Cell cycle checkpoints:

The sequential events of the cell cycle are directed by distinct cell-cycle control system. The signals are transmitted within the cell by signal transduction pathways. Cells have their built in stop signals that halt the cell-cycle at check-point untill overridden by go ahead signals. A cell cycle check-point is a critical control point where stop and go ahead signals regulate the cycle. There exist a series of check-points, which refers to the points of monitoring of cell-cycle events such as DNA replication, DNA damage repair, spindle assembly, congression of chromosomes and separation of chromatids / chromosomes to opposite pole. generating signals in case of errors in process and halting the cell-cycle at specified points. Thus in cell-cycle there are three main types of check-points

i) DNA-damage checkpoints (ii) DNA-replication check-point (iii) Spindle check-points. A large number of cell cycle check-points mutants have been isolated for the checkpoints.

In the cell-cycle, 2 check-points have been identified at three stages.

(a) G_1 - check-point: Transition from G_1 to S Phase (start)

(b) G_2-chak-point: Transition from G_2 to M-phase

(c) M-phase-check-point: Within mitosis/meiosis itself

Recent studies indicates the presence of four check-points G_1 - check-point (DNA-damage check-point) of is also called the restriction point and is most important in cell-cycle start phase.

If the cells receives a go-ahead signals at G_1-check point it usually completes the cell cycle and divides. If it does not receive the go-ahead signals, the cell exits from the cell-cycle and switches to a non-dividing state, the G_0 phase. This check-point monitors damaged DNA which detects DNA damage and does not allow entry of the cell into S-phase by inhibition of S-CDK complex. Damage DNA stimulates transcription of many genes which encode the proteins that binds to S-cdk inhibits their activity and thus blocks the entry into mitosis. In mammals, a protein of p53 gene causes delay in entry of cells with damaged DNA into S-phase and mutation in p53 gene, therefore causes cancer due to increase in frequency of cancer promoting genetic alterations

G_2-check-point (DNA-replication/DNA damage check-point). It is the controlling point involved in driving the cell to M-phase. This is triggered by MPF [maturating promoting factor] which is a cyclin-cdk complex [cdk-cyclin dependent kinase]. It promotes mitosis by phosphorylating a variety of other proteins kinases. This check-point monitors unreplicated and damaged DNA which delays mitosis until DNA damage is repaired. Mitotic entry of G_2 cells is delayed in yeast by the rad-9 gene.

The damaged DNA sends a signal to a series of protein kinases that blocks the de-phosphorylation and activation of M-cdk blocking entry into mitosis

MPF and its role in regulation of cell-cycle.

A proteinaceous factor termed as maturation promoting factor (MPF), subsequently re-named as mitosis promoting factor is identified and purified. MPF is a heterodimer complex between kinase a catalytic subunit and cyclin a regulatory subunit. Kinase enzymes brings about phosphorylation of specific target proteins at different stages of cell-cycle which is necessary for its progression. Cyclin regulates the kinase activity by binding to it. In the absence of cyclin the kinases are inactive and are therefore called cyclin dependent kinase. cyclin confers basal kinase activity to the cdk due to conformational changes. A number of cdk's are known - cdk-1 and cdk-7. Different members of cyclin family appear at different level points of the cell-cycle — G_1-cyclins, S phase-cyclins and mitotic cyclins (M). cdk is attached with specific-cyclin (G_1-cdk, S-cdk and M-cdk) at specific phase of cell-cycle for its progression.

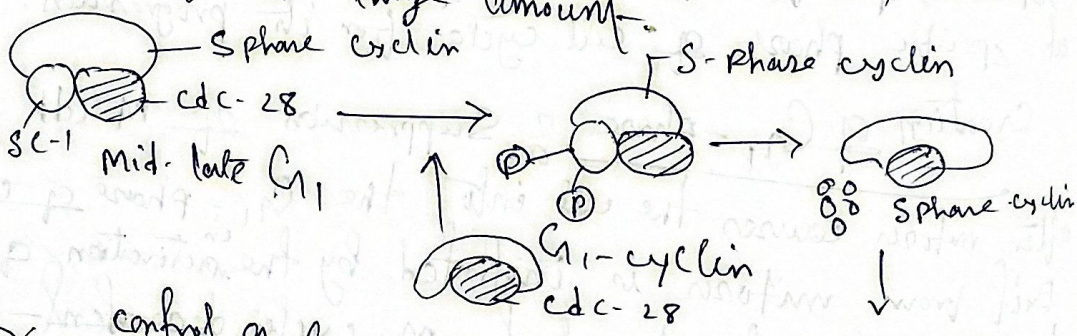
Creation of G_1-Phase :- Suppression of M-cdk activity after mitosis causes the cell into the G_1-phase of cell-growth. Exit from mitosis is initiated by the activation of M-cdk through ubiquitin dependent M-cyclin dependent degradation. proteolysis of cyclin through ubiquitination is triggered by APC which is activated by cdc-20 and Hct-1 protein. Accumulation of M-cyclin for reactivation of cdk to initiate mitosis requires a specific time and is thus delayed to cause the entry of cell into G_1-phase for cell growth. Exit from mitosis is initiated by the inactivation of M-cdk, cyclin degradation. proteolysis of cyclin through ubiquitin dependent M-cyclin dependent

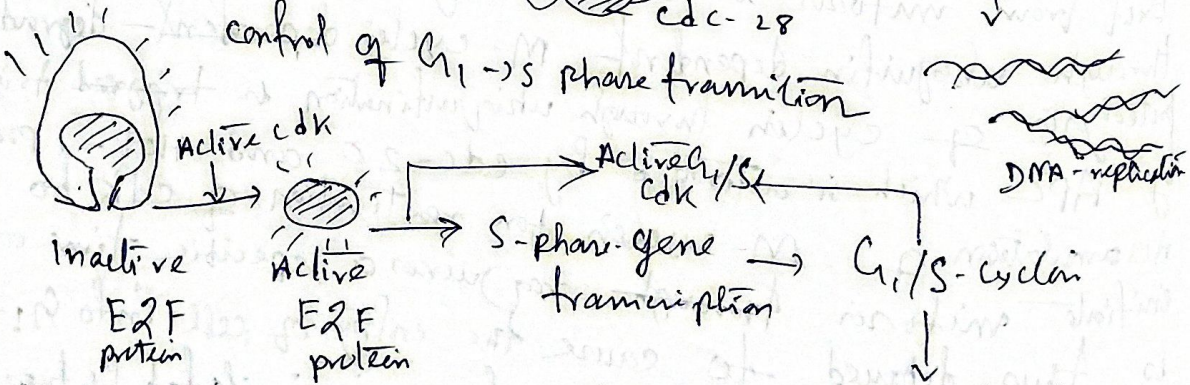
Suppression of cdk activity also occurs through increased production of cdk-inhibitor protein

Initiation of S-phase: Escape from stable G_1 occurs through the accumulation of G_1-cyclin leading to the G_1-cdk activity. G_1-cdk triggers the transcription of S-cyclin genes mediated through E2F regulatory protein and synthesis S-cyclin. Thus S-cdk-activity resumes to cause the cell to enter S-phase. S-cdk initiated DNA replication after complexed with cdc6 and Mcm proteins. S cdk-triggers origin firing assembly of DNA-polymerase and other replication proteins and activates the DNA helicase to initiate DNA-replication. Dissociation of cdc-6 from ORC and export of mcm from nucleus terminates replication.

Passage through G_1-2 phase:

After completion of DNA replication in S-phase accumulation of M-cyclins promotes gradual accumulation of M-cdk complex. But M-cyclin complex remain inactive due to kinase activity wee-1 on tyrosine residue where phospho-rylation is inhibitory. At the end of G_2, inactive M-cdk is present in large amount.



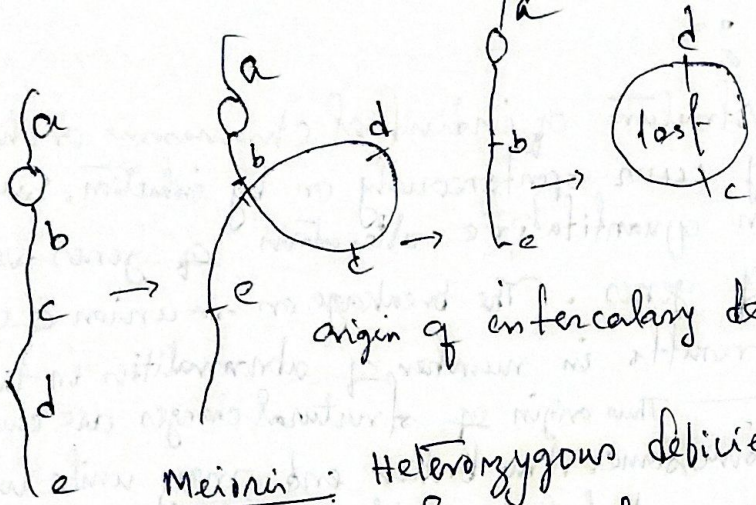


Chromosomal Aberration :

Alternation in the structure of individual chromosome or chromosomal aberration may occur spontaneously or by induction. Such changes may result in quantitative alteration of genes were re-arrangement of genes. The breakage or re-union of chromatid segments results in number of abnormalities in the chromosome structure. Thus origin of structural changes are caused by breaks in the chromosome. Any broken end may unite with any other broken end, thus potentially resulting in new linkage arrangements. Depending upon the number of breaks, their locations and the pattern in which broken changes are possible. The first cytological re-arrangement in plants were made in maize by B. McClintock.

Types of different kinds of structural changes of chromosomes have been demonstrated in Fig. - ① Deficiency - parts of chromosome lost or deleted. ② Duplication - parts of chromosome duplicated or added ③ Inversion - section of chromosome detached and reunited ④ Translocation - parts of chromosome detached and joined to non-homologous.

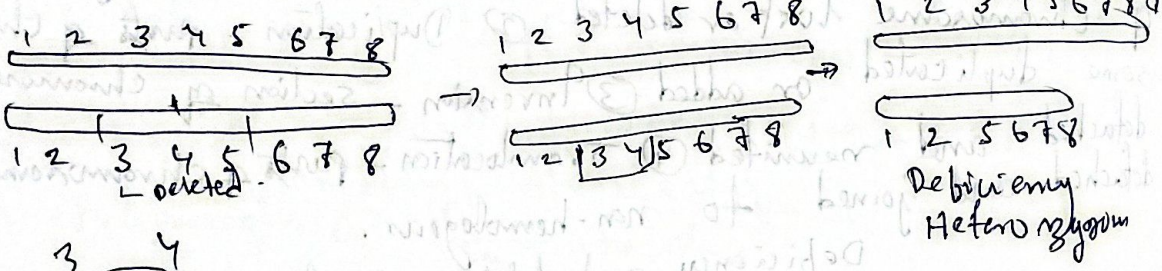
Deficiency and deletion : When a chromosome terminal segment of one end due to single break, the phenomenon is known as deficiency and the chromosome is regarded as deficit chromosome. Loss of an intercalary segment due to two breaks and single rejoining is known as deletion. In the original chromosome having gene-order a, b, c, d, R, t.. a loop may form due to folding. This deficiency was evidence presented by Bridges 1971 in Drosophila melanogaster, showed deletion of x-chromosome that included the bar-locus. Deficiency or deletion are of two types - ⓐ Terminal deletion — A single break near the end of chromosome would be expected to result of terminal deficiency. ⓑ Intercalary deletion - if two breaks a section may deleted and an intercalary deficiency is created.

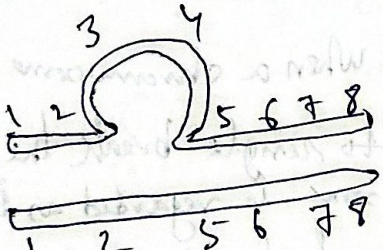


origin of intercalary deficiency

Meiosis: Heterozygous deficiencies during meiosis form a loop in a bivalent and it can be observed in pachytene

Effect:- Deficiencies have an effect on inheritence also, in presence of deficiency a recessive allel will behave like dominant allel and this phenomenon is called pseudodominance exhibited by deficiency heterozygotes has been utilized for location of genes on specific chromosome in Drosophila. Thus chromosome deficiencies have greatly facilitated the checking of linkage map.





pachytene configuration

Chromosome pairing in deficiency heterozygote. A somatic cell that has lost a small chromosome segment may live and produce other segment cells heterozygous like itself, each with deleted section of chromosome. Phenotypic effects sometimes indicate which cells or portions of the body have descended from the originally deficient cell. If the gamete deficient cell is a gamete that is subsequently fertilized by a gamete carrying a non deficient homologue, all cells of the resulting

organism will carry the deficiency in heterozygous condition. Recessive genes on the non-deficient chromosome in the region of deficiency may express themselves. Heterozygous deficiencies thus usually decrease the general viability.

Duplication :- Duplication represents additions of chromosomes parts. A chromosome segment is present in more than two copies.

origin :- Duplication originates out of unequal crossing over.

Meiosis :- If duplication is present only on one of the two homologous chromosomes, at meiosis (i.e pachytene) a characteristic loop is obtained.

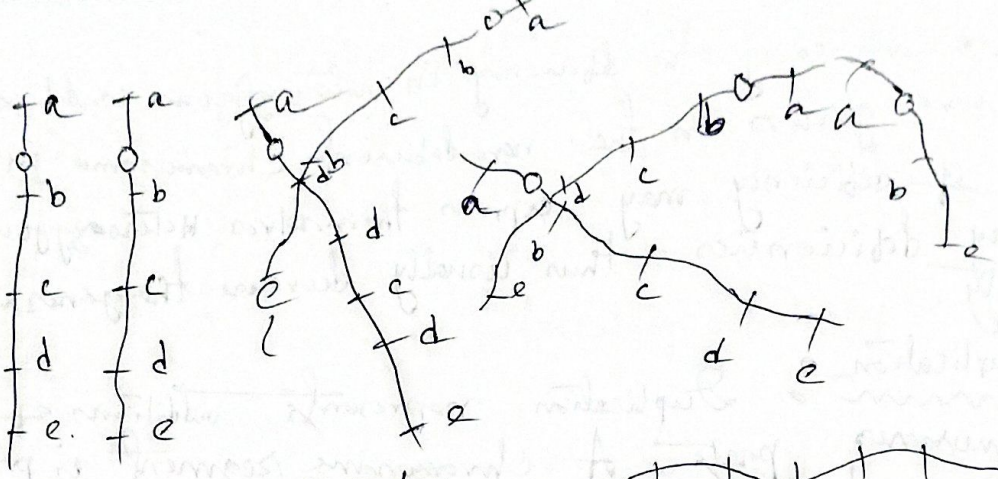
Effect : The duplication was critically examined in the 'B' (bar) locus of the 'X' chromosome of Drosophila. 'Barred eye is a character where eyes were narrower as compared to normal eye shape. This phenotypic character is due to duplication of a part of chromosome. The Bar character is due to duplication in region 16A of 'X' - chromosome. Barred eye individuals (16A 16A) gives rise to ultrabar (16A 16A 16A) and normal wild type due to unequal crossing over. Barred eyes have different phenotypes in homozygous bar and heterozygous ultra-bar individuals although in each case number of 16A segments remain the same. This was called position effect.

Types :- Duplication are of different types on the basis of position of duplicated segment.

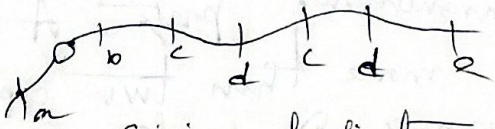
(a) Tandem duplication - Adjacent region.

(b) Displaced homobrachial duplication — at displaced position of the same arm.

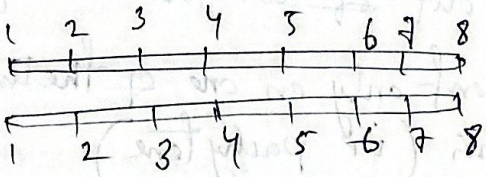
(c) Displaced heterobrachial duplication — on the different arm of the same chromosome.



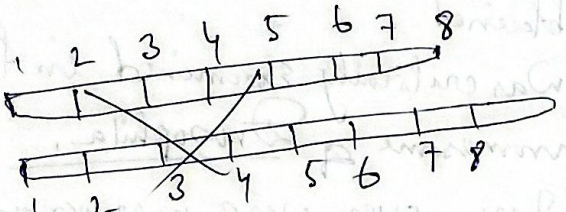
The origin of duplication

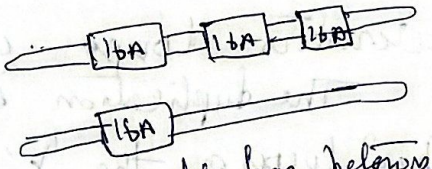


origin of duplication

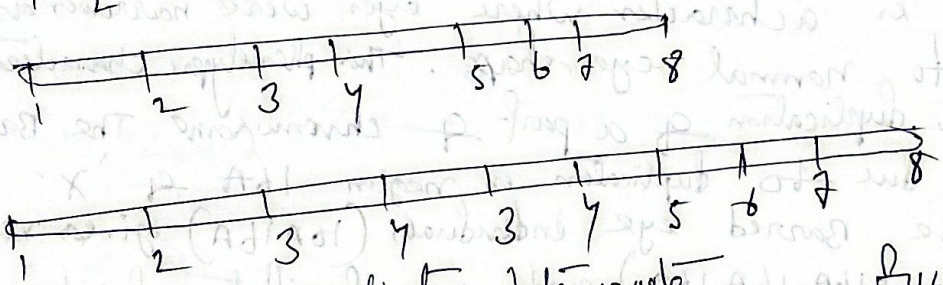


A pair of homologous chromosomes

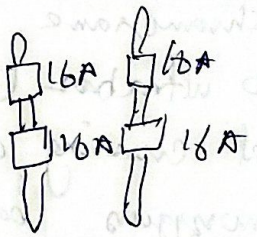




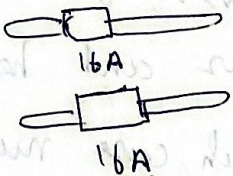
Double bar heterozygous



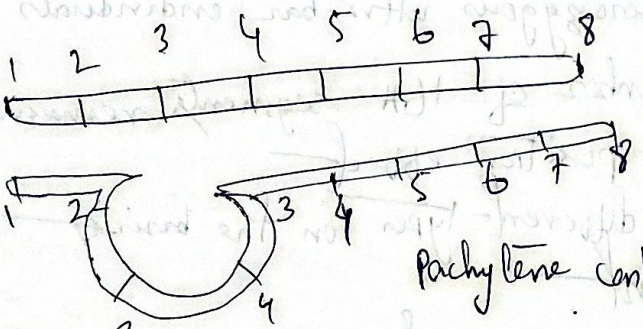
Duplication heterozygote



Bar homozygous



Normal Bar



Pachytene configuration in duplication.

(d) Transposed duplication ——— on a different chromosome.

(e) Reverse tandem duplication — duplicated segment found a reverse repeat at adjacent region.