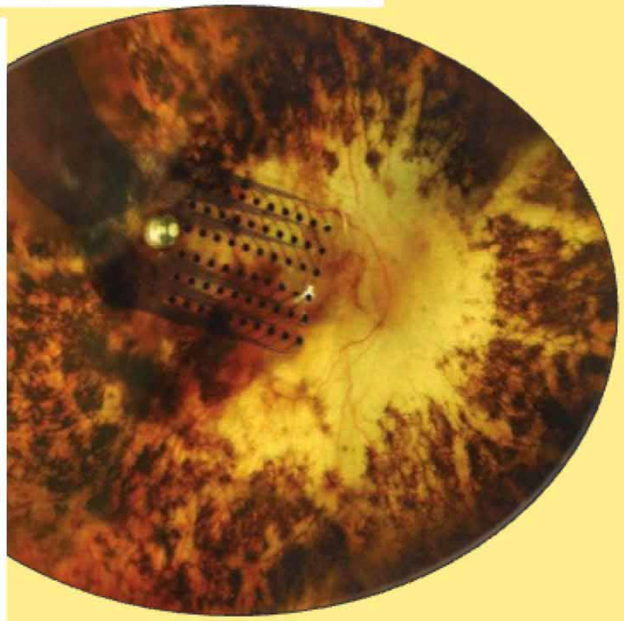
Inkjet!

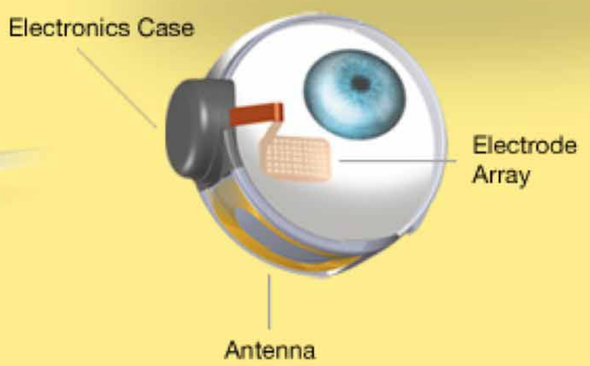
Prof Keith Martin Cambridge: Piezoelectric printed retinal ganglion cells and glia to create a living pattern network.

Possible to print a retina for transplantation









THANK YOU

Thanks to patients
Colleagues and staff at Hospitals
Gresham College Staff
Museum of London AV

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