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BI 2233 - GENETICS

Mitosis and Meiosis Review

Somatic cells divide by mitosis to create identical daughter cells
No recombination occurs, but can be induced

Meiosis occurs in diploid cells to form 4 haploid cells.
Recombination occurs, so each of the cell has different DNA. Also called reduction division. Responsible for giving rise to variation.

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History of Genetics

Ideas about cell theory, plant hybridisation, classical genetics and germ plasm theory were coming together

⇒ Hippocrates – Each organ produces something (gemmule - Darwin) that is collected in semen
~400 BC

⇒ Aristotle (350 BC)
He noted that children looked like their parents and grandparents
He said that not the character but the potential to generate them is inherit
He also noted the hybrid animals – mule, (something new always coming out of water spots in Libya)

⇒ Joseph Kolreuter, Germany (1760s)
Systematic study of plant pollination
Said that 2 pollens fertilise but concluded that mixing of fluids resulted in pollination ∴ he studied pollination in water, the pollen plasmolysed almost immediately
Hybrids were intermediate b/w parents

Carl Friedrich von Gärtner, Germany (1800)
Studied plant hybridisation
F_2 generation has more variation than F_1 generation
They both did a huge no. (10,000) of ~~vari~~ crosses in plants.

(2) → Charles Darwin (1800s) Discontinuous

- Experimented with plants and pigeons. Noted (discrete) and continuous variation – discontinuous were the heritable traits that skipped a generation.
- He was interested in developing unifying principles
- Crossing has a unifying effect (hybrids are intermediate) & keeps populations uniform.
- (?) Inbreeding results in differences between population
  Right words?

History of Cytology

→ Robert Hooke (1670s)
30x microscope – observed cork and coined the term 'cell'
Hadn't seen the microbial world yet.

→ Anton von Leeuwenhoek (1677)
200x – 300x magnification – observed sperms & guessed that 1 was enough for fertilisation

* Gerlach discovered nuclear stain in 1850s (accidentally)

These kinds of observations give rise to Cell Theory –

→ Schleiden & Schwann
All organisms are made of cells (fundamental unit)
All cells come from pre-existing cells
Integrates to form tissue.

Schwann – coined "metabolism"
Debate over development of organism by cell division vs cell growth

Robert Brown (1831) – discovers Nucleus.

→ Robert Remak (1860s)
Cell division is discontinuous and associated with events in the nucleus.

Rudolph Virchow (1860s)
Studied tumors and extended principles to living organisms
Cell doctrine: Omnis cellula e cellula

→ Walter Fleming (1879) – observes mitosis in salamander tail fin

Heinrich Waldeyer (1888) – coined 'chromosome'
Interpreted longitudinal splitting of chromosome to be of importance in heredity

Earnst Haeckl (1890s) – nucleus contains hereditary substance
males and females contribute equally
Egg large & sperm has just nucleus.

Oscar Hertwig (1876) – observed fertilisation in sea urchin
observed the fusion of nuclei & said it was most important.
Coined 'male/female pronucleus'.

Edward van Beneden (1883) – Chromosome no. in Ascaris = 4 in diploid
Observed reduction division in meiosis and attainment of diploid state in fertilisation.

History of Developmental Biology

→ * Preformation vs. Epigenesis – Spermists/Ovists – organisms develop from a miniature version that exists in the sperm/egg
– the opposite idea – form of living things comes into existence.

* Malphigi Mario (1693) – chick embryo drawing
Nicholas Hartsoekar (1694) – Spermist drawing

* Some studies caused confusion –
Charles Bonnet (1760s) – aphids – progeny found in female's abdomen
(?) Regeneration – presuppose a hereditary role for cells in wounded tissue

Siebold – parthenogenesis in bees and aphids

→ Theory of Germ Plasm – Weismann (1890)
Reproductive tissue is set apart at birth
Changes in somatic cells don't translate to the germ line
Germ line cells produce haploid cells (gametes) by a 2 step process – reductional & then equational division

Gregor Mendel (1822 - 1884)

* Mendelian genetics - developed a set of rules which govern heredity
  - based on statistical observation of few visible 'trait's from one generation to next

Non-mendelian genetics deals with cases where his rules can't be applied generally.

* Pea plant - Pisum sativum

Diploid
Sexual reproduction
Large no. of seeds
Self & Cross fertilisation
Short generation time
Inexpensive - easy to obtain & maintain

Chose to study 7 pairs of contrasting traits.
To ensure accurate results, he first made two-breeding strains by self-crossing them for 2 years

* 3 stages - Planted in spring
  Flowers appear in summer - fertilisation
  Pods with peas appear in the fall

He had excellent controls, large numbers, grew plants indoors to avoid insect infestation & bad weather

* Crosses

- Cross b/w two purebred pea plants which differ by a single trait is considered a Hybrid Cross
- Reciprocal cross - to determine if trait depends on the sex of the ♀ organism.
- Monohybrid : Phenotypic :- F_2 :- 3:1
  Genotypic :- F_2 :- 1:2:1
  (recessive)
  The trait disappears in F_1 and reappears in F_2 (mystery?)
  Classic example of discontinuous trait.
- Dihybrid cross : Phenotypic :- F_2 :- 9:3:3:1

For a smooth seed, S - dominant allele codes for Starch Branching Enzyme I (SEBI) that's required to produce amylopectin - which helps in shrinking regularly as they lose water, while wrinkled ones lose water irregularly.

- Genotypic ratios can be identified through Punnet square
- Mendel's Postulates
  1. Unit factors in pairs
     Genetic characters are controlled by unit factors in pairs
     They don't mix / contaminate each other.
  2. Dominance / Recessiveness
     Two alleles of genes are present — one dominant, one recessive (only expresses itself when present homozygously).
  3. Segregation
     During gamete formation, unit factors segregate randomly
     So the outcome can be predicted statistically.
  4. Independent assortment
     During gamete formation, segregating pairs of unit factors assort independently
- In a way, Mendel was fortunate to choose traits that are present on different chromosomes / not linked
- Nageli, a botanist in Munich, working on heredity failed to recognize the importance of Mendel's work while he lived.
- Hugo de Vries & William Bateson were proponents of Mendel's laws. Bateson coined 'genetics' and co-founded the Journal of Genetics along with Reginal Punnett. They also discovered linkage
- R.A Fisher in 1931 considered Mendel's data fraudulent because of the accuracy of his results.
  Mendel had excellent controls & discarded experiments affected by bad weather & technical problems.
  Ultimately, this is only a minor criticism

What about whittling down a list of 20+ traits to 7?

Meiosis - source of variation
↳ Independent assortment of sister chromatids
Crossing over

A lot of diversity is created from independent assortment alone - we can have 2^x combinations of chromosomes, where x is the no of haploid chromosomes

On top of this, these chromosomes can be different because of recombination

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Lecture 3

After the period of oblivion, Hugo de Vries & others re-discovered Mendel's work.

Morgan, Muller and Sturtevant were majorly the ones who established the chromosomal theory of inheritance by working on drosophila.

It was a huge leap to use fruit fly as model organism to study genetics, development & so on.

It has won many Nobels.

T.H Morgan

He was a developmental & evolutionary biologist in Fly Room in Columbia, where he carried out many mutants

When he discovered a white-eyed mutant, he studied its mode of inheritance & discovered that it was sex linked

When white was crossed with wild red type -

WW x ww

F_1 Ww

F_2 WW Ww ww 3:1 phenotypic ratio

But: all females were red whereas half of males were red and other half white

White-eyes lack a transporter protein that would pigment their eyes.

Scute · proneural gene of achaete-scute complex — encodes a Tx factor involved in nervous system development

* If the daughters had dominant lethal mutations on other chromosomes, they'd die

Hermann Joseph Muller

He looked at the role of X-rays in creating mutations.

In 1907, Bardeen had observed that fertilised toad egg exposed to X-rays developed abnormalities that prevented development.

Muller worked more systematically & found that X-rays gave rise to mutations that were heritable and were akin to mutations already existing in the population. AIM: To establish that lethal mutations on X-chromosome are inherited from ♀ & ♂

→ Experiment 01

- bb (bobbed bristles) located on X-chromosome of male
- sc v f / sc v f homozygous females
  ~~skewed~~ scute vermillion (eye) forked (bristles)
- Expose them to X-rays (∃ some mutations)
- sc v f/bb females & sc v f males were formed in F_1
- [sc v f/bb]* females were crossed with wild type

In F2 generation, males were counted from 1000 such cultures to see if there were any mutations on sc v f or bb genes

Observation:

- Control group: 1 mutation in 947

X-ray treated group: 88 lethal mutations in 758 cultures.

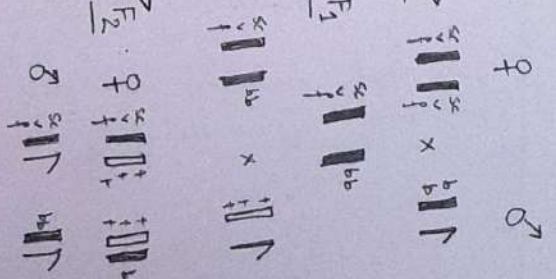
Mutations in these genes shouldn't be lethal right?

⇒ Let's say they are lethal: less than 50% males would have sc v f or bb

But how do you know they died because of loss of function on these genes and not others?

In F_2, if sc v f/Y is missing ⇒ ♀ mutation in P
bb/Y is missing ⇒ ♂ mutation in P.

* Revolutionary idea to use visible genes to track lethal mutation.



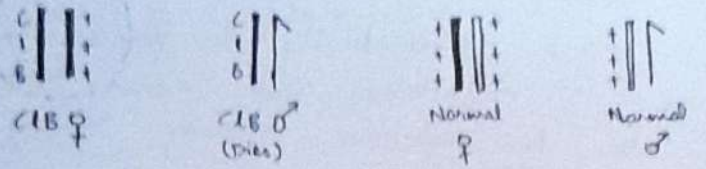
CONCLUSION

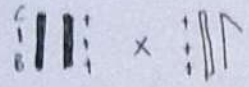
X-ray causes mutations in both males & females & they can be inherited across generations

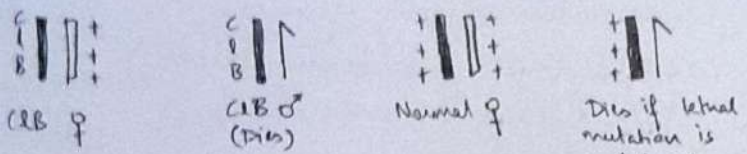
(8) → Experiment 02 : ClB method

ClB X chromosome
- Crossover suppression due to inversions on X
- Recessive lethal l
- Dominant eye mutant B – bar eye

P  female of interest

F1 
ClB ♀ | ClB ♂ (Dies) | Normal ♀ | Normal ♂

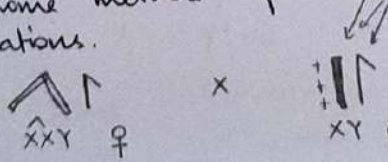


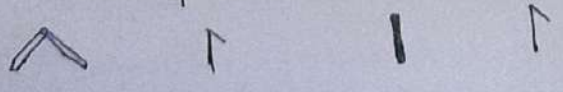
F2 
ClB ♀ | ClB ♂ (Dies) | Normal ♀ | Dies if lethal mutation is induced

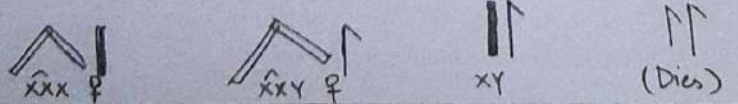
- Mutation is induced in male only – produces a lot of sperm ie. large no. of mutations. + hemizygous
- ClB – marked chromosome : marked with a visual, distinct phenotype so you can track it easily
- The only males in F_2 that survive are those that have the mutated chromosome from ♂ P.
  If that chromosome carried a lethal mutation due to irradiation, there would be no males in F_2
- Helps us quantify likelihood of lethal mutation on X-chromosome if we irradiate males

⟹ Experiment 03

Attached X-chromosome method for detection of X-linked visible mutations.


$\hat{X}\hat{X}Y$ ♀ × XY ♂

Gametes 


$\hat{X}\hat{X}X$ ♀ | $\hat{X}\hat{X}Y$ ♀ | XY | (Dies)

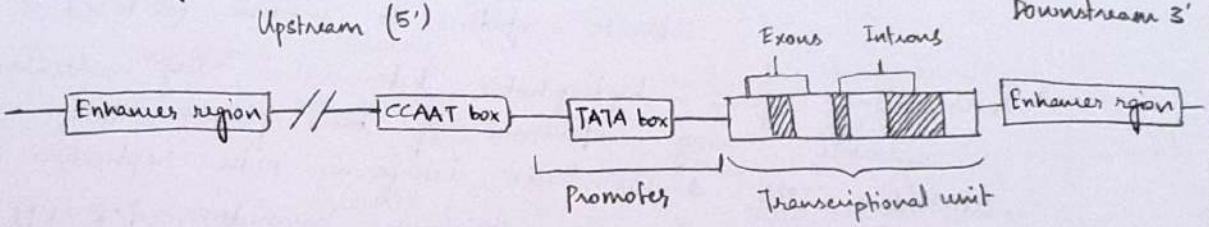
To detect mutations on X-chromosome that are visible, Muller developed this.

In P, we use a $\hat{X}\hat{X}Y$ female and an irradiated male so the progeny obtained in F_1 will have males that contain the irradiated, mutated X-chromosome

(?) Is there any way to ensure that the progeny that dies is only because of lethal genes we expect

For most genes, in animals, they can survive with just one copy of the gene

Gene Organisation



Transcription of the gene is regulated by the promoter, where the T_x factor binds

The enhancer region (can be present upstream, downstream or several kbp away) folds onto promoter region to increase transcription. i.e. regulate the occurence, timing and amount of transcription.

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Lecture 4

Alleles & phenotypes by Muller : Muller's morphs

Allele : One gene may exist in several different forms

Eg: Smooth and wrinkled seeds due to presence/absence of starch branching enzyme

A gene is directly regulated by the promoter but the tissue specific, temporal regulation is done by the enhancer region

Mutation

Heritable alteration of genetic material

- Maybe gross at the level of chromosome or point mutations that result in base pair substitution or frameshift mutation

- Creating mutations

Spontaneous : DNA replication error & polymerase accuracy
base alterations & damage, frameshift mutation in areas of repeat rich DNA.

Mutagens : Physical or chemical, man-made or natural agent which can alter DNA.

- Chemical analogues — Mutagenic chemicals that can be substitute for

* Base analogs : Bromouracil = Thymine, Aminopurine = adenine ATGC
* Chemicals which alter structure & pairing properties:
  Nitrous acid - deaminates adenine & cytosine to an ether group, thus altering pairing
  Converts cytidine to uridine (C to U)

* Intercalating agents - hydrophobic heterocyclic ring molecules that resemble ring structure of base pairs - they distort DNA double helix, interfering with replication etc
  Eg: Acridine ~~org~~ orange, ethidium bromide, ICR-191

Radiation

Ionizing radiation - X-ray or Gamma rays
Breaks DNA leading to :
- huge deletions
- chromosome loss
- loss & damage of bases
- crosslinking of DNA to itself or proteins

Ultraviolet radiation - less energetic - UV C : 180-190 nm - energetic + lethal
UV B : 290-320 nm - lethal + mutagenic
UV A : 320 nm - visible pyramidine dimers

Examples of useful mutagens
Bromouracil, aminopurine - base analog
Nitrous acid - oxidative deamination
Acridine orange, ethidium bromide, ICR 191 - Intercalating agents
Hydroxylamine, MNNG, EMS, DES - Alkylating agent

MNNG : N-methyl-N'-nitro-N-nitrosoguanidine
EMS : Ethylmethane sulfonate
DES : Ethylethane sulfonate

Classification based on impact on a protein

Ethylmethane Sulfonate (EMS)

- This can be fed to organisms & easily incorporate them into germlines.
- Like other alkylating agents, this converts guanine → O6-Ethylguanine So instead of GC, Thymine is paired with guanine
  Some alkylating agents may also cross-link DNA resulting in chromosome breaks.
- Rate of mutation : 5×10^{-4} to 5×10^{-2} per gene
  Concentration & rate of uptake maybe specific for different organisms (to get 1 mutation per gene).

Types of Mutations

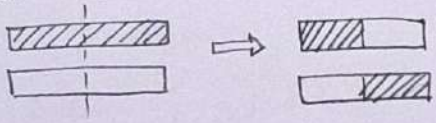
⇒ Based on structure of gene or chromosome

* Point mutations – change in a single base pair

1. Silent – no change in amino acid due to redundancy of genetic code
2. Missense – results in a codon that codes for diff. amino acid
3. Nonsense – mutation results in stop codon, truncating the protein early
4. Frameshift

* Insertions & deletions – frameshift mutations

* Translocation



* Inversion

⇒ Based on effect of gene
- Loss of function
- Gain of function
- Dominant negative

Lethal mutation
Reversion (mutated → normal)

⇒ Based on fitness in a population
- Harmful
- Beneficial
- Deleterious
- Neutral
- Advantageous

Any real difference b/w Advantageous and Beneficial?

Note: The reason we can see mutations because they have some physiological consequence that's not lethal

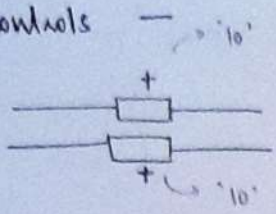
See slide 10 for examples

Muller's morphs

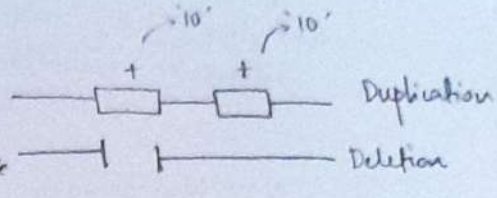
Classification of mutations based on their behaviours in various situations and based on gene interactions between them

TYPES OF MUTATIONS

Controls —

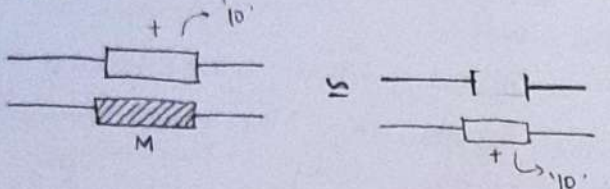


Homozygous wild type



Phynotype (P) : Wild type

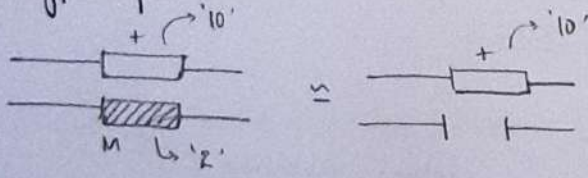
1. Null allele



- Loss of function mutation by null/amorphic allele
- Mutant phenotype
- Compensated by duplication

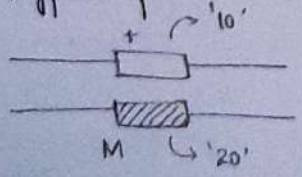
Eg: Blood group O is mutation in A antigen and is inactive

2. Hypomorphic allele



- loss of function mutation caused by hypomorphic/leaky mutation
- Mutant phenotype
- Compensated by duplication

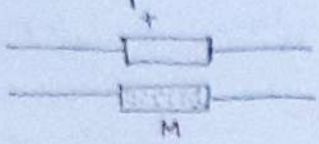
3. Hypermorphic allele



- Gain of function mutation caused by hypermorphic mutations — maybe due to loss of regulation i.e. increased transcription
- Mutant phenotype
- Compensated by deletion, gets worse due to duplication.

Could also happen when proteins/features are modified

1. Neomorphic allele



- Gain of function mutation due to neomorphic allele which produces an entirely new kind of phenotype
- New mutant phenotype
- Not compensated by duplication or deletion
- Example : BCR-ABL mutation — BCR & ABL genes break off and translocate, which results in ABL (a kinase) being constitutively expressed ie its active in an untimely manner.

Complementation Analysis

★ Here, we are considering that mutants are recessive. So —

$\frac{m}{m}$ → Paralysis $\frac{m}{+}$ → WT $\frac{+}{+}$ or $\frac{Dup}{Del}$ → WT

$\frac{m}{Del}$ → Paralysis $\frac{m}{m}$ + Dup or Transgene → WT

Britannica. Complementation test is used to determine whether two mutations associated with specific phenotype represent two alleles or are variations of two genes.

A mutation in which the homozygous form and mutation + deletion form are giving rise to same intensity of phenotype is called NULL Mutation.
This can be compensated by duplication.

★ Consider if this were a hypomorphic mutation—

$\frac{m^h}{m^h}$ → Paralysis < $\frac{m^h}{Del}$ → Stronger paralysis (as compared to $\frac{m^h}{m^h}$)
Probably walks better

When there's a gradation of severity i.e. combining mutation and deletion gives more intense phenotype than homozygous mutation — then HYPOMORPHIC mutation.
This can also be rescued by duplication

Intensity of phenotype : $\frac{m}{m} > \frac{m^h}{m} > \frac{m^h}{m^h}$

↳ Can be understood in terms of no. of protein product molecules formed

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* Hypermorphic mutation

$\frac{m^{hyper}}{m^{hyper}}$ → Mutant (more sensory hair) $\frac{m^{hyper}}{Del}$ → WT hair

Intensity of phenotype : $\frac{m^{hyper}}{m^{hyper}} + Dup > \frac{m^{hyper}}{m^{hyper}} > \frac{m^{hyper}}{Del}$

So the phenotype can be rescued by a deletion.

* In case of neomorphic mutation, we can't change the phenotype by duplication or deletion or heterozygosity

So based on the results of the crosses, we can classify these various kinds of mutation

Genetic complementation

- This is used to characterising mutations – which locus they affect.
- When we make crosses between two different alleles for a particular kind of mutation, its called G. Comple.
- When mutations belong to different complementation groups, they generally affect different phenotypes.
- The mutant X is crossed individually with organisms that have deletions in 3 different regions.

mutx		
Del1	Del2	Del3

- Del1 and Del3 fail to produce the phenotype along with mut X (like paralysis) so they complement X.
- mut X + Del 2 results in the phenotype ie paralysis. So, if fails to complement ⇒ Del2 has deletion of genes which mutx is altered in.

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Lecture 5

Complementation

Use this to analyse bristle formation

Temp-sensitive paralysis

* Irradiation gives rise to point mutations and these are more interesting

Tool kit
- Mutants in process of interest
- Visible markers & phenotypes on different chromosome
- Chromosomal deficiencies / deletions
- Chromosomal duplications

Bristle mutations

Wild-type (WT) Stubble (sb) - shorter bristles

Getting bristle mutants in flies - ClB method

P ClB/+ ♀ × +/Y ♂

F1 ClB/m × +/Y

m/Y ClB/Y ClB/+ m/+

Recessive bristle defective mutant

In such a screen, 100s of mutants were obtained and those males were used.

Complementation analysis between $m_1, m_2 \ldots m_{100}$ will give complementation groups. For example -

$\frac{m_1}{m_3}$ → WT $\frac{m_2}{m_3}$ → loss of bristles $\frac{m_3}{m_4}$ → WT

This shows that m_2 & m_3 are most likely alleles of the same gene & fall in same complementation group.

Deletion analysis can be used to say whether phenotypes in m_2 and m_3 are uncovered by the same deletion. For eg: $\frac{m_1}{del1}$ → WT $\frac{m_2}{del1} = \frac{m_3}{del1}$ → bald.

This allows for an estimation of mutants which are alleles of the same gene.

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Temperature sensitive paralytic mutants (tsp)

- In nature, paralysis is a debilitating conditions — it might not be able to eat, mate, migrate or anything.
- And this paralysis might be caused due to different physiological reasons.
- It might be difficult to propogate such flies.
- Shibire mutation: The flies become paralysed at ~30°C i.e. in the vial, they fall down & become unconscious. When temperature is restored, they will recover and move up again (∵ negatively geotactic)
- These mutants are produced by CIB method and complementation analysis is done to narrow down the region of chromosome where the affected gene lies.

Dominant negative mutation

It reduces the function of wild type. Its similar to neomorphic mutation — the phenotype can't be rescued by crossing with deletion, hypo or null mutation.

As they're not recessive, they can't be mapped using deletion.

Semi-dominant - non-Mendelian, partially reduces the function of wild type

Example - Shibire ts1 tsp (s)

s/s	-	27°C / 3min
s/del	-	27°C / 3 min
s/+	-	38°C / 3min
+/+	-	>42°C / 3 min
+/Del	-	> 42°C / 3 min ⟹ its not just reduction in no. of proteins.

Shibire gene codes for Dynamin which hydrolyses GTP.

The functional unit of this protein is a ~~tetratem~~ tetramer.

This explains the semi-dominance of the mutation.

The gene produced different units which come together to form the tetramer. ←

Dominant null mutation – haploinsufficiency
The organism will not survive with just one working copy of the gene

+/M – lethal
+/del – lethal

Dup. +/M – viable
Dup. +/del – viable

Dup/M when hapo-insufficient will rescue more easily when compared to Dup/M when M is dominant negative

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Lecture 06

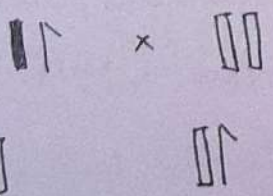
Recall : Muller's method for detection of X-linked lethal mutation – CIB method.

C: Crossover suppression through inversion of genes.
It has the phenotype of WT but it doesn't allow recombination to occur.
If recombination occurs, then it results in chromosome with two centromeres or none which would result in inviable progeny
For recombination to happen, the similar regions will have to align, for which the DNA strands would have to twist & contort.

l: lethal recessive mutation

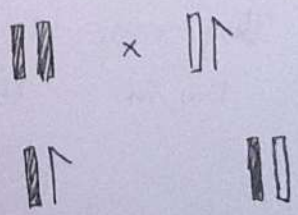
B: Bar-eye – dominant marker.

Mutant male with WT female



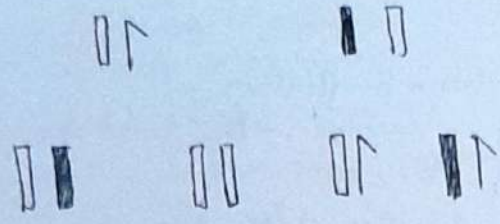
Carries female

WT Male x Homozygous female



Mutant male

WT male × Heterozygous female



This pattern of inheritance tells us that the mutation is X-linked.

Mapping mutations onto autosomes.

Generally only 2 pairs of autosomes are considered for mutations. This mapping is done by having multiple markers on each ~~chromosomal~~ autosomal chromosome

$$w/w\ ;\ +/CyO\ ;\ +/TM3 \quad \times \quad w/Y\ ;\ +/+\ ;\ +/+$$

$$w/Y\ ;\ \frac{\quad}{CyO}\ ;\ \frac{\quad}{TM3} \quad \times \quad w/w\ ;\ \frac{CyO}{Sp}\ ;\ \frac{TM2}{TM6}$$

$$\frac{\triangledown}{CyO}\ ;\ \frac{TM3}{TM6} \qquad \frac{Sp}{CyO}\ ;\ \frac{\triangledown}{TM6}$$

Two visible markers on 3rd chromosome ⇒ not on 3rd

Two visible markers on 2nd chromosome ⇒ mutation not on 2nd

By looking at the segregation of markers, we can assign mutation to a particular chromosome

CyO - dominant curly
Sp - dominant, increased bristles
TM6 - tubby - smaller flies
TM2 - bristle mutation
TM3 - short bristles.

Balancers

They can be used to identify heterozygous / carrier females

Bar/+ : Kidney shaped eye Bar/Bar or Bar/Y : slit eye

† They discovered that there were many more genes (that followed Mendelian genetics) than there were chromosomes.

Lecture 7

Linkage and Recombination

Not all genes are independently assorted. Some genes on chromosome are linked

This discovery was associated with that of diplody and recombination. Constancy of chromosome no. was being appreciated in early 1900s.

Rabl & Boveri – each chromosome had a distinct localisation in nuclei & they didn't lose their individuality at the end of division.

Montgomery (1901). Sutton (1902) – chromosomes occur in pairs, can be seperated based on size and shape, one is maternal, the other paternal.

Wininwarter (1901) – side by side pairing of chromosome

Sutton (1901) – linked x chromosome to sex-determination.

Sutton & de Vries (1903)†

→ Linkage – first shown by Correns (1900) in Matriola

Two strains – Anthocyanin in petals & rough stems, leaves
White in petals & smooth leaves

F_1 gave dominant heterozygous i.e colored, rough

F_2 gave progeny in CR : Cr in 3:1 ratio and not 9:3:3:1.

He said that there was absence of recombination between two phenotypes – complete linkage

Tschermark later dissected that it was 4 genes that were linked

→ Bateson and Punnett (1905)
Also found lack of independent assortment

TH Morgan found a similar deviation from Mendel's laws –

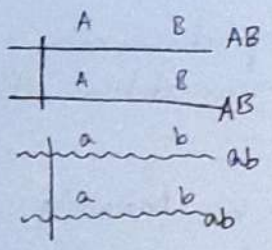
Mutant traits : pr - purple eye color → dense pattern – pseudopupil not visible vg - vestigial wing

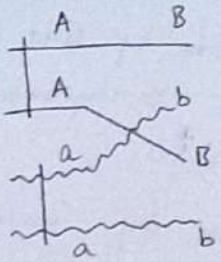
P $pr/pr\ vg/vg \times pr^+/pr^+ . vg^+/vg^+$ $\qquad pr^+/pr^+ . vg/vg \times pr/pr . vg^+/vg^+$

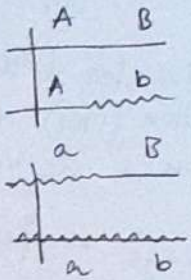
F_1 $pr^+/pr\ vg^+/vg$ $\qquad pr^+/pr\ vg^+/vg$

T.C $pr^+/pr . vg^+/vg\ ♀ \times pr/pr . vg/vg\ ♂$ $\qquad +/pr . +/vg \times pr/pr . vg/vg\ ♂$

pr^+	vg^+	1339		pr^+	vg^+	157	
pr^+	vg	151	Parental > 50%	pr^+	vg	965	
pr	vg^+	154	Recombinant < 50%	pr	vg^+	1067	
pr	vg	1195		pr	vg	146	







It was observed that frequencies of recombinants b/w linked loci is always < 50%.

Three point cross

Note: Recombination is always studied in females because the process is absent in males

v: vermillion cv: crossveinless ct: cut (serrated wing)

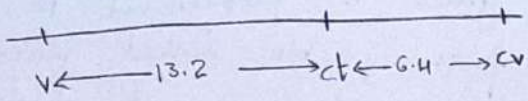
P: $v^+/v^+\ cv/cv\ ct/ct \times v/v . cv^+/cv^+ . ct^+/ct^+$

F_1: $v^+/v . cv^+/cv . ct^+/ct$

Test cross : $v^+/v . cv^+/cv . ct^+/ct \times v/v . cv/cv . ct/ct .$

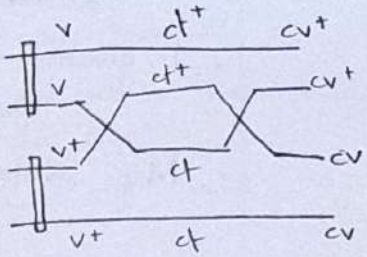
Total : 1448

Recombinants for v-cv : 268 18.5%
v-ct : 191 13.2%
cv-ct : 93 6.4%



$13.2 + 6.4 = 19.6 \neq 18.5$

The values will match if you consider double recombinations



For v-cv recombinants, we need to include $v-ct-cv^+$ & v^+-ct^+-cv genotypes. Then we get — $\frac{284}{1448} = 19.6\%$

Linkage – tendency of DNA sequences that are close together on a chromosome to be inherited together during meiosis phase of reproduction

Interference
Cross-over at one locus interferes with crossover at the adjacent locus.

$$I = 1 - \frac{\text{Observed no. of double recombinants}}{\text{Expected no. of double recombinants}}$$

In the above example —
Observed no. = 8

$$\text{Expected} = \underbrace{0.132 \times 0.064}_{\text{linkage}} \times 1448 = 12$$

$$I = 1 - \frac{8}{12} = \frac{1}{3} \text{ or } 33\%$$

∴ The two crossover regions are not independent and single crossover is favored over double

Recombination

- It's the process by which pieces of DNA are broken and recombined to produce new combination of alleles
- Recombinations occurs through independent assortment - in non-linked genes - and crossover in linked genes.
- Sturtevant quantified it - centimorgan (cM) = 1 map unit
  - The distance b/w genes is proportional to the recombination frequency b/w linked genes.
  - 1 genetic m.u = distance b/w genes for which recombinant freq was 1%
  - Map distances are additive

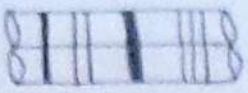
Recombination / linkage map can be superposed on chromosome map in flies.

The mutation linkage map was being mapped to X-chromosome
Getting multiple deletions with overlapping sequences made it possible to do complementation analysis.

Polytene chromosomes

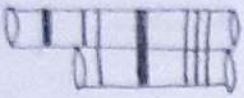
- Chromosomes from salivary gland of flies shows a banding pattern when dyed. This is because DNA is replicating and staying next to the original strand because the cell doesn't divide
- They provide high level of function in certain tissue They're strongly amplified form of interphase chromosome
- Banding pattern gives us some info about loci. Loci that have increased transcription i.e more RNA stain lighter while those with decreased transcription stain darker.
  So this pattern is very regular and reproducible
- Experiment by Demerec and Hoover showed positions of genes can be determined on chromosome by comparing with deficient chromosomes.

y yellow
ac achaete
sc scute

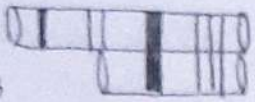


- They considered 3 recessive mutations on X-chromosome. When heterozygous with WT, the flies were normal.

- When mutant chromosome was crossed with def 1, the fly was still WT



- Through complementation analysis, we can say that —



- these genes are not in first 4 bands
- y, ac are in 4-8th band
- sc is in 8th - 10th band.

Linkage analysis between these genes can confirm if our mapping is correct.

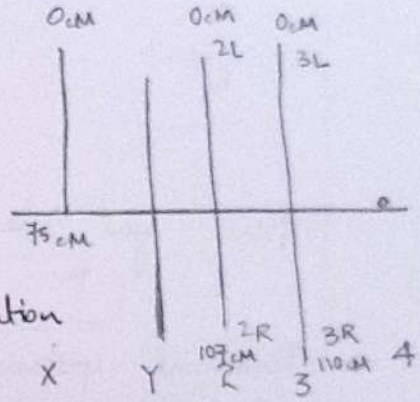
Chromosomes

Drosophila just has 4 chromosomes.

X - acrocentric - asymmetrical arms

2, 3 - metacentric

We get the length as >100 cM because of multiple recombination hotspots leading to increased recombination events



Database of drosophila - Flybase

Lecture 10

Epistasis - interaction of non-allelic genes to control a phenotype in a different gene

- One or more loci interact to create new phenotypes
- One allele masks the effects of the other

(29) → Bateson and Punnet found an example of gene interaction in comb shape in chicken (Rose, single, pea, walnut)

P	Rose (RR_{pp})	×	Pea (rrP_P)
F_1	Walnut		

F_2	9 Walnut	3 Rose	3 pea	1 Single
	R_ P_	R_ pp	rrP_	rrpp

So the shape is determined by interaction of alleles in two loci

→ Flower color in peas (Duplicate Recessive epistasis)

Precursor → Step 1 → Step 2, Anthocyanin

P

C

P	Purple	×	White
F_1	Purple		

F_2	9 Purple	:	7 White
	P_ C_		cc__/__ pp

Here you need both gene products to produce the color i.e its a multistep process

→ Dominant and recessive epistasis

Primula blue color – Malvidin pigment production

K ---- X ---> malvidin

D

K produces malvidin but dominant D suppresses this phenotype

P	Blue	×	White
F_1	White (KkDd)		

F_2	13 White	:	3 blue
	__D_		K_dd
	kk dd		

→ Kernel color (Duplicate dominant epistasis)
Here two alleles have same function i.e one substitutes the expression of genotype at a distinct locus

Gene A
Precursor → Colored kernel
Gene B

F2. 15 Colored : 1 Colorless
A___/__B_ aabb

→ Mouse coat colour (Recessive epistasis)

	Agouti	Black	Albino
	A_	aa	cc (no pigment)

P Agouti Albino

F1 Agouti

F2 9 Agouti : 3 Black : 4 Albino
A_C_ aaC_ __cc

Precursor —Gene C→ Black —Gene A→ Agouti

Penetrance vs Expressivity

- Degree of phenotypic expression varies from individual to individual and this property is called expressivity (0 to 1)
- Penetrance - variability of expression is so that it doesn't always give a phenotype (0,1)

Eg: Familial periodic fever - autosomal dominant inheritance with reduced penetrance.
Coat variegation in dogs - varying expressivity
Polydactyly - dominant disorder with variable expressivity.

→ Homozygous lethal yellow allele in mice
So, YY animals don't survive to adulthood.
Yellow is only present in heterozygous form.

P Yy × Yy

F_1 1 YY : 2 Yy : 1 yy ⇒ $\frac{2}{3}$ yellow : $\frac{1}{3}$ black.

Dead Yellow black

→ Incomple dominance in Mirabilis jalapa
R: Red r: white

RR × rr

1: RR 2: Rr 1 rr

Red Pink white

→ Co-dominance – blood group in humans
A, B, O, AB (no antigen)

AO × BO

AO BO AB OO

→ Sickle cell anaemia
Hb^S/Hb^S – severe, often fatal anaemia
Hb^A/Hb^S – no anaemia – selective advantage in geographic locations where malaria is prevalent

Hb^S also pleiotropic effects on development of many tissues such as lungs, kidneys, spleen & heart.

→ Multiple alleles
When more than a few forms of genes exist in a species, they give different phenotypes
Eg: eye pigment in drosophila.

Usually in organisms, we can see both pleiotropy and multiple allele effects
⇒ Very few genes show Mendelian inheritance

Lecture 11

Fungal Genetics – Deciphering metabolic pathway.

Linkage, recombination and metabolic pathways were discovered in fungi

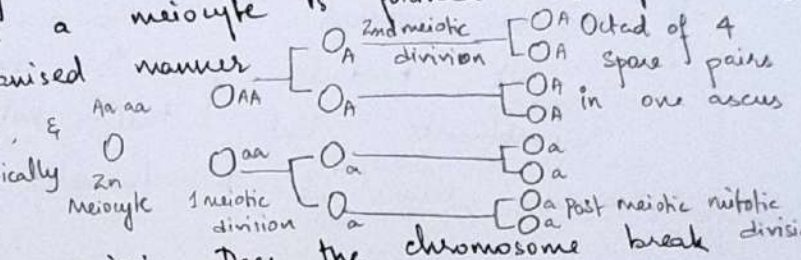
Model organism – Neurospora – spores are arranged in nerve-like fashion

Ascomycete, grows on rotting stuff – spores are present in one sac.

Haplodiplontic life cycle

Has 7 chromosomes in haploid state

- All the spores present in one sac are the result of one meiotic division.
- In neurospora, when abundant nutrients are present, the haploid organism grows vegetatively and forms hyphae
- In lack of nitrogen, cross fertilisation occurs b/w two strains and a meiocyte is formed and it divides in a very organised manner



Octad of 4 spore pairs in one ascus

very conveniently placed, & can be directed microscopically & seperated

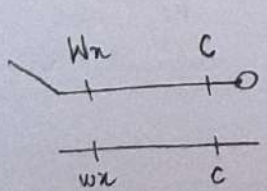
Genetic Recombination

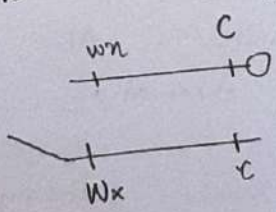
It occurs during meiosis. Does the chromosome break and at which stage does it occur?

Crossing over due to chromosome breakage and rejoining

→ Barbara McClintock and Harriet Creighton in 1931 found that the 9th chromosome in corn plant has dissimilar chromosome – it has a long arm & a knob.

They considered two traits – Wx (waxy vs starchy) C : colored





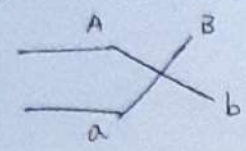
The recombinants always occured in jn conjunction with the long arm (Waxy) or knob (colored)

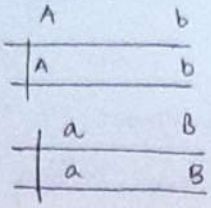
∴ Breakage is responsible for recombination to occur

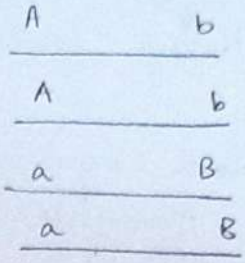
Breakage occurs at 4 chromatid stage
Organisms with haplodiplontic life cycle helped decipher it

Cross AB × ab → AB, ab, Ab, aB

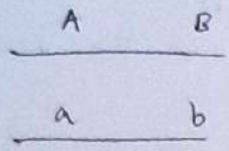
Two chromosome

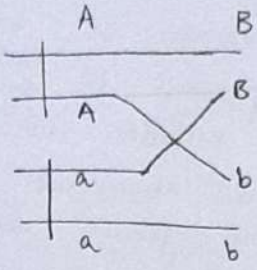


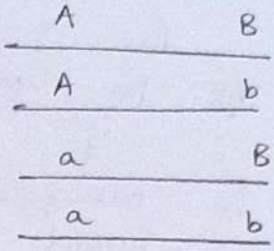




4 chromatid stage

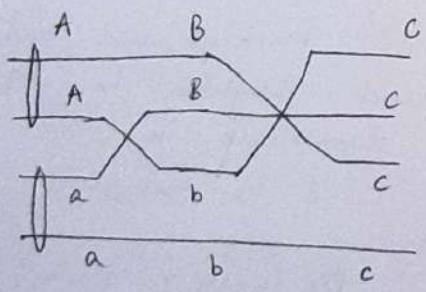


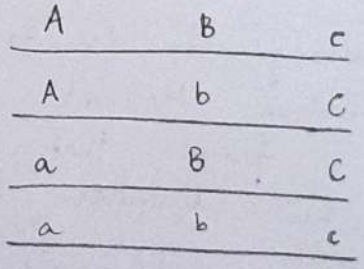




If crossover occured at 2-chromosome stage, then all products of the meiosis would be recombinants. But that's not what was observed.
∴ Crossover occurs at 4 chromatid stage.

Multiple crossovers can involve more than one chromatid —





If we get gametes : ABc Abc aBC abc
⇒ All chromatids were engaged in recombination

Recombination repair pathway
Involves many steps are each are intricately regulated by complex proteins. So recombination is a precise pathway

1. Cohesins bring together homologous chromosomes pair and attach them at centromere
2. Spo11 – causes a break in the double stranded DNA and one of the strands is chewed up, so the counterpart becomes the invading strand
3. The invading strand invades the homologous chromosome and initiates crossing over. There are different set of proteins which faciliate the process and code for the extension of chewed up strand
4. The junction formed is resolved i.e the strands of sister chromosomes are exchanged

Recombination occurs more away from the centromere For this to be initiated correctly, the machinery is closely regulated

Beadle and Tatum's dissection of metabolic pathways

- Experiment: Neurospora were subjected to X-rays to create mutants and each spore was seperated through microscopic dissection.
- They can be grown individually in test tubes with complete and minimal medium.
- The aim was to identify enzymes involved in metabolic pathways by growing mutants in complete medium and replicas in minimal (some nutrients missing) medium
- If a mutant doesn't grow in minimal medium, we can check what nutrient it isn't able to process / synthesize

Complete (G) Minimal + amino ā. (G) Minimal (O)

↓

Min + Ala (O) Min + Gly (O) ..... Min + Arg (G)

They concluded that the mutant had loss of function mutation in Arginine metabolism.

They found more mutants

Mutant 1				Mutant 2				Mutant 3			
M	M+Arg	M+Cit	M+Orn	M+Arg	M+C	M+O	M	M+A	M+C	M+O	M
0	1	0	0	1	1	0	0	1	1	1	0

Defect in: Enzyme X Enzyme Y Enzyme X

Ornithine - citrulline metabolism:

Precursor $\xrightarrow{\text{Enzyme X}}$ Ornithine $\xrightarrow{\text{Enzyme Y}}$ Citrulline $\xrightarrow{\text{Enzyme Z}}$ Arginine

Example:

	A	B	C	D	E	G
1	−	−	−	+	−	+
2	−	+	−	+	−	+
3	−	−	−	−	−	+
4	−	+	+	+	−	+
5	+	+	+	+	−	+

$$E \xrightarrow{5} A \xrightarrow{4} C \xrightarrow{2} B \xrightarrow{1} D \xrightarrow{3} G$$

8/3

Lecture 13

1900 – Rediscovery of Mendel's work by DeVries, Correns and von Tschermak

1910 – Sex limited inheritence in Drosophila
Eg: White eye.

Mutation – variation due to change in individual gene
artificial transmutation of gene

Hermann J Muller – discovered that increasing background radiation increases mutations
X ray is a powerful but crude tool – creates random mutations.

* Neutral theory, negative selection, epigenetics, evolvability embryology, Genomics revolution, HGT, Behavioural inputs (Baldwin effect)

Modern Synthesis (1930s - 1940s)

Combines Darwinian selection and Mendelian inheritance (genetics), with a focus on statistics.

This 'solved' the discrepency in both fields

Population genetics — emphasis on quantitative characters.

Post modern synthesis* — whole bunch of concepts in biology that have advanced our understanding

Mendelian genetics — only applicable to organisms that follow his laws of genetics.

There a lot of concepts that are non-Mendelian.

Horizontal Genetics — exchange of genetic material in same generation within or across species.

Observed in prokaryotes extensively and even eukaryotes

This has dramatic effects on evolution

& C. Diver

Maternal Genetics

First observed by A E Boycott, (1920s). He studied water snails and inheritance in them.

True breeding snails of Limnea — one dextral (c) and other sinistral ())

(♀ ×)♂ Reciprocal cross)♀ × (♂

((( )))

This contradicts Mendelian pattern of inheritance

" impressed on the egg by the mother" — Sturtevant

Extranuclear inheritance – mechanism.

There is genetic material outside the nucleus in mitochondria and RNAs are also present in cytoplasm.

⇒ All the mitochondria in a person is coming entirely from the mother.

The ovum also has a lot MRNA deposited by the mother and all the initial development is directed by the proteins synthesized from these mRNA.

MRNA inheritance only affects the phenotype in the first few hours of development. Its very transient.

Going back to Limnea –

Consider there are two alleles – S^+ (Dextral) and S (sinistral). $S^+ > S$ – S^+ active WT allele

P S^+S^+ ♀ dextral × SS ♂ sinistral

F1 S^+S

Self cross S^+S ♀ dextral × S^+S ♂ sinistral

F2 S^+S^+ S^+S SS

All dextral

(?) SS ♀ Sinistral × S^+S Dextral

SS S^+S

Sinistral

SS ♂ sinistral × S^+S ♀ sinistral

S^+S SS

Dextral

Because of dominant allele in genotype – MRNAs expressed are S^+

Cross 1	Cross 2
P Right × Left	P Left × Right
F_1 All Right	All left
F_2 All Right	All left
F_3 3 Right : 1 Left	3 Left : 1 Right.

9/3/24

Lecture 14

Genetics began when Mendel's work was rediscovered
TH Morgan developed the fruit fly as his model system. Other models are E.coli, yeast, C elegans, and mice.

1910 - Columbia - Fly room
He used hand lenses to observe mutations
He also trained a dozen students (including Sturtevant, Muller and Bridges) who dominated the field
1928 - Division head at Caltech
1933 - Nobel prize
1940 - George Beadle takes over as division head

In Limnea Peregra, the shell and internal organs can be dextral or sinistral determined by the cleavage pattern of egg after fertilisation.

D : Dextral (active) d : Dextral (inactive)

For parental generation, we take pure lines -
The coiling (?) is determined by the genotype of the mother because the nurse cells deposit mRNAs in the oocyte and this determines the coiling

P dd ♀ (s) × DD ♂ (d)

F1 Dd ♀ (s)

Self Dd ♀ (s) Dd ♂ (s)

F2 DD Dd dd

dextral

So we can find out if a female is homozygous or heterozygous by looking at its progeny.

In flies, 1-3% of genes are maternal.

Oocyte is formed in female animals. Its nourished by surrounding diploid maternal nurse cells. They provide nutrition, energy and gene products to the oocyte.

So the phenotype of oocyte is determined by the genotype of nurse cells.

Maternal to Zygotic transition

Graphs of developmental stages in various animals

In sea urchin, the maternal influence lasts for 1-1.5 hours (red shade)

Grey shade - zygotic genome takes over

The slope of red comes down suddenly because there are mechanisms that degrade maternal mRNA in the embryo so zygotic genome can take over.

≠ A chunk of maternal genes expressed in the early development overlap with the zygotic genes expressed later by similar-working (homologous) genes.

Mitochondrial inheritence

→ Unlike maternal mRNA, the mitochondria are permanent.

- Mitochondria have a haploid genome — mtDNA. - 17,000 bp
- They can undergo fusion and fission. When they fuse, there are multiple genome in the fused mitochondria — so even if some genomes are mutated, most of mRNAs produced will be effective ones
- Carries few genes: Genes coding rRNA and tRNA 13 genes encoding proteins involved in ATP generation via oxidative phosphorylation.

Lecture 15

Correcting the linear pedigree chart

Nurse cells deposit maternal determinants in oocyte which pattern A/P and D/V axes after fertilization.

After dumping nutrients and mRNA the nurse cells undergo apoptosis

Some fraction of mRNA are deposited in a pattern

Eg: Bicoid mRNA is stuck to anterior side. So when goes into nucleus ← Bicoid protein shows a gradient in A/P axis.

This dictates the development of head - thorax.

Similarly Dorsal protein has a gradient in D/V axis.

This asymmetry is entirely maternal inheritence and determines early development.

This is manged by scaffolding cytoskeletal elements, certain signals ~~and~~ from nucleus and the surrounding follicle cells.

Read : Maternal to Zygotic Transition.

Mitochondrial inheritence

Most mitochondrial proteins are encoded by the nuclear genes. Proteins are synthesized in cytosol and transported into mitochondria.

Often, multiple nucleoids are present per mitochondria varying with organisms. This reduces the effect of mutation (even dominant one) on the organism.

Some neurodegenerative diseases are caused by mitochondrial mutations.

Eg: Leber's hereditary optic neuropathy, Parkinson's, (LHON)

Dystonia.

No recombination in mitochondrial genes.

(36)

Pedigree chart — All children of an affected female are affected, and only the female children can pass it on.

LHON — disease in which defects in mitochondria's ETC. degeneration of retinal ganglion cells

X-linked dominant inheritance is different from mitochondrial pedigree charts.

Fusion / Fission and Signalling

They can keep happening regularly and mitochondria exchange alleles.

It can also be a signal for DNA replication and cell division

Mirabilis - Chloroplast

Pigmentation of leaves in Mirabilis is determined by chloroplast genes —

- Green (WT) - green pigmentation is formed
- White (mutant) - synthesis of green pigment in chloroplasts

Cells containing both types of chloroplasts display green coloration.

(?) Heterotroplasmy —

white ♀ × Green ♂ — All white

Green ♀ × white ♂ — All green.

≠ Basal (minimal) transcription occurs in the oocyte

Lecture 16

Drosophila genetics

When you look at a model organism — everything we can observe in us can be observed in them.

There's a strong overlap b/w ~30k genes in humans and ~14k genes in drosophila. They function the same way.

Recall: Drosophila has 4 pairs of chromosomes. Y and 4th chromosomes are heterochromatinised and have very few genes.

Genetic screens — a method to figure out the function of a gene. It was developed by Nusslein - Volhard and Weischaus & they got a Nobel in 1980

It can also tell us what 2 or more genes do together.

In the ~14k genes, some are present on the same chromosome or a 'packet'

A few genes are activated all the time. They're called housekeeping genes. They've been conserved throughout many organism

When a gene is discovered its first christened as 'CG2368', computed gene_number. We know nothing about it.

But when its knocked out, then some function is lost. So, the gene is named after the loss of function Eg: eyeless, wingless etc.

Elements of Heidelberg Screen

Embryonic cuticle as a readout of 'patterning'

- They took an embryo at ~18 hrs after fertilisation.
- They put it in acid and heated it for a few hours. The inner mass is dissolved and only the outermost cuticle remains.
- When it's observed in dark film microscopy. All wild type embryos have the same pattern — dorsal hair, ventral lines, anterior head part, tail part etc.
- They fed EMS to male, enough to cause mutation (random) in 1 bp. This male was mated to normal female and the progeny were screened
- The mutations (27,000 lines) were stabilised using (?) balancer genes — a gene that suppresses the repair of mutated genes
- Out of this, ~18,000 were lethal mutations (homozygous recessive)
  - ~4,300 — embryonic lethality
  - ~580 — embryonic phenotype
  - ~140 — genes (complementation groups)
- At the end of this long screen, they found (discovered) ~140 genes implicated in development.
- In some mutations, the cuticle had denticle belts all over (ventralized embryo)* and another where the embryo had dorsal hairs all over (dorsal embryo) i.e only development of one side.
- Similar mutations with development of either head or tail.
- This means that the APDV axes are determined very early in development and crucial for development of later organs.
- The maternal genes (through nurse cells) are critical to establish axes (Among Bicoid), so that the zygote can the build up on it.

* Knocking out the 'Dorsal' gene gives us a ventralised embryo

So the drosophila is patterned by multiple protein concentration gradients

Zygotic screen

P ♀ $\frac{DTS}{Bal}$ × — ♂ (EMS)

F1 ♀ $\frac{DTS}{Bal}$ × $\frac{mut}{Bal}$ ♂

F2 $\frac{mut}{Bal}$ ♀ × ♂

F3 $\frac{mut}{mut}$ $\frac{mut}{Bal}$ (ignore) ~~$\frac{Bal}{Bal}$~~ lethal

Maternal Screen

Unless, to check for maternal effect on embryo, the zygotic screen is taken one step further by crossing mutant female with wild type. the mutation is lethal, the phenotype of progeny is observed.

F3 $\frac{mut}{mut}$ ♀ × WT ♂

F4 Progeny - Test phenotype.

≠ DTS - Dominant Temperature Sensitivity

Dominant markers used to keep track of the chromosome They're also sensitive to temperature - when given a heat shock, they die off leaving only the desired mutants alive - no hassle of seperating each fly seperately

(40)

Lecture 17

16/3

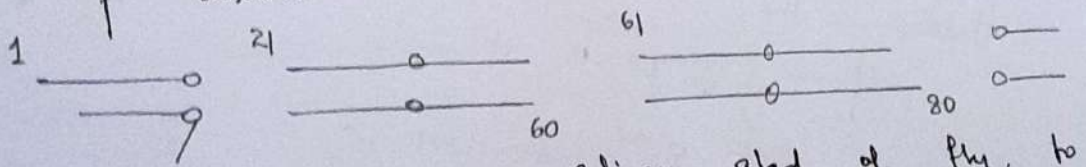
Egg + embryo hatches as 1st instar larva on day 1.
Eats a lot and lives as larva for 6-10 days
Then becomes and adult. life span ~ 3 months.
But males mostly for first month.

Balancer chromosome
Eg. clB, TM3 (*) ; CyO, SM6 (2) , TM6 (3)
They're chromosomes with large inversions so it suppresses recombination and recombination based repair

(*) Bar mutation - Dominant : kidney shaped eye (heterozygous)
as homozygous - ultrabar, slit like eye
(2) CyO - Curly wing - Balancer 2
(3) Ser - serrated wings

* The centromere and region near it is made of polynucleotide sequences and heterochromatinised

How did they figure out the inversions?
It was done by mapping the chromosome and placing the gene of interest on different regions of chromosome.



Polytene chromosome — in salivary glad of fly, to produce a lot of enzymes, the cells have closely packed, multiple (4n?) chromosomes for efficient signalling and transcription. These chromosomes are have banded pattern — heterochromatin and euchromatinised regions
Calvin Bridges (1935) drew the banding pattern quite accurately using camera lucida

This led to discovery of first signalling pathway in development known as Toll-Dorsal pathway

The banding pattern changes significantly after giving a heat shock - proteins required for it are transcribed.

Genes are mapped in relation to these bands.

Fly genome was sequenced by 2000 and all the mapping was pretty accurate.

The Heidelberg screen was done for all 3 chromosomes.

This allowed us to uncover genes/proteins that control early development and same/similar genes act in human development also

Eg: Dorsal, Bicoid, Nanos, Antennopedia

Dorsal - gene(s) that regulates the ventralisation of the embryo

'Dorsal' group

It was observed that many lineages gave rise to Dorsalized embryo. This was because there were mutations in many genes involved in Toll pathway. #

Ventralized embryo is formed because of mutation in cactus gene which acts as a negative regulator of toll signalling.

So same mutated phenotype might mean there are mutations in genes responsible for different elements of a signalling pathway.

Overview of Genetic Screen

Loss of function - Mutagenesis (EMS or transposon), Reverse genetics screen

Gain of function - UAS Gal4 system (hyperactive)

Mosaic - FLP-FRT (zygotic), DFS-FRT-OVOD (Maternal)

Combinations - UAS Gal4 with FLP, Crisps screen

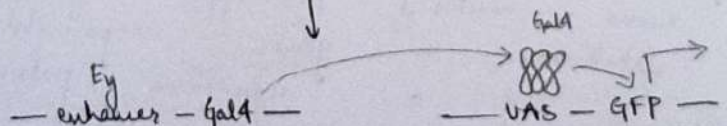
Enhancer/Suppressor screen

UAS - Gal4 System (Brand & Perrimon)

The goal is to increase the expression of a certain gene in a targeted manner, in a spatiotemporal context.

* They took 2 genes from yeast —
  Gal4 : makes Gal4 protein — it looks for an upstream activating sequence (UAS) and binds to it. It then recruits transcription elements i.e increases the transcription of genes downstream to UAS.
  UAS : DNA sequence (acts as a promoter)
* They created transgenic animals so that UAS was added upstream of the gene of interest, say gene X. (GFP)
  So this line of flies has UAS - gene X sequence (GFP) in every cell.
* In another fly, a genomic enhancer, say Eyeless promoter (Ey) is integrated with Gal4 sequence. Both lines were maintained repeatedly and had WT phenotype
  This line had Ey - Gal4
* When crossed,

Enhancer - Gal4 ♂ × UAS - GFP



In F1 flies, UAS and Gal4 were brought together. Gal4 protein activated UAS which in turn started the transcription of GFP gene

* The result is that these flies have iridiscent eyes when observed in UV light
* You could make Gal4 that expresses in different times (maternal or development) and spaces (eye, wing).
  By crossing, we could force the expression of any gene in the domain of our interest.

Eg: Gal4 : Argos (impedes development of eye)
Gal4 : Argos-Sprouty (sprouty inhibits argos so eye is normal)

Ventral nerve chord has a ladder like structure
Roundabout mutant - Axons coiled instead of crossing and innervating muscles
Commissureless mutant - no ladder, rungs - just parallel lines.

→ FLP technology

FLP is a sequence of DNA that's as close to the centromere as possible. When FLP recombinase is added, the arms of homologous chromosomes exchange or 'flip'.
For this to occur, mitosis is an important step. It can give rise to WT/WT, mut/mut or WT/mut cell.
After this, the cells will keep producing cells with some genotype as them.
Eg: While forming an eye, if FLP recombinase is introduced, some homozygous mutants are formed (white) and they don't have pigment. Because of this, white patches are formed on eye
This lets us focus on a tissue and study mutations there instead of the entire organism
Mosaic patches are formed in that group of cells
Eg: warts (tumor suppressor), piopio (breaks adhesion between 2 layers of the wings)
lar (formation of R7 layer in the eye)

PP

Lecture 18

→ RNAi Screen

Any gene

Sox 1 gene forms a dsRNA, interacts with mRNA and degrades it, not allowing that gene to express.

So when UAS is paired with Sox 1, the same UAS-Gal4 system can be used as a loss of function screen.

The gene is sequenced so that its MRNA folds over itself and suppresses the mRNA of a particular gene

Paired with the Gal4 you want, you can use it to knock down genes.

→ Enhancer / Suppressor screen.

Scabrous promoter: expresses in neural cells, macrochaete

VAPB: gene that causes neurodegenerative diseases when mutated

—sca — Gal4—

—UAS — VAPB—

Overexpression of VAPB also decreased the no. of macrochaete from 10 to 4-5.

If you increased the dose by increasing temperature, then macrochaete were absent.

If RNAi was done, then gene was not expressed and macrochaete recovered - so kinda reversible.

Taking the line of sca-Gal4; UAS-VAPB/+, 2635 genes were knocked out one by one

Sometimes # macrochaete went to 10, sometimes 0 & sometimes remained 5.5

Any gene that took #m to 0 was called an enhancer (a protein that worked on same pathway that enhanced the effect of VAPB) and gene that took #m was called a suppressor.

↳ 10

Very easy to count the no. of neuroblasts so it was a very quick screen.

Through this 58 suppressors and 45 enhancer genes were identified. Many genes formed clusters that helped understand how VAPB works.

An analog of VAPB in humans is implicated in a neurodegenerative disease called ALS (Amyotrophic lateral Sclerosis)

In drosophila, they found interactors of other ALS loci - ALS1, ALS2, ALS11 and ALS14. This way they could connect 6 ALS loci through a single genetic network (↳ of 24) and show mutations in this can cause neurodegenerative diseases.

2nd example: Cancer : uncontrolled cell-proliferation and metastasis

Genome wide screen : to discover tumor suppressor genes and other genes involved in regulation of cell proliferation.

Imaginal disc - In larval stage, there are small patches of ~1000 cells ($\frac{1}{10}$ - $\frac{1}{20}$ mm) that grow over the pupal stage to form wings.

They took two genes that cause tumors in humans (oncogenic?) and expressed them in small imaginal disc in flies using UAS-Gal4. What they saw was huge overproliferation

They expressed GFP in these cells to visualise the overproliferation.

apterous enhancer : expresses in one part of imaginal disc

— apt enhancer — Gal4 — —UAS - EGFR— —UAS — GFP—

EGFR or activation causes proliferation.

Yorkie (Yki) also causes the same in a slightly different way.

When GFP is expressed they can be seen quite clearly in the larva

In the absence of 2 other genes, the Yki & EGFR went nuts — crazy overproliferation. So, KK107955 and KK103055 were identified as tumor suppressor genes — i.e when active, they suppressed the action of Yki and EGFR.

So this becomes a discovery method to discover analog genes in humans that suppresses tumors.

They also found a enhancer genes.

The found many genes, 5 of which were involved in coding for same mechanism — transcription pausing — something that happens during development, in transcription.

Gal80 — a negative regulator of Gal4 that's active at 18°C, binds to Gal4 and doesn't let it express the relevant gene.

History
Mendelian Genetics
Morgan (x-linked), Muller's expts (3)
Mutagenesis
Muller's morphs (hypo, hyper, neo), Dominant -ve
Complementation
Mapping mutations
Linkage & recombination
Interference, Expressivity, Penetrance
Epistasis (revise with slides)
Fungal Genetics
Maternal genetics (MET, MTDNA)
Genetic screen — Heidelberg. UAS-Gal4, FLP-FRT. Enhancer - Suppressor.

PART 2

1/4

Lecture 19

Transposons - Horizontal Gene Transfer

Period : Post-Morgan - 1930s - 1940s

Barbara McClintock - Maize chromosomes

She was studying the pattern of seed colour

1921 - UG Genetics, Cornell

1920s - PhD - Cytogenetics (set it up with Sharp & Collins)

1931 - famous Crossing Over PNAS paper

1950 - Ac/Ds Paper (PNAS while she was in CSHL)

Crossing over

Morgan in 1916 had predicted this phenomena based on recombinant fruit flies.

B. McClintock and Harriet Creighton saw that there was a distinct architecture to one of the chromosome in maize lines.

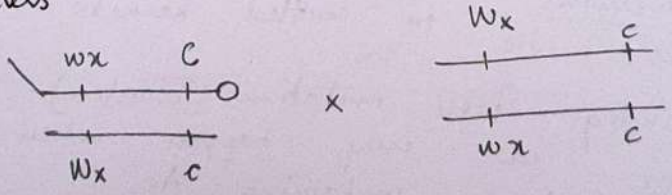
It had a knob on one end and a bent arm on the other (9th chromosome)

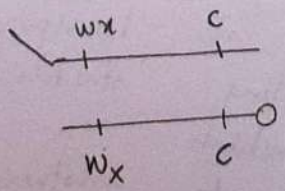
Wx : starchy

wx : waxy

C : colored

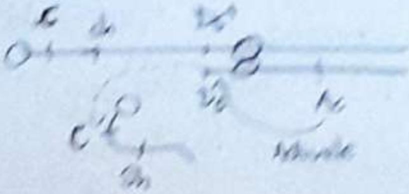
c : colorless





Detection of Chromosomal breakage*

Sometimes, the broken chromosome without region at the Ds locus. The small fragments would remain active in the cell, but it wouldn't be replicated or passed on.

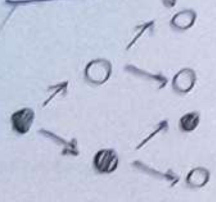


Ac Activator

Ds: Dissociation

* It's common in experimental papers when using organic solvents and all

She also observed that a gene at the Ds locus would 'jump' and insert into the colour gene, thus deactivating it.



This kind of transfer gives rise to a mosaic tissue giving rise to patchy seeds

Mostly but not always

She said that another gene, Ac would activate the Ds gene to jump and insert itself somewhere else

Transposable elements (Ac/Ds)

- Ac causes (initially?) mutations in gene for kernel pigmentation
  Anthocyanin (WT) ⟶ colorless (mut)
  But reversions occur at a high freq which gives rise to mottled kernels
- Ds: causing stable mutations (colorless)
  Reversion can only happen when crossed to a strain containing Ac

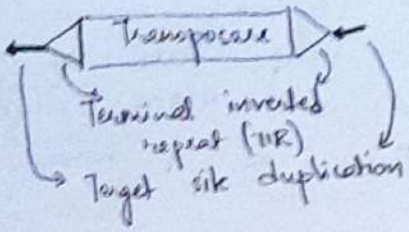
when Ds is removed from in b/w C gene and put somewhere else

Ac & Ds both are transposable elements

Ac in its coding stretch codes for a transposase which can make Ds and Ac genes jump to another locus. Ds has lost its transposase, so its non-autonomous element whereas Ac is autonomous

Ac : 4.5 kb flanked by inverted repeats

Autonomous



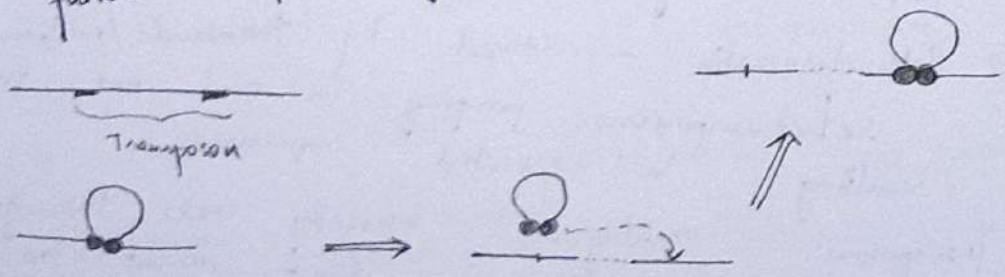
Class 1 : RNA transposon (retro transposons)

Class 2 : DNA transposons

Class 2 transposons are singular – they remove themselves and insert in some other locus but its still a single copy

Class 1 transposons are developed through reverse transcriptions – thousands of copies of dsDNA can be produced and introduced into different positions in the genome, potentially deactivating thousands of genes.

50% of human genome is made of transposares and debris left behind by transposons. And there are several (40 - 100s) families of different kinds of transposons.



5/4

Lecture 20

Her work was not understood or appreciated.
: her paper was very complex and her conclusions were dramatic despite the fact that she was well known in 1950.
By 1970 - 1980, the idea was accepted when it was observed in bacteria & other organisms.

1983 - Nobel prize.

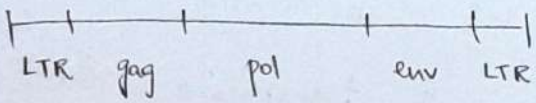
3D structure of transposare – its a dimer

The transposons have inverted repeats at the ends where the transposare cuts the DNA in a staggered manner (ATGCA)

Then this piece inserts itself into the gene, while leaving behind an ATGCA sequence as many no. of times as it moves.

Retrotransposons

Retrovirus MoMLV



gag - coat protein

pol - polycistronic DNA - Protease + Rev. transcriptase + Ribonuclease + Integrase

env - protein coat

This sequence is found in yeast, drosophila & humans with varying similarity.

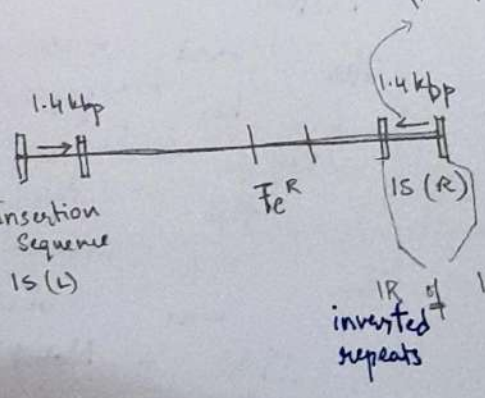
* In L1 in humans, gag & env are lost, so this is actually a DNA particle that's just sitting in our genome.

Heterochromatin – caused by thousands/millions of retrotransposons jumping in and out thus resulting in repeated sequences.

Transposons – mutagenic : basically make the genome dynamic but you don't want too much dynamism, so transposons are tightly regulated

Tn 10 transposon in bacteria

9.3 kbp



This transposon contains tetracycline resistance gene and this is present on a plasmid.

Terminology

Replicative : results in multiple copies of transposons

Conservative : a single transposon moves around

Composite : Tn10 - has IS (insertion sequence) at the end

Simple : Tn3 - has IR (inverted repeats) at the end

Transposable elements in yeast: Ty

They can cause gene inactivation (by insertion into coding or regulatory sequences) or activation of previously silent genes

Structure : 6 kbp containing 334 bp direct repeats (delta elements & long terminal repeats) which carry strong promoters for RNA pol II

Almost 50% of human genome corresponds to interspersed repeats — debris left behind by transposons

SINEs : v. small 100-300 bp - 1.5 M copies - don't have the transposase gene (↓3%)

LINEs : > 20% - 1-5 kbp - 20k-40k copies

LTR elements : > 8%

DNA elements : > 3% → 80-3000 bp 300k copies

(Autonomous & non-autonomous)

As can be expected a host of diseases (genetic) are caused by these.

2-3 kb ←

Insertions — L1 insertions cause Duchenne muscular dystrophy

Type 2 retinitis pigmentosa, β-thalassemia and chronic granulomatous disease

Human	~ 50%	Maize	~ 75%
Fly	~ 3%	Barley	~ 85%
Worm	~ 7%	Iris	~ 98% [thugel]

Maize, rice, barley, sorghum – all have evolved from a common grass ancestor.

They have ~ 5-10% coding genes out of ~ 2.5 Billion bp of genome, in 70 mya of evolution

This is possible because they have 4n – 8n (even 16n – 32n) sets of gene.

Thus, transposons become a powerful mutagenic tool that works internally. Its tightly regulated to express only during certain stages

Colorful effects – varieties of blood oranges# and grapes are the result of there transposable elements.

Refer lecture for more info.

Other examples – corn, peppered moth, placenta 6/4

Lecture 21

Genetic diversity is caused by –

Deletion, duplication, inversion, translocation

From invertebrate → vertebrate evolution, there have been 2-4 cycles of whole genome duplication.

Eg: There are ~ 200,000 Alu elements 10% of which are within genes.

A recent study reports that transposons are v. important in implantation and uterine placental development.

Transposons & Selfish gene concept (1970s)

Selfish gene – ultimate goal is to maintain itself

	Drosophila	Human
Mariner	0-5	53,000
piggyBac	0-10	500
p-element	0-15	0

↳ DNA transposons entered the gene recently (~1950) & spread throughout world populations.

Blood oranges – normal variety with transposon in the anthocyanin gene responsive variety

Rio – 350 MB
Maize – 2500 MB

These transposon numbers can be used to track the phylogeny i.e. when the species diverged and how long ago.

p-element : Example of eukaryotic TE
Flies collected before 1920s (fly lines) lack this transposon. But in the WT populations, this new TE spread rather quickly, all over the world.

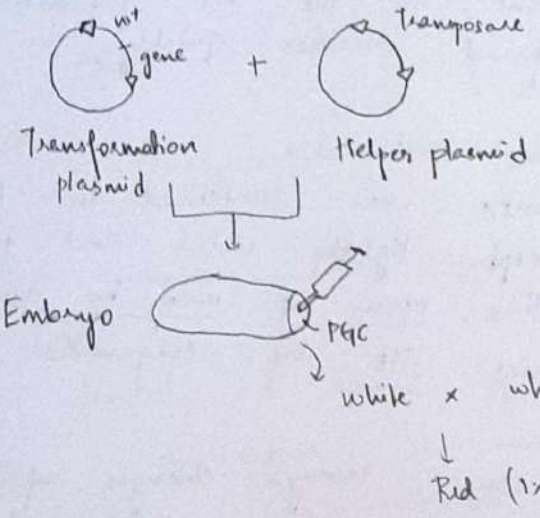
How are transposons contained?
Transposon elements are contained in the heterochromatin which is kept tightly coiled and packed. most of the time, opens up only in germline stage
The host silences TE by epigenetic mechanism.

Transposition can cause enough changes of in organism — it can induce speciation by selective mutation of transposons post-vicariance (allopatry).

P-elements
- ~ 4-5 kbp. Has a huge 'transposase
- 4 exons called 0, 1, 2, 3
- Full length protein can act as a transposase but can be spliced to block transposition.
- Rubin and Spradling
  p-element + bacterial plasmid — circular DNA.
  Plasmid part had tags. By transformation into bacterial DNA.
  In drosophila, since p-element will hop, it can create transgenic drosophila
- R&S added a p-element (with WT white-eye wt gene) to the primordial germ cells in the embryo which go on to form germline.

1% of next generation showed red-eyed flies.
Transposon did work to transfer the genes.

- They used a second vector with transposase that cannot integrate into genome, which ensures non-autonomy of hopped element but the genes inside the element remain in the germline.
- The transposed piece reaches PGCs and the rest of the plasmid is degraded.



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Lecture 22

Horizontal gene transfer

In bacteria this can happen in 3 ways —

1. Transformation : A bacterium picks up naked DNA from its surroundings

Griffith's expt and transforming principle

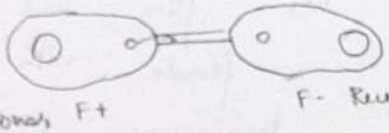
Strain	S (virulent)	R	Heat killed S	Heat killed S + Living R
Mouse	×	✓	✓	×

So Griffith suggested that bacteria are capable of transferring genetic information through a process known as transformation.

Advantage of lysogenic – all hosts (bacteria) are not killed off at once.

Avery, Macleod & McCarty proved that the transforming principle is DNA that transforms R strain to non virulent S strain.
They did it by mixing S strain extract and removing one component at a time (lipid, protein, DNA) and mixing with R and seeing what happens to mice – the mouse lived only when DNA in S strain was destroyed

2. Conjugation
Two bacteria exchange genetic information by forming a conjugation tube (sex pili) and donating/receiving a fertility F plasmid.



3. Transduction
This is transfer of genetic material through bacteriophages through the lytic or lysogenic cycle.
In lytic cycle (eg T2), viruses break down host DNA and hijack the machinery to replicate their own genome. When packing viral DNA into capsids, some random bacterial DNA fragments are also packed. This is called generalised transduction
In lysogenic cycle, the viral DNA is inserted into bacterial chromosome (prophage). The genes flanking the phophage are sometimes transduced when the DNA is excised. This is called specialised transduction

12/4

Lecture 23

Horizontal Genetics

Recall : Creating transgenic drosophila

The transposon can enter a population and its survival depends on its interaction with the genome and ~~the~~ its effects on fitness.

Lab drosophila – flies maintained in lab since 1910

WT ♀ × lab ♂ — Fertile

WT ♂ × lab ♀ — Sterile [Ovaries didn't develop properly]

WT flies had p-elements. When crossed with a female with no p-element, in the hybrid, the transposons jumped around and interfered with genes responsible for development of ovaries

This one-side sterility phenomena is called Hybrid Genetic dysgenesis.

In the first cross, there are p-element suppressors in the egg (of WT ♀) which prevents random transposition. This is lacking in the second hybrid.

Another eg: Mule & Hinny

Similar dysgenesis is observed in different species of drosophila.

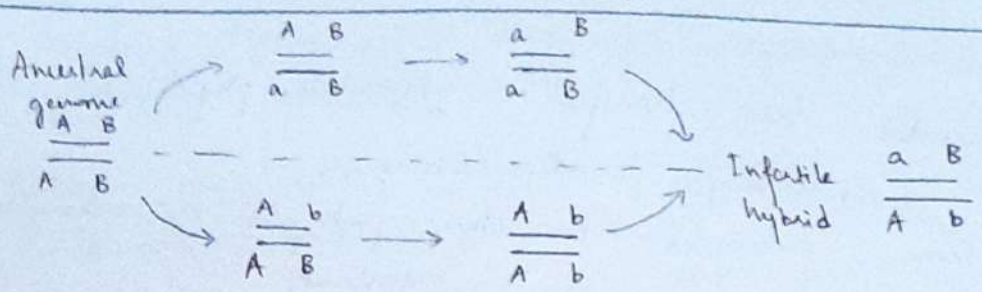
Hybrid - Incompatability and Speciation

One of the models – Dobzhansky - Muller model

They suggested genetic incompatability (due to isolation) is the main reason for speciation.

* Analogous to human heterochromatin protein 3

Two-locus version



Experiment studying this model

A study in 2006 : D. melanogaster ♀ x D. simulans ♂ gave rise to a infertile hybrid.

They induced mutations in D.m. and crossed with D.s ♂. In one of the crosses, they cross gave rise to fertile hybrid. Because of mutation in ~~Hybrid~~ Lethal mutation rescue (Lmr/HP3)* in Drosophila. So this is an example of new allele in Dobzhansky-Muller model.

Similarly, another mutation was discovered in D.s called Hybrid Male rescue (Hmr) that is another gene/allele ~~in~~ that would've played a part in speciation. Suppressing/mutating these genes makes the hybrid viable

This gives us insight into the genetic basis of speciation.

Recall : Bacterial gene exchange

Transfection : Experimentally forcing desired DNA to enter the cell and integrate with the host genome.

2 types - Stable & Transient.

Read reviews - GR17 & GR18, about HGT

Horizontal Gene Transfer — Reticulate phylogenetic tree

It could happen intra/interspecies
From prokaryotes to other prokaryotes or eukaryotes

Endosymbionts are very important
* Protection / Germ cells

HGTs are rare and improbable events, but once they occur and incorporate into genome & get passed down through germ cells, these changes become permenant.

Examples
- Antibiotic resistance / Toxin resistance
- Insertion sequence
- Pathogenecity islands
- Agrobacterium Ti plasmid
- Viruses and viroids
- Organelle to nucleus transfer

Evidence for lateral/Horizontal Gene transfer
Prokaryote genomes are mosaics of
- 'Backbone' or 'core' genes of common history
- Island of genes of alien origin
- Phage genes
- Single alien gene

Role of mobile DNA in evolution of Vancomycin-Resistant Enterococcus faecalis
- 25% of genome is mobile element related, which play a role in development of resistance

→ 7 phage regions
30 insertion sequences
Transposons & plasmids

Compound transposon can hold various antibiotic genes within it

Human microbiome - hotspot of microbial HGT

Airways, mouth, gastrointestinal, oral, skin, urogenital - hotspots of microbiomes.

There have been coexisting with us for a very long time. So HGT between bacteria and somatic cells is not uncommon.

Total no. of species: 308 Avg: ~44 HGT genes per microbe

There's a high frequency of HGT in the ocean

HGT is also found between parasites and their hosts

Viruses are also key players

15/4

Lecture 24

Chen et al (2016) - Approx 51 genes in human genome have seq. homology with viral genes.

Lim et al (2008) - bacterial conjunction in cytoplasm of mouse cells.

HGT in prokaryote > eukaryote. Simple method for inclusion of prokaryotic genome into eukaryotic DNA -

- Start as an endosymbiont
- Integrate into the genome over time

Wolbachia - Gram -ve bacteria that infects arthropods

Lecture 25 19/4

Wolbachia

18% of insects/worms infected - mutualism to parasitism

Maternal inheritence

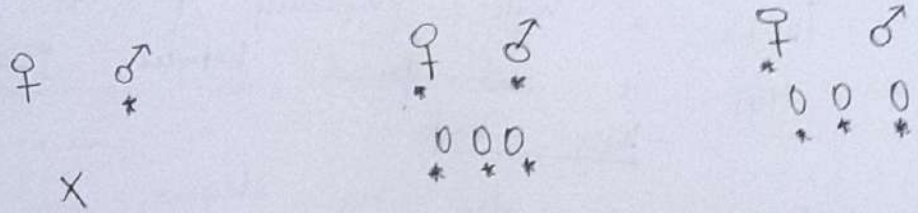
Affects reproduction *(selectively kills males via cytoplasmic incompatibility)*

Spiroplasma - similar parasite

These bacteria also protects these insects against other pathogens.

Wolbachia is in the cytoplasm of egg/oocyte.

If it releases its DNA into the cytoplasm through lysis or Type IV secretion, and its possible that this integrates into host DNA.



Through this cytoplasmic incompatibility, mosquito populations (dengue, zika, chikanguinya) have been controlled by releasing thousands of infected ~~by~~ mosquitoes.

DNA Sequencing

The $1000 genome - the idea that human genome can be sequenced in 1000 USD.

Sequencing human Genome

2001 - 2.7 G$ - 11 years - HUGO

100 M$ - 3 years - Celera

The ideas of sequencing began in 1980s with collaboration of multiple countries

Craig Venter - Celera

By 2017 - Archon Genomics Prize - 1000 $ sequencing

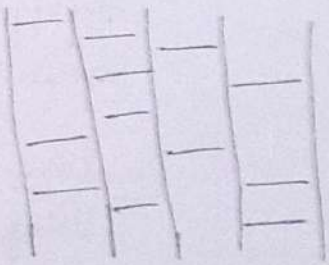
1980 - λ virus
1994 - H. influenzae
2000 - Drosophila genome
2007 - Global ocean sampling expedition
~ 3,000 microorganisms

All this was done through Sanger sequencing.
Venter took the heirarchal shotgun approach

BAC libraries - each segment of DNA is cloned and transformed into bacteria or transfected into yeast and these libraries are maintained in freezers.

Venter successfully managed to pair the fragments of DNA after sequencing them individually. He had super computers and used the public database to progress.

Dideoxy (Sanger) Sequencing - Result.



Reading a real gel
Read downward moving back and forth for each nucleotide in order
Complement of this will be the original required DNA sequence.

Development - automated sequencing by using dideoxy nucleotides differently colored fluorescent dyes in a single reaction instead of 4 different reactions.

Next Generation Sequencing (NGS)
Methods developed after Human sequencing
ABI, Heliscope, Roche, Illumina etc.

Movies

1. Introduction

3 components in NGS – sample preperation & data outputs

sequencing reactions mainly differ in sequencing reaction methods

Different

a) Pyrosequencing
b) Sequencing by synthesis
c) Sequencing by ligation
d) Ion semiconductor sequencing

Important aspects

1) Accuracy - 1000 bp without errors
2) How many runs can be done in parallel? Speed
3) Cost of sequencing
   Machine & /Nucleotide
4) Speed of sequencing

Problems

1) Amount of DNA extracted is vv less - has to be amplified
2) BAC library - maintaining unique sequences
3) Make sure its reproducible

20/4

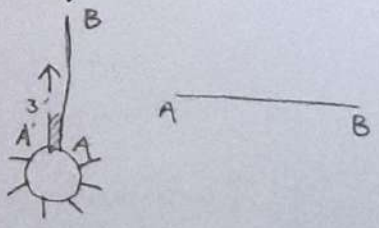
Lecture 26

Workflow of early NGS techniques

- Cut a genome to DNA fragments 300 to 500 bases
- Immobilize single strands on a small bead
- Amplify the DNA on each bead
- Seperate each bead on a plate with upto 1.6M wells

Emulsion PCR

Using beads ⇒ 1 piece of unique DNA on each bead



Extending the complement sequence can tell us the nucleotide being added and that can be read

This process works better if some oil is added to solution → Emulsion PCR

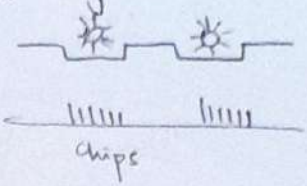
Movies

1. Introduction – Sample preperation, sequencing methods and data outputs

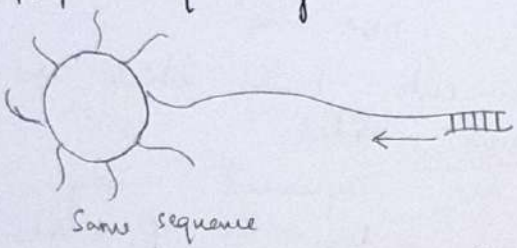
* Pyrosequencing

Illumina Workflow

1. library preperation – in vitro adaptor ligation (beads)
2. Cluster sequencing – polony array
3. Analysing / Detecting the sequence
4. Reading sequence parallely



454 Sequencing – Roche

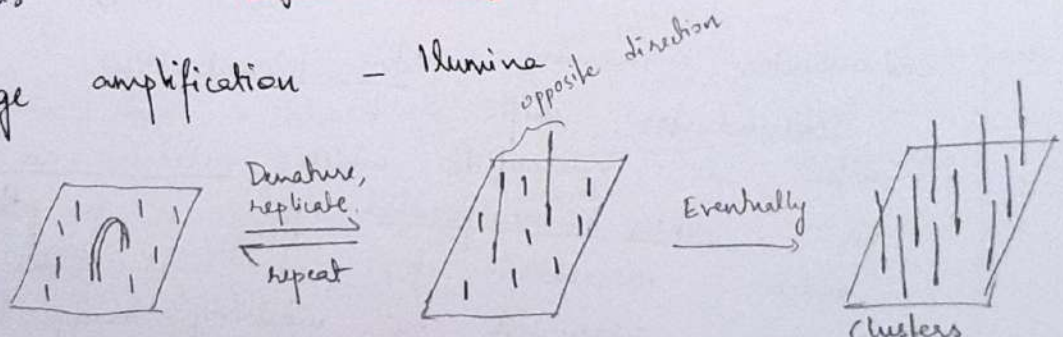


Everytime a correct nucleotide is added, there's a flash of light because the reaction involves luciferase

A is added, flashes are recorded, then its washed away & G is added & flashes are recorded again

This is pyrosequencing

Bridge amplification – Illumina



Sequencing by synthesis

Bridge amplification results in clusters that have forward and reverse sequences seperately

Sequencing both (seperately in time) reduces error in space

Novies

2. Illumina

Genetic material fragmented, adenylated, adapter oligose are attached on both ends, then repeated by size and purified

Cluster generation through bridge amplification

Sequencing using fluorescent nucleotides

3. Helicos

Add fluorescent nucleotides and image and wash away and repeat.

Done for unique sequences of 100-200 nucleotide long sequenced independently

DNA sequencing (Polony sequencing)

4. SOLiD

Fragment library - 1 DNA seq

Mate paired library - 2 DNA seq

Fragments are cloned onto beads which are spread on glass slides

Then the fragments are sequenced using (fluorescent) → di-base probes (5 bp) - done for 7 rounds (35 bp)

Primer is reset for by 1 bp and the whole process is repeated for 5 rounds

~ 99.94% accuracy ↳ Gives a dual measurement of each base

5. Semiconductor Sequencing - Ion Torrent NGS

Semiconductor chips - beads - each well flooded with a nucleotide which releases an H^+ ion when incorporated. There is a pH sensor which measures the pH and notes it

This is repeated by washing away that nucleotide, calling the next and measuring pH.

Massive parallel sequencing - takes lesser time

6. Intro to NGS

a) Pyrosequencing

Sequencing is regulated by release of Pyrophosphate which in turn generates a flash of light which is recorded

Sequence progresses one nucleotide at a time by incubating sample with

(-) High reagent costs and ↑ errors rates for homopolymers

b) Sequencing by Synthesis

Incorporates a single nucleotide at a time, fluorescence is recorded & dye + terminator cap is cleared and washed away so that further segment can be sequenced

This overcomes homopolymer issue but ↑ errors with increased read length ∴ noise

c) Sequencing by ligation (SOLiD)

Doesn't use DNA polymerase.

Uses 16 octamer oligonucleotide probes (Universal base - can bind to any base)

Probes have 2 specific bases and 6 degenerate bases and a dye that's cleaved off one (DNA) is attached. There probes are attached by ligase.

The dye + 3 bases are cleaved off and next probe is attached - done with 7 probes

Then the primer is offset by 1 bp and the process is repeated for 5 rounds.

(-) Very short read lengths

d) Ion semiconductor sequencing (5)

Semiconductor transistor can record the ph of the cluster when addition of nucleotide causes release of H^+ ion.

7. SOLiD

- Fragmentation – Nebulization (N_2), Sonication, Digestion
- ligation of Adapter Sequences
- PCR – amplification by hybridization to bead.
  Each bead – unique fragments – polymerase colonies (polonies)
  Gather all good beads & centrifuge them out
  Attach to glass slide
- Sequencing Reagents – Template strand, primers, octamers dibase probes, ligase.
  1. Primer binds to template strand
  2. Probe hybridisation & ligation
  3. Fluorescence measured
  4. Dye-end + 3 nucleotides cleaved, next 6 probes hybridized
  5. Repeat the process 5 times by offsetting the primers by 1 base.
- Data-analysis
  A base and a color define the next base in the sequence
- Issue: Palindromic sequences ⇒ ss DNA twists and hybridizes itself forming local hairpin bends and ~~not~~ making that seq inaccessible

22/3

Lecture 27

Oxford Nanopore technology – measures changes in ion flow through nanopore as ss DNA is paired through a channel in a membrane

Technology Summary

SOLiD sequencing

Elements of Hybridisation – 3 H-bonds are most stable.

SNP - Single nucleotide polymorphism - occur every 1 kbp
eg. Eye color in humans
23 x 10^6

Array Experiment
1) cDNA of each mRNA is sequenced and
stretches are attached to a chip.
There is an array - which might have
This is more than 1 copy with a stretch of each gene probe
Then cDNA from a particular set of cells
is isolated and spread over the array.
The DNA will try to hybridise with its
complement when we can find which mRNA
Through this is being expressed in lives cells and
based on intensity. how many copies of the gene

2) Expt 2 : Normal cell - green Cancer cell - red
Then both cDNA sets are spread over
the array. If green - that mRNA is expressed
in normal cell. If red, cancer cell and if
yellow, then its expressed equally in both

Microarray technology
Go through last 10-15 mins of lectures
Photolitography. Injekt. Robot spotting
Image analyses and data visualisation of microarray

Process of making array - Building the chip + RNA preparation
+ then expt + analysis

Used to be cheaper to carry out array expts -
gives an idea of transcriptional library
of the cell Now everything is sequenced

Utility - Gene expression. Alternative splicing. micro RNA
expression. SNP genotyping

Exon arrays - alternative splice variant detection

Lecture 28 26/4

Crispr Cas 9 Technology

When bacterial genome was sequenced, a specific kind of repeated sequences which were conserved — 90% of Archaea, 40% of bacterial genome

It seemed possible that these were transfered by HGT

Review : Doveau et al. 2010

Repeats and spaces sequence : 60 bp - 15 kbp

The spacer sequence was found at 3' end of Cas genes — nucleases, helicases, DNA / RNA binding, polymerases

Cas genes + repeat-spacer sequence : CRISPR sequence

2010 It turns out that entire Crispr sequence was translated to one long mRNA which were then cut into smaller RNA peices.

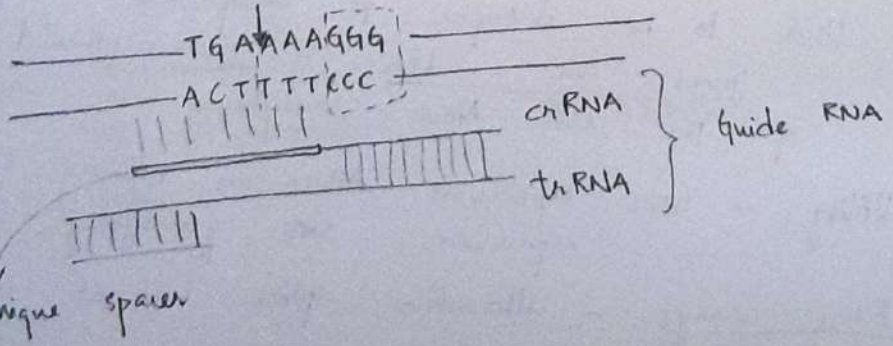
The palindromic repeat would fold the strand while the unique spacer sequence hybridised with viral genome so it can be neutralised (reminiscent of RNAi method)

So this is actually a host defense mechanism.

2012 — Cas 9 protein was identified

2013 — Crispr - Cas technology

PAM MOTIF



→ guides Cas9 mediated cleavage

Guide RNA (gRNA) has Crispr (crRNA) and trans-activating crRNA (tracrRNA). crRNA region hybridises to the sequence of interest.

GGN – Protospacer Adjacent motif – the enzyme will cut 3 bp after this sequence

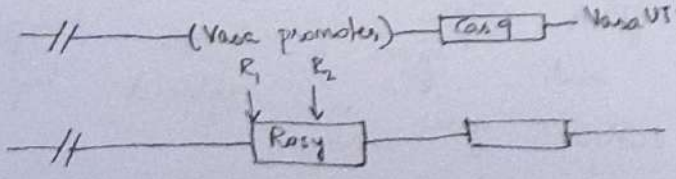
The cut is made by Cas9 protein.

Genome engineering (2013 – 1st demonstration)

- One there's a nick, the DNA repair machinery of cell tries to reseal/repair it. Once in a while, an extra bp is added or removed ⇒ frameshift mutation in a functional gene
- Or, the machinery tries to repair by comparing with analogous chromosome. So, we insert multiple copies → stretches of dsDNA with analogs of existing gene and desired sequence in between. So, the cell repairs by inserting that sequence where the cut was made formed template DNA.

This was shown in vivo & very soon incorporated into other model organisms, especially drosophila

Crispr-Cas9 in flies.



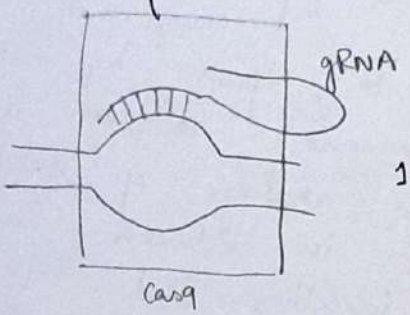
They put Cas9 downstream of vasa promoter so that its only expressed in germline cells.

He created gRNA that would bind to sequence in Rosy gene - he found that cuts do occur, and visible mutations occured in 16 fly lines.

Crisps Cas9 can be used to generate mutations in the gene of your choice by engineering gRNA and expressing Cas9 in primordial germ cells. (crossing transgenic flies or injecting the embryo).

The efficiency depends on many factors including the locus, the design, machinery etc

A large gene will have a considerable no. of PAM site



Once the nick is made, two types of repair -

1. Non-homologous end joining (NHEJ)
   Reattaches broken ends
   Error prone
   Creates mutations

2. Homology Directed repair (HDR)
   Repairs broken DNA by comparing to sister chromatid or an inserted donor repair template, so there's a precise incorporation of required DNA sequence

Example: Gene replacement by HDR
Insert gRNA for 3' and 5' end of a gene and give a donor template s.t. the entire gene is replaced by another.

* ssODNA based replacement
To replace lysine at position 951 to arginine.
She inserted a template with required change and gRNA so the required mutation would occur through HDR.
3 out of 100 lines had K551R mutation in the Caspar gene.

* pHD - ScarlessDsRed
ScarlessDsRed - if successful, the animal will have red fluorescent eyes.
Targeted gene : Ira Mutated version of Ira (along with DsRed) was inserted as a vector in the fly and gRNA + Cas9 to make the necessary mutations.

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Lecture 29

Many applications of Crispr-Cas9 system
- To insert fluorescent protein
- Transcriptional regulator
- DNA editing enzyme

Gene Drive - Mutagenic chain reaction
Say Cas9 + gRNA is inserted into genome using Crispr HDR method.
These two genes are expressed ~~indeed~~ independently.
They then 'attak' the other homologous chromosome, make a nick & then repairs it by HDR so that both chromosomes now have Cas9 and gRNA. This is called gene drive.

Mendelian inheritence

♀ ♂

♀♀ × ♂♂ y^-

♀♀ ♀♀ ♂♂ ♂♂

\+ \+ \+ \+

y^+ (WT) : Brown

MCR inheritence

♀♀ × ♂♂

♀♀ ♀♀ ♂♂ ♂♂

\- \- \+ \+

y^- (mut) : yellow.

Creating mutants through MCR inheritence — crisps will give mutants (unintentionally). making homozygous

Normal inheritence — Altered gene : one copy inherited from parent, so 50% chance of passing it on

MCR inheritence — > 50% chance of passing it on because heterozygote gets converted to homozygote So mutations can be spread throughout the population rapidly

SNP - Single nucleotide polymorphisms

To study ancestry, liabilities, abilities and other predictions.

23 and Me - HapMap Project

Variability in human genome

lots of variability — insertions, deletions, mutations

lots of alleles contribute to diseases

There are many de novo mutations in each generation

1000 human genome — 14 population, 38 M SNP, 1.4 M small insertions & deletions, 14k large deletions and lots of analysis of human genome, distribution & change

De novo genome seq
Plastic / oil digesting bacteria's genome sequenced, genes identified and used

Metagenomics
Sequencing the total DNA of an ecological niche, and analysing that.
Eg: Rumen of cattle, nice to understand obesity

Haplogroups of humans - to understand populations and migration routes.
Genetic variation in Indian population and comparing to caste

Microbiome ecology is critical to health
It has 1M+ genes whereas humans have 23,000 genes. The health & nature of microbiome changes with age and disease.

Sequencing ancient genome - cave bear, mammoth, neanderthals
Through this we've understood the history and evolution of human societies.

Genetics and Society
Genetic Information Non-discrimination Act - prevents insurance and employers from discriminating based on genetic information.

GATTACA
Government has access to a huge database that can be used without much regulation

Lecture 30

Human Gene Therapy

Crispr is relatively new — hasn't been used in human genome editing even though its very powerful

Targeted medicines, GMO, new industrial products are some developments assisted by genome editing. Ethical regulations & laws are just falling into place.

Recent advances: Allelic drive in drosophila, neutralizing elements to halt gene drive, gene drive in mice

1990s - first successful use of gene therapy

2002 - Sickle cell treated in mice

X-SKIDS treated in children, but it triggers leukemia

2007 - Inheritable blindness treated in human

First successful case - Ashati DeSilva (1990)

Dr. Anderson treated Severe Combined Imune Deficiene caused by a mutation in ADA gene

Gene therapy using adenovirus vector - a good gene was tranfected into her RBCs and the blood was tranfused back.

It worked — probably her bone marrow was also fixed

But a few children died because of faulty vectors. Now private companies do it successfully with better vectors.

Other diseases targeting other organs (liver, lungs) have also been treated through this.

Recent experiments using Crispr to edit human genome to treat diseases. Ethical?

Lecture 31

Sex determination & dosage compensation

Sex is determined due to difference in chromosome numbers

Evolution of sex chromosome — how do males survive 1 copy of X? Sex chromosomes evolved along with dosage compensation

Assumption in Mendel's theory (heredity is equal from both parents) doesn't hold.

Dioecious organisms — seperation of sex

If gametes in both sexes are similar, then its called isogamous.

Eg: Reproduction in Chlamydomonas

It undergoes sexual reproduction (through gametogenesis) when nutrients are scarce

It forms mt+ and mt- gametes, which form sex pili, fuse and form diploid cell

This zygote undergoes meiosis and gives rise to free chlamy.

Other than genetic, sex determination can occur based on environmental cues — temp and photoperiod.

There is MF, FM, FMF and FMFM patterns
↳ male at lower T, F at higher

Some genes in the zygote, proteins in the yolk are T sensitive. They're hormonal, so they affect the gonads formed

~~They're~~ There's a breif period when the zygote is sensitive to T.

Sex chromosomes have
- reduced recombination
- specialized gene content (sex-specific)
  SRY gene in Y-chrom
- dosage compensation
- heteromorphic size

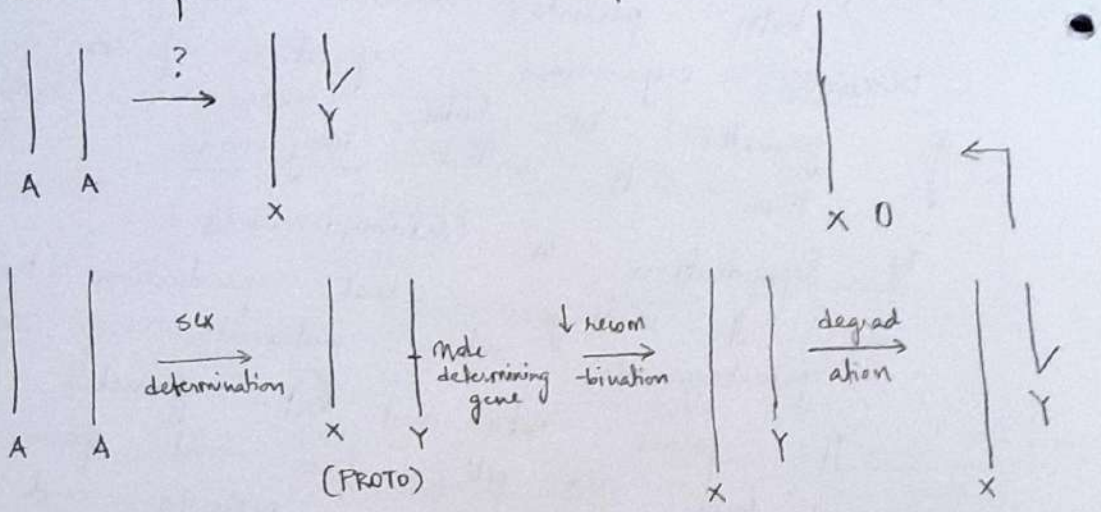
Sex determination system

XX - XY
Mammals

XX - XO
Insects

ZW - ZZ
Birds, fish

Evolution of sex chromosomes from autosome?



Reduced ~~sex~~ recombination ⇒ accumulation of genes (especially Y) because there's no corrective mechanism.

This led to degradation of Y chromosome, its genes transferred to autosomes. So Y chromosome gets shortened and totally disappears.

X chromosome has a homolog gets repaired because it when it goes through the female. This mechanism/pattern has been decoded through genetic mapping – sequencing and signals and so on.

Sex differentiation has independently occurred multiple times — so its clearly advantageous. It can arise from monogenic heterogametic sex determination. Ultimately, it results in different types of chromosomes, or environmental through lack of recombination, sexually antagonistic alleles, and degeneration + dosage compensation in Y chromosome.

Sex determination can also occur in plants.

11/5

Lecture 32

Evolution of sex chromosome —

1. Acquisition of sex-determining gene
2. Loss of recombination
3. Shrinkage of Y-chromosome
4. Coevolution of dosage compensation.
   Males can live with single copy of X.
   To make this possible, we need dosage compensation

Y-chromosome

X has 2000 - 3000 genes, whereas Y has ~~vv~~ less.
Both X and Y have a similar pseudoautosomal regions which allow for pairing b/w X & Y.

Models of the human Y

1. Dominant Y : presence of Y gives maleness
   so those genes must be dominant.
2. Selfish Y - Some selfish sequences in Y
3. Wimpy Y - relic of X & most of its genes are relics of X chromosome genes/ taken up genes from other autosome that have other function.

~~shrink~~ will disappear in ~5 Mya

Marshall Graves argues that SRY is younger Y chromosome, so its not the original sex determining gene. It arose from a gene on proto-sex chromosome pair with a function (probably brain-determination) in both sexes.

Y has been lost twice, independently, in rodent lineages.

Epigenetic inheritence for X

Dosage compensation
- Offsets differences in no. of sex chromosomes
- 1 sex chromosome is altered

Genomic Imprinting
- Occurs during gamete formation
- Involves single gene / chromosome
- It modifies the genetic material through histone regulation so that the chromosome is marked & can be recognised by the zygote
- Governs whether offspring express maternally or paternally derived gene.

Epigenetic inheritence
- Modification occurs to a nuclear gene or chromosome
- Occurs during gametogenesis & embryogenesis
- Gene expression is altered (not sequence) Maybe fixed during an individual's lifetime
- Expression is not permanently changed over multiple generations

Dosage compensation

There are different no. of sex chromosome in some species. To deal with this, organisms make use of Barr bodies.

Barr bodies

Condensed structure in interphase nuclei of somatic cells of female cats, absent in males.

Described by Barr & Bertram (1949)

Later identified as condensed X-chromosome, attached to nuclear lamina

Mary Lyon used this knowledge to explain the variegated coat color of female calico cats

Hypothesis: Calico cats are heterozygous for X-linked alleles determining coat color. Their somatic cells inactivates one of them randomly, thus giving rise to variegation

They have a white underside due to another dominant gene

X-chromosome inactivation

DNA becomes highly compacted (Barr body), so that most genes can't be expressed

This becomes interesting when genetic disorders are studied in females — some can inactivate WT, then disorder is expressed

Chromosonal aneuploidy

Failure of pair chromosomes to seperate during gamete formation (non-disjunction)

If it occurs in meiosis I, then all 4 gametes have aneuploidy. If it occurs in meiosis II, the 2 gametes have aneuploidy

Down's (#21, 3n)

Turner (XO)[0]

[1] Klinefelter (XXY)

[0] Jacob's male (XYY)

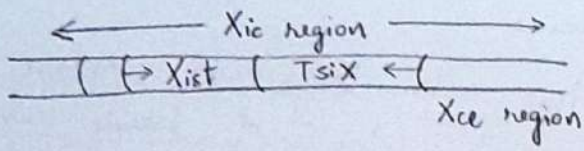
Metafemale (XXX) [2 barrs]

(80)

If a cell has >1 X-chromosome, then there's a method of recognition & inactivation

The mechanism of silencing is on other autosomes, triggered by the ratio of expression of certain genes.

Portion of X



Inactivation is controlled by non-coding RNAs of genes on X-chromosome

RNA of Xist block further transcription of any genes on that X-chromosome & results in condensation. There's another gene Tsix.

In the active X, TsiX RNA gets coded first/early and it blocks the actions of Xist.

So one chromosome remains active, while other is condensed

There are also lot of other changes in the inactive X —

- ↑ DNA methylation
- ↓ histone acetylation
- ↓ histone H3-K3 methylation
- ↑ histone H3K9 & H3-K27 methylation.
- macroH2A histone variant

During development One X-chromosome is chosen randomly in some cells in the embryo to be suppressed. The progeny cells somehow remember and suppress the same X-chromosome.

Blot of Xist RNA

Image : Xist coats the X chromosome

Individuals with >2 X-chromosomes are infertile and face mental health issues. This is because not all genes can be effectively suppressed.

During development (in eutherians & marsupials where mother rears the kids) the cells seem to differentiate between maternal and paternal X.

Marsupials suppress paternal X in almost all cells

Even in eutherians, paternal X is expressed only in some patches.

Paternal X inactivation (Theories, not well differentiated)

1. De novo inactivation
   there, X_p is suppressed in the trophoblast (surrounding layer) and randomly inactivated in the inner cell mass.

2. Pre-inactivation
   Zygote recognizes X_p and silences it. X_p is reactivated in epiblast & then randomly inactivated in developing tissue there too, its kept inactive in trophoblast.

More info ↳ at ~35:00 mins of recording of different experiments

Factors required for inactivation / reactivation are present at v. early development.

Jacob's Male (XYY) – Cartoon : Clinical karyotype
1 : 1000 males – quite frequently
Asymptomatic condition, no different physical features, no medical problems ; normal development & fertility.
Not inherited unless there's non-disjunction during spermatogenesis.

Metafemale (XXX)
Fertile, low IQ, not inherited unless there's a non-disjunction

Consequences of loss of dosage compensation
Placental mammals may have evolved random X-inactivation to alleviate burden of * X_m mutations. Eg: Rett's syndrome
XXX & XXY are individuals display developmental defects even though extra X is suppressed, some genes are not.
X has highest density of immunity genes. Autoimmune disorders in females are linked to activation of genes from inactivated X-chromosome

13/5

Lecture 33

Dosage compensation in Drosophila
Principle of X-chromosome to Autosome ratio
Different mechanisms of dosage compensation in different animals.
Mammals – In ♀, 1X is silenced
C elegans (hermaphrodites) – both X's down regulated
Drosophila males – hypertranscription of X-chromosome

Mouse X inactivation centre

Xist : inactivates X

Tsix : acts antagonistically, so activates X (one q'em)

Deletion of Xist repressor & other deletions makes the chromosome active

TH Morgan – sex linking in drosophila.

Recall modified phenotypic ratio of red/white eyes

Drosophila : XX / XO

	X X	XY	XXY	XO
Drosophila	♀	♂	♀	♂
Humans	♀	♂	♂	♀

Chromosome	3X/2A	3X/3A	2X/2A	3X/4A	2X/3A	X/2A	XY/2A
X : A	1.5	1.0	1.0	0.75	0.66	0.5	0.5
Sex	← Female →			← Intersex →		Male	Male

There are a few sex-determining genes on autosome which interact with X-chromosome.

SXL : sex lethal regulates sex determination & dosage compensation

SXL → sex determination
SXL → msl expression } Splicing regulation
SXL → X-linked genes } Translational repression

X:A = 1 Leads to femaleness

X:A = 0.5 No SXL → msl 1, msl 2, msl 3, mle, mof

msl 1, msl 2, msl 3, mle, mof → rox 1 RNAs, rox 2 RNAs → complex on X-chromosome

Both proteins & RNAs colocalize on X-chromosome to increase transcription

Evidence of dosage compensation : Apricot eye-color

- It occurs due to partial expression of X-linked gene
* Homozygous females resemble hemizygous males *
  2 copies of allele in female produces a phenotype similar to 1 copy in male
- Heterozygous females have paler eye color
∴ Difference in gene dosage is being compensated at level of gene expression

H J Muller - Apricot - w^a

Light → Dark

♀ 1 dose w^a/w

♀ 2 doses w^a/w^a

♀ 3 doses $w^a/w^a w^a$

♂ 1 dose $w^a/-$

♂ 2 doses $w^a w^a/-$

Dosage compensation of apricot mutant

Dosage compensation doesn't occur for all X-linked genes. not even all eye colors

Eg: Eosin eye color
Conferred by X-linked gene
Homozygous females have dark eosin shade whereas * hemizygous males have light eosin *
Heterozygous females - w^e/w - light eosin

So, most X-linked genes show dosage compensation whereas some donot and can cause developmental defects like infertility, congenital heart problems

Reasons for differential or incomplete ∞ inactivation are not understood

~~Random~~ Dosage compensation in birds

ZZ ♂ : ZW ♀

Z chromosome is large, contains most sex-linked genes

W - microchromosome - has large amt. of non-coding repetetive DNA

Dosage compensation occurs, but not for all genes

Molecular mechanism is not understood

- Highly compacted Z chromosome is not seen in ♂.
- Maybe both are downregulated in ♂ or one Z is upregulated in ♀

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Lecture 34

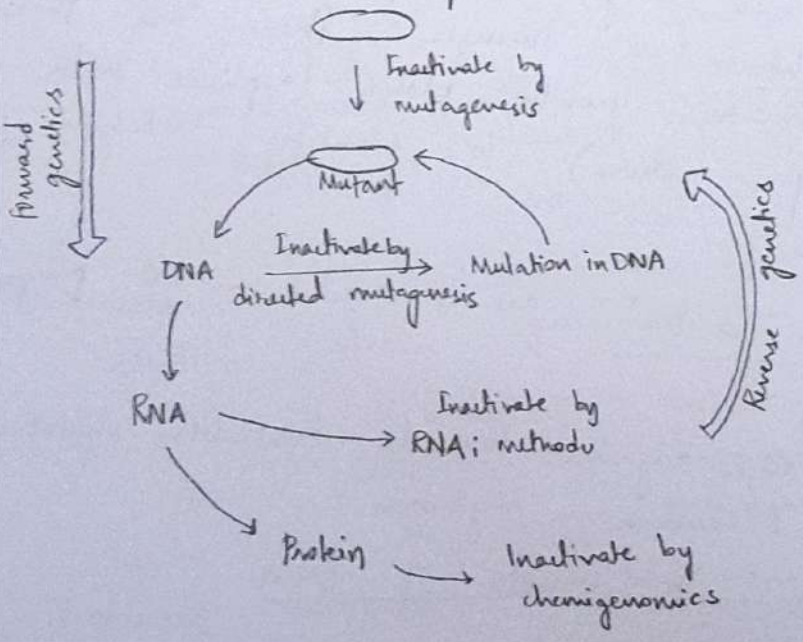
Dissection of Gene function

Ch 16 of Griffiths

Design of assay for screens in flies & non-flies

Dissection of genetic pathway in C. elegans

Forward vs Reverse genetics



(8)

Forward genetics

1. Standardize an assay system
2. Mutagenize plants/animals in which process of interest is disrupted
3. Complementation analysis
4. Map the mutants to chromosome & linkage markers
5. Deletion / duplication mapping.
6. Identify putative genes in chromosome region
7. Sequence the genes to uncover the mutants. Generate rescue transgenes to see which one gains functions back.
8. Study gene by tagging with fluorescent markers
9. Make protein and characterize it - structure, localisation & binding properties
10. Study expression pattern using promoters.

→ Determine whether clustered mutations are in same gene or nearby genes

Examples —

1. Bacterial cell with different growth requirements
   Prototrophic (WT) can grow to big colonies in minimal medium, so they get filtered out, while auxotrophs pass through. There are then transferred to growth plates (replicas with different kinds of medium) to identify metabolic pathway and so on.

   $ad^+ leu$ x $ad\ leu^+$ are crossed - 20 progeny

2. Blind flies
   WT - all migrate towards light (+ve phototaxis)
   Mutant - random migration
   Mutations in circuits of brain.

3. Different growth morphology of neurospora
Turns out, different colonies are enriched in different members of cytoskeleton – actin, dynein dynaction,

4. Eg Cell cycle mutants in yeast
Mutants only so that cell division defects occur at a certain temperature.
This screen gave rise to discovery of proteins necessary for proper cell division. Studying a basic organism means this is conserved in all eukaryotes.

5. Dissection of nuclear division in aspergillus.
Different mutations – no division, lenses division, no transportation.

6. Zebra fish
Great model system to study development, behavior
A mutant can be studied by crossing with UV treated sperm (genetic material is scrambled) so a haploid fish develops upto larval stage
Eg. development of red blood cells.
mutants – reduced haemoglobin + altered stripe

Enhancers and Suppressor are powerful tools to identify other genes in the same pathway.
An enhancer mutation + mutant will enhance the phenotype & suppressor mutation results in less severe phenotype.
Suppressor mutations can be intragenic or extragenic.

(11) * Intragenic suppression : true revertants
A reversion could restore the original protein sequence

* Partial revertants : Isoleucine is chemically similar to original leucine (WT) than phenylalanine (mut), and maybe less disruptive of the protein function.

* Compensatory mutations : intragenic mutation at a second place which offsets the damage created by first mutant through interaction in 3D structure
This also gives us insight into protein folding

In pathways involving negative regulation, loss of function mutation in downstream genes can suppress mutation in upstream genes.

Consider A → B

a ⊣ B

a ⊣ b

Mutation in A ⇒ B is active

Mutation in B reduces / eliminates its function ⇒ suppresses mut A.

Eg: ced 9 ⊣ ced 4 → ced 3 → Apoptosis

LOF mut. in ced 9 (lethal) can be suppressed by mutations in ced 4 or ced 3.

In positive regulation (signalling) pathways, usually only GOF mutation in downstream gene can suppress mutation in upstream genes.

A → B

B is active in presence of A

a(lf) ⊣ B

B is inactive

a(lf) ⊣ b(gf) → B is active without A

b(gf) suppresses a(lf)

There are extragenic mutations.

Ordering a genetic pathway.

ced9 → ced4 → ced3 → Apoptosis

We can order this using artificially GOF mutation in ced3 or ced4 by overexpressing the genes in specific cells.

Eg. MEC-7 is a specialised β-tubulin expressed in subset of mechanosensory neurons (eg. ALM)

It has a strong promoter

So we invert ced3 and ced4 (in separate lines) downstream of mec7 promoter.

High levels of either ced3 or ced4 cause ALM neurons to die

But which activates which?

Case I: ~~ced3 is overexpressed~~, LOF ced4 mutation

~~ced4~~ → ced3 survival

Case II: overexpressed ced3

ced4 → ced3 Apoptosis

Case III: overexpressed ced3 + mutant ced4

~~ced4~~ → ced3 Apoptosis

Case I: ced3 mutant — cd4 → ~~ced3~~ survival

Case II: Overexpressed ced4 — ced4 → ced3 Apoptosis

Case III: Case II + I — ced4 → ~~ced3~~ survival

Thus we can conclude that ced4 is upstream of ced3.

Lecture 35 18/5

Genetic tricks to dissect gene function.

Transgenic insects – mosquito, silkworm & beetle

Using P-element : We remove the transposase gene in the p-element and replace it with required gene, say GFP.

Donor plasmid : Eye enhancer - promoter - GFP

A mixture of donor & helper plasmid (transposase) is injected into the embryo. If they're incorporated into germline cells, then the progeny will have green eyes.

They're injected into posterior of early embryo (syncitial) using a microinjection

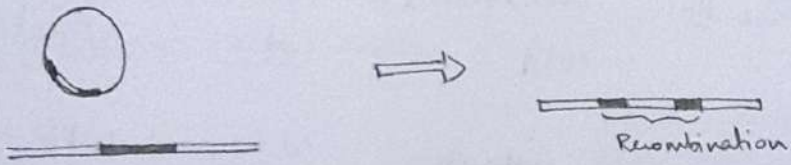
We can use this to see if this transgene rescues a particular mutant.

Homologous recombination to mutate a gene

This is used in bigger organisms like mice

The plasmid has flanking regions around mutant gene which is homologous to gene in chromosome.

It doesn't work as efficiently in all organisms.



Inserting GFP construct of gene into animals

GFP – a protein from jelly fish. Aequorea victoria

A similar protein is mRFP. These fluorescent proteins have been modified for use.

Applications –

- Localization in cell biology
- Tracing of molecules & cells

- pH of endosomes, membrane potential in neurons
- protein-protein interaction Eg. FRET
- highlighting tissues, following lineages
- visualising promoter activity

Received Nobel prize in 2008.

pEGFP-N1 vectors from Clontech

4.7 kb

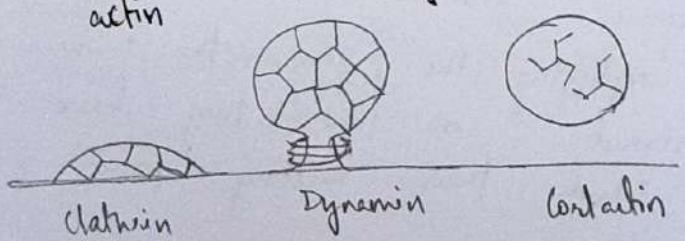
The vectors has a multiple cloning site (MCS) where a protein of interest can be inserted. Then GFP is protein coding region is present. So the GFP protein is present at the C-terminus of the protein of interest.

There are restriction sites after GFP also, so that protein + GFP can be taken out and inserted elsewhere, for example, in the Drosophila transgenic vectors for somatic tissues - pUAST ~9kbp

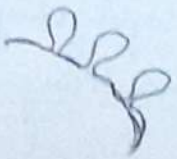
These vectors have selection markers, like some antibiotic resistance (for bacteria) or white [+] gene for Drosophila.

Clathrin-mediated endocytosis

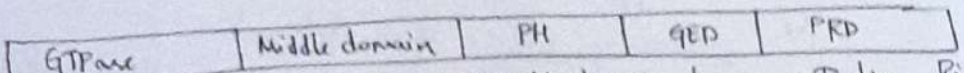
Dynamin is a ubiquitously expressed protein which helps in endocytosis. Clathrin causes budding. dynamin pinches it off into a vesicle and cortactin helps move vesicle away from membrane using actin machinery.



It was discovered when trying to isolate temp-sensitive paralytic flies i.e. it can't walk at higher T.
This shibire mutation caused the formation of electron dense endocytic pits in the neurons (in synapse).
This was due to mutation of dynamin - a cleaving protein that helps in endocytosis in metazoan cells.
It highly conserved



Dynamin domains



GTPase + GED: GTP binding & hydrolysis
PH: Membrane binding
GTP Effector Domain
Proline Rich Domain

- GED folds back of GTPase and they use GTP to actually cause fission
- PRD: binds to many other proteins such as actin polymerisation (cortactin) & membrane bending (endophilin)

Making a Dynamin - GFP
When making a construct, the stop codon of dynamin. We can use restriction enzymes that DO NOT CUT dynamin in the middle.
We can obtain cDNA of gene of interest, and compare and make sure
While amplifying the gene, the primers are designed so that they have unique restriction site and their melting point > 48°C.

Overhangs

Forward primer - ag - EcoR1 site - 20bp of dynamin

Melting pt : 60°C

Reverse primer : Its nuc needs to match that of forward primer. Synthesis happens from 5' to 3', So reverse complement is taken i.e.

Reverse seq : ... caag

Complement : ... gttc

Reverse complement : gcttg ...

Reverse primer has - homologous region of gene, delete stop codon, add unique restriction site, add overhangs for enzyme to cut, adjust frame by eg adding 3 bases b/w primer and restriction site

Reverse primer : ag - Kpn 1 site - frame rate adjustment base - homologous bases.

After amplification, we get a DNA that's like

EcoR1 - Dynamin - Kpn 1

Then we take fluorescent construct, digest with same enzymes, for instance the GFP forward primer can have EcoR1 whereas reverse primer can have Xba1 restriction site

We then ligate the two, transform into organism, and screen for required individuals.

Before transformation, the two genes should be transferred to a vector.

PCR can be used to generate mutants in a plasmid.

Oligonucleotide directed mutagenesis — the modified oligonucleotide (base pair change, insertion or deletion) binds to ssDNA plasmid. When its polymerised and later replicated in cell, then the plasmids would have mutated in required spare.

Eg: Dynamin-ts2 GFP
Glycine to Serine mutation at 141st amino acid
i.e. 421st base GGC → AGC.

To enrich the mutant strand, DpnI can be used which digests the template / parental methylated and hemi-methylated DNA
Then these plasmids are transformed into competent cells.

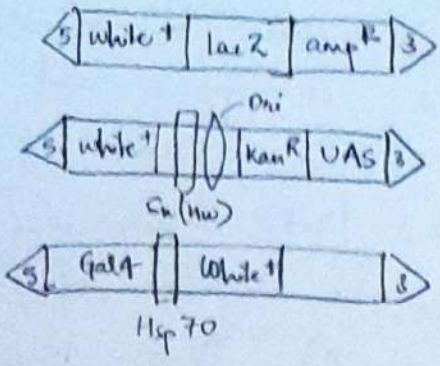
Video 36 - Reverse Genetics

E. coli analysis of protein secretion

* Translational fusion: The gene of protein of interest and LacZ are fused so that the two proteins are translated together so they're synthesized fused. So this tells us protein activity.
(Xgal $\xrightarrow{LacZ}$ Blue color)

* Transcriptional fusion: Essentially terminator of pol is removed so that both proteins are transcribed onto same mRNA but they're still synthesized as separate proteins
This is an indicator of promoter activity.
This can be inserted downstream of a tissue specific protein.

P-element mutagenesis



The p-elements have markers
- white+ is WT allele which can be used in white mutants.

These p-elements have lots of variation in terms of proteins they synthesize. Their expression will vary based on their upstream promoter.

Localisation of proteins: Protein trap screen.

In higher organisms, mRNA are spliced to remove introns and keep exons. A p-element with GFP flanked by splice-acceptor (SA) and splice-donor (SD) sequences. Such a p-element can incorporate into an mRNA such that there's a GFP domain in the middle of our POI.

SA | GFP | SD

This gives an idea of promoter activity and localisation of protein - when protein is synthesised, so is GFP i.e. its "trapped".

Enhancer trap screen - the transgene is inserted near the enhancer of a particular gene (say which is expressed in wing/thorax), so organs can be tracked through development.

Heat-shock promoter: it triggers transcription when the animal is given a heat shock.

Due to these two, we can express any gene, anywhere at any time!

Generating protein trap lines

A male with GFP transposon is crossed with female containing transposase. This hybrid animal has GFP over its genome ∴ transposons hop. So larvae (glowing green) can be visually screened and used to figure out which proteins have been trapped.

Mapping p-element to 2nd / 3rd chromosome by crossing (different mutations i.e markers on different 2nd & 3rd chrom of ♀ & ♂). Then targeted sequencing will allow us to identify the protein trapped

with balancer strain

Reverse Genetics

Find a gene which has interesting domains but no known function

Knock it down

Look for phenotype in the process of interest (eg. cell division, behavior etc).

RNAi – RNA interference

It can be genetically expressed in a specific promoter, downstream of UAS element.

This inhibits the expression of a particular gene.

So new proteins can't be made

⇒ It doesn't affect long-lived proteins.

dsRNA can be injected into a cell or a transgene w/ reverse repeat is introduced into genome or a transgene containing promoter on either side can be introduced into the genome

Dicer (protein) chops dsRNA into 21-23 long & one stranded guide RNA bound to RNA Induced Silencing Complex (RISC) attaches to mRNA of complementary sequence and destroys it.

This technique is NOT knocking out the gene.

This dsRNA can be expressed using the UAS-Gal4 system to control the time & location of expression.

Chemicogenomics

* Drug screening which inhibits process of interest

A library of compounds can be added to wells where yeast colonies are growing.

Then find compound that produces phenotype of interest; then identify protein target of the interfering compond

* Reverse chemical genetics: Start with protein of choice in process of interest and look for drugs which will specifically bind to that.

Lecture 37 - tutorial

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Lecture 38

Genetic Screens

Eg. 1: Genetic control on eukaryotic cell division

Paul Nurse, Leeland Hartwell & Tim Hunt

Nobel 2002 - Cyclin & cydin-dependent kinases.

Though this they understood genes and proteins involved in cell cycle division, which are widely conserved & v important.

knowledge in feild was going along with developing methods

Develop mutants — for cell size & nuclear material copy
WT was treated with nitrosoguanidine to 30% survival
i.e. GC → AT transitions

100 survivor plates. After 4 days, colonies are replica plated at 25° (grow comfortably) and 35° (poor growth
⇒ dead cells can be estimated using Phloxine B)
Enters & labels cytoplasm of dead cell

Observations — cell number
DNA and RNA — diphenylamine reaction
Protein — lowry et al method
Nuclear staining — Giemsa
Cell plate staining — primulin

Nuclear division, early/late cell plate mutants — these could be visualised & confirmed

Mating to determine complementation groups — haplodiploid life cycle.
Different plots for different mutants

The cell size and division are interlinked — wee mutants — smaller cells.
Wee mutant lets Cdc2 active which makes cell divide early. In Cdc2 mutant, when it's inactive, there's mitotic delay & hence cell growth

Universal control of M phase by Cdc2 was confirmed in 1990
↑ Details, Details — everything took ~20 yrs to figure out

Eg.2 — Screening for genetic control of behaviours
Neuronal control of Drosophila walking direction
Backward walking — a trait used when backing away from a threat.
To screen this, we should be able to switch on and off neurons which control this.

Promoter → UAS-TrpA1 trans-gene — open Ca^{2+} [Activated at 30°C] so a neuron is forcefully fired

Crossed to

3470 Gal4 lines expressing in different neurons in the brain

Only backward walking in - VT50660 Gal4
This Gal4 could be used to label the neurons or inhibit them (UAS - Tetanus toxin) or refine the behaviour, so it could be quantitatively assayed

The distance walked in different mutants & control was measured

VT50660 Gal4 UAS-INT : these flies can't walk backwards.

Identifying the neurons : VT50660 Gal4 x UAS-mCD8-GFP
7 neurons were highlighted - Moonwalkers

Activating individual neurons - you can figure out the influence of each of them.

Implicated : MDN + MAN-1 and MDN + MAN-2 neurons majorly control backward walking. Also MDN1