GENETICS

Lab 3

(2016 - 2017)

Please read and make sure you understand the following instructions and knowledge before you go on.

Revised the Lectures:

- The Cell cycle
- Mitosis phases

Revised the Lab 2

Chromosomal Aberrations in the mitotic cell cycle

Objective:

Upon completion of this lab, the students should be able to:

1. Understanding why we use Onion as test organism.
2. Identify the various aberrations in stages of mitosis from slides and sheets.
3. Evaluate chromosome aberrations and disturbances in the mitotic cycle.
4. Sketch each aberration.
5. Summarize the cause of each stage.
6. Assess a great number of genotoxic/antigenotoxic agents, which contributes to its increasing application in environmental monitoring.

Introduction:

There are number of toxic chemicals in the environment, they are mostly discharged by industries into water, air and soil. The chemicals enter in our environment through both natural and anthropogenic ways. Once they enter in our biological process, it's really difficult to eliminate them from the environment and disturb various biochemical processes, leading to fatal results.

Numerous potentially mutagenic chemicals have been studied because they can cause mutagenic, damaging and inheritable changes in the genetic material. Many thousands of toxic chemicals including pharmaceuticals products, domestic and industrial

wastes, pesticides and petroleum products are present in the environment and new chemicals are being introduced every year.

Environmental biologists are presently concerned to safeguard the human beings from exposure to chemicals. **Genotoxicity** is to determine the magnitude of genetic risk to man by an environmental agents/ chemicals under a specified level of exposure. Unfortunately, the direct assessment in human is not feasible because of ethnic, logistic and practical considerations.

There are many employing wide variety of organisms ranging from viruses, bacteria, plants and insects to human cell cultures (recently stem cells) and intact mammals (rats, rabbits,….) to evaluate the mutagenicity (point mutation to chromosomal aberrations) of environmental chemicals. In order to identify the harmful effects of substances in different concentrations and time of exposure, a variety of tests have been employed, such as cytogenetic tests. These tests are commonly used for biomonitoring the extent of pollution and to evaluate the effects of toxic and mutagenic substances in the natural environment.

Higher plants constitute an important material for genetic tests to monitor environmental pollutants. However this feature is due to the possibility of assessing several genetic endpoints range from point mutation to chromosomal aberrations in cells. Among the higher plants species, the most frequent ones used to evaluate environmental contamination are *Allium cepa*, *Vicia faba*, *Zea mays*, *Tradescantia*, *Nicotiana tabacum*, *Crepis capillaris* and *Hordeum vulgare*. But, still among these species, *Allium cepa* (Onion) has been considered an **efficient test organism** to indicate the presence of mutagenic chemicals due to its kinetic characteristic of proliferation and the presence of small number of chromosome (2n=16) suitable for this type of study. *A. cepa* root chromosomal aberration assay was described as an efficient test system routinely used to evaluate the genotoxic potential of chemicals in the environment, because it is cheap, easily available and handled as well as its sensitivity and good correlation with mammalian test systems. Thus, *A. cepa* is an efficient test organism for environmental monitoring, especially in contaminated aquatic environments (Table 1, read only).

Table 1. Summary on use of *Allium cepa* root chromosomal aberration assay for environmental monitoring.

S. No	Agent/s studied	Nature	Type of aberrations	Reference
1.	Hospital effluents	Chemica l mixture of pollutants	chromosomal disruptions, anaphasic bridge/s and micronuclei	[19]
2.	Coal fly ash	Mixture of chemicals	root growth and mitotic indices inhibition; binucleated cells formation.	[20]
3.	Industrial wastewater	Wastewater	mitotic division reduction; mitotic anomalies	[21]
4.	Lead	Heavy metal	decrease root growth and mitotic index; Induce chromosome bridge/s, laggard chromosome/s and micronuclei.	[22]
5.	Nano-silver	Anti-bacterial	mitotic index decrease, c-metaphase, stickiness, bridge/s, laggard/s and micronuclei	[23]
6.	Magnesium sulphate	Fertilizers	cytostatic and clastogenic properties	[24]
7.	Industrial effluents contaminated with azo dyes	Mutagenic chemicals	mitotic index reduce; bridge/s, laggard/s, c-metaphase, binucleated cells; loss of chromosomes	[25]
8.	Lead	Heavy metal	root growth and mitotic index reduced; chromosome bridge/s, laggard chromosome/s and micronuclei	[22]
9.	Maleic hydrazide	Herbicide	chromosomal aberrations like bridge/s, laggard/s etc.	[26]
10.	Petroleum hydrocarbon	Complex chemical mixture	nuclear bud, micronuclei, mini cells, polynucleated cells, chromosomal bridge/s, c-metaphase and break/s	[14]
11.	Extracts of *Psychotria* (*P. myriantha* and *P. leiocarpa*)	Herbal medicine	chromosomal aberrations, inhibition of cell division was more in *P. leiocarpa* than *P. myriantha*	[27]
12.	Quizalofop-P-ethyl	Herbicide	stickiness, bridge/s, vagrant/s, c-anaphase, multipolarity, micronuclei	[28]
13.	Cadmium	Metal	inhibition of mitotic index; CA, MA	[29]

			and micronucleus	
14.	Maleic hydrazide	Metal	mutagenic events reduce and induce translocation of chromosomes	[30]
15.	Atrezine	Herbicide	inhibit mitotic index; micronucleus, chromosomes and mitotic aberrations	[31]
16.	Aluminium	Metal	oxidative stress, damage DNA and cell death	[32]
17.	Aqueous extracts of *Azadirachta indica, Morinda lucida, Cymbopogon citratus, Mangifera indica* and *Carica papaya*	Medicinal plants	mitotic spindle disturbance, inhibitory, mitodepressive, turbagenic and inhibition of root growth	[33]
18.	Curcumin	Antimutagen	chromosome break/s, gap/s and fragment/s	[34]
19.	Potassium metabisulp-hite	Food preservative	mitotic index reduce; break/s, gap/s	[35]
20.	Sodium benzoate, boric acid, citric acid, potassium citrate and sodium citrate	Food preservative	mitotic division reduce, anaphase bridge/s, c-mitosis, micronuclei, break/s, lagging, stickiness, and unequal distribution	[36]
21.	*Plantago lanceolata*	Medicinal plant	decrease mitotic index; induce breaks, bridges, stickiness	[37]
22.	Vanadium	Metal	chromosomal aberrations	[38].
23.	Avenoxan	Herbicide	abnormal cell increased, stickiness, bridge/s, laggard/s	[39]
24.	Acetaminophen	Analgesic	roots did not grow at high concentration, mitotic index declined	[40]
25.	Fumonisins	Toxic	genetic damage occurs, chromosomal aberrations, sister chromatid exchanged	[41]
26.	Lechates from solid waste	Heavy metal contamination	mitotic index inhibition, chromosomal aberrations and	[42]

			micronuclei	
27.	Heavy metal contaminated river water	Heavy metal	decreased cell reproduction; bridge/s, fragment/s, laggard/s, c-mitosis, micronuclei	[43]
28.	Dinocap	Fungicides	stickiness, c-mitosis, laggard/s, multipolarity, micronuclei, polyploidy fragment/s	[44]
29.	Air pollution	Cytotoxic substance	mitotic cell division decreased, genotoxic substance found	[45]
30.	Diuron contaminated soil	Urea herbicide	break/s, micronucleated and binucleated cells; mitotic index declined	[46]
31.	Atrazine	Pesticide	break/s	[47]
32.	BDE-99	Flame retardant	chromosomal aberrations	[48]
33.	Industrial wastewater from Shawa, Meet EI, Akrad, Telbana, Belgay	Industrial wastewater	mitotic division inhibition, chromosome ring/s, fragment/s, bridge/s, disturbed metaphase	[49]
34.	Sewage water	Toxic metals	growth inhibition occur, wilting appears on root tip/s, abnormal dividing cell increased	[27]
35.	Aqueous extract of *Aristolochia triangularis, Cayaponia bonariensis, Solanum granulsolepros-um,*	Antihypertensive agents	micronuclei, asynchronic divisions	[49]
36.	Sodium metabisulfite	Food preservatives	mitotic index decreased, c- mitosis, stickiness	[50]
37.	*Azadirachta indica*	Insecticide	micronucleus, multinucleated cells, bridge/s, stickiness, laggard/s	[51]
38.	Lead	Metal	mitotic activity inhibition, level of DNA synthesis declined, c-mitosis	[52]
39.	Sewage and industrial effluents from the Amritsar	Domestic and industrial wastewater	high number of micronuclei and anaphase aberrations	[53]

40.	Cypermeth-rin and fenvalerate	Insecticides	mitotic index inhibition; chromosomal and mitotic aberrations	[6]
41.	Cs and Sr	Radioisotopes	germination rate of onions decrease; aberrations like stickiness, vagrant	[54]
42.	Waste, surface and ground water	Toxic substances	root growth inhibition; metaphase and anaphase aberrations	[55]
43.	Polluted water sample	Industrial and municipal wastewater, water from treatment plant	fragment/s, c-mitosis, stickiness	[15]
44.	Alkyl benzene, sulphonate and citowett	Surfactants	root length declined; mitotic index decreased; chromosomal aberrations	[56]
45.	Wastewater samples	Mixture of toxic substances	inhibition of mitotic activity, chromosomal and genomic aberrations	[57]
46.	Phosphine gas	Fumigative agent	root length and viability of seeds reduced, frequency of aberrated cells increase	[58]
47.	Carbetamide	Pesticide	c-mitosis, break/s and bridge/s	[59]
48.	Chlorophenoxy acids	Herbicide	c-tumors, stickiness, vagrant/s, fragment/s; mitotic index decreased	[60]
49.	Carboxin, Oxycarboxin	Pesticide	micronuclei	[61]
50.	2, 4, 5-T	Herbicides	cell enlargement and chromosome aberrations; duration of mitotic cycle increased	[62]

Evaluation:

The mitotic index, % cells in mitosis, % cells in phase and the frequency of chromosomal aberration are used to evaluate genotoxicity.

Mitotic index (mentioned previously in lab 2)

% cells in mitosis = total number of cells in all phases of mitosis x 100/ total number of cells

% cells in phase = total number of cells in phases x 100/ total number of cells

Chromosomal abnormalities/aberrations (in slides and sheets)

Frequency of chromosomal aberration (CA) = number of aberrant cells x 100/ total number of cells

Note:

Mitotic index (MI) is considered as an indicator of cell proliferation biomarker so, the cytotoxic level of a test chemical/compound can be determined based on the increase or decrease in MI. Significant reduction in MI seen by most of the treatments may be due to the inhibition of DNA synthesis or the blocking in the G2 phase of the cell cycle while, other chemicals have been reported to inhibit mitotic activities.

The cytotoxic level can be determined by the decreased in MI and % cells in mitosis: decrease below 22% of negative control (MI= 0.22) causes lethal effects on test organism while a decrease below 50% (MI < 0.5) has sublethal effects and is called cytotoxic limit value.

Chromosomal aberrations (CAs):

CAs varies at the chromosomal level by change in either total number of chromosomes or in chromosomal structure which occur as a result of the exposure of chemical treatment. Some of those chromosomal changes could be seen at Mitotic cell cycle. To evaluate the different chromosomal abnormalities, several types of CAs are considered in different phases of cell cycle (Interphase, prophase, metaphase, anaphase and telophase).

Note: Normal phases are previously mentioned and seen in Lab 2.

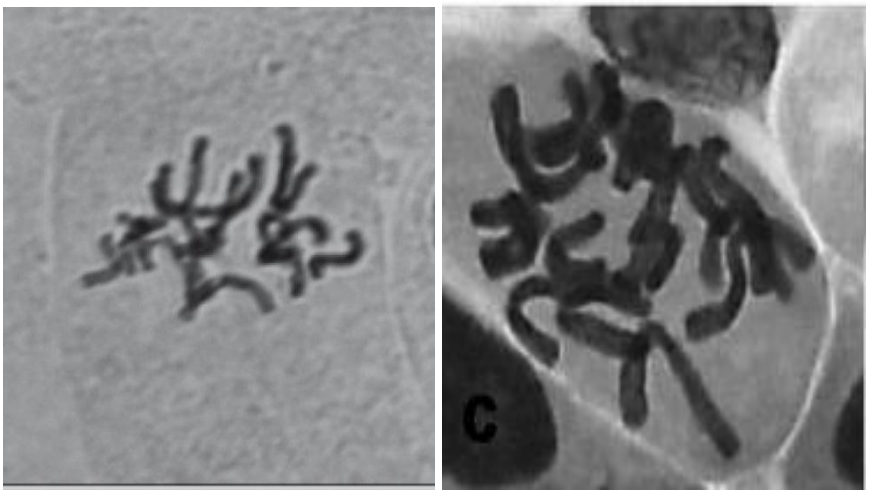
CAs were grouped into 2 types, clastogenic and physiological aberrations. physiological aberrations (انحرافات فسيولوجيه) include c-mitosis, vagrant/s, stickiness, delayed anaphase and laggard/s.

Clastogenic aberrations (انحرافات تكسيريه) have one of two types of structural changes due to breaks in chromosomes. It happens during DNA replication and includes chromatin bridge/s (sister chromatid exchanges, interchanges and reunions), chromosomal break/s (result in the gain, loss, or rearrangements of chromosomal segments), ring chromosome/s (loss of chromosomal segments) and micronuclei. The causal of this process is called clastogen.

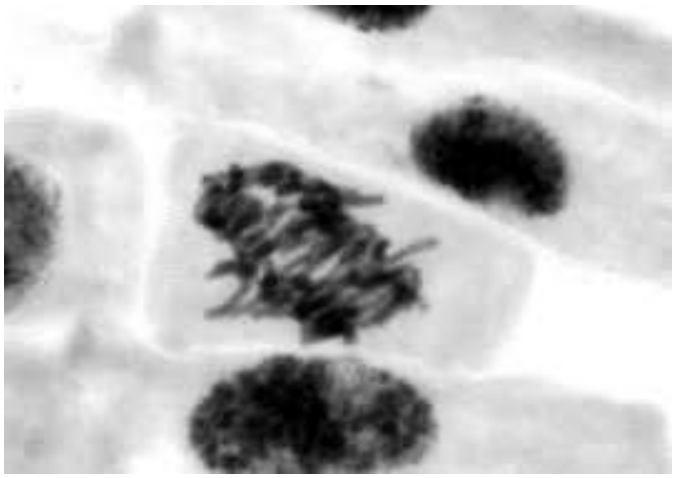
Note: Chromosome stickness, briges, c-mitosis and vagrant chromosomes may be due to the inhibition of spindle formation and thus resulted in cell division disturbances.

Physiological aberrations:

c-Mitosis (c-Metaphase): It appears when the treatment prevents the assembly and function of the spindle fibers resulting disorientation and scattering of the chromosomes over the cells. This process is followed by delay in the division of centromere. It indicates a weak toxic effect which may be reversible.

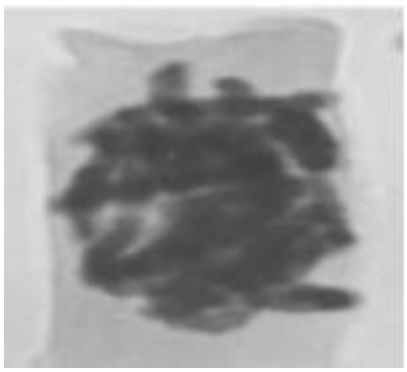


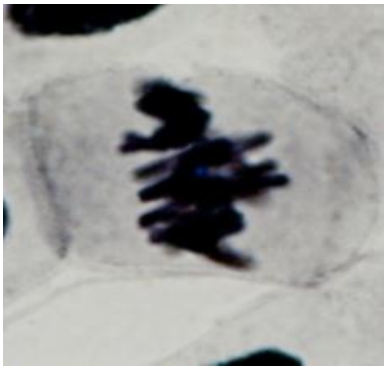
Delayed anaphase: It appears when the two anaphasic chromosomal groups lie close to each other near the equatorial plate.

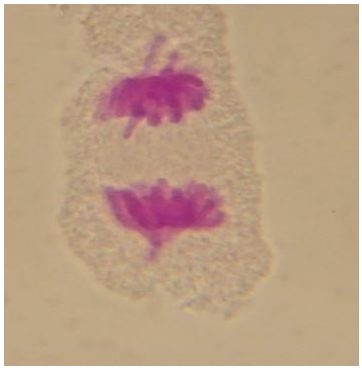


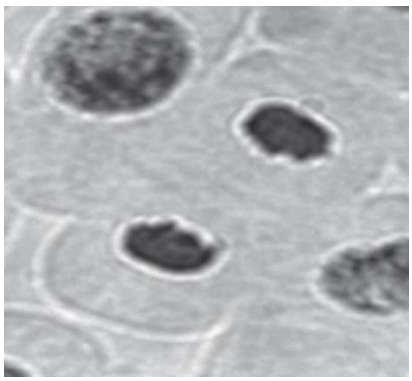
Lagging chromosomes: It results due to failure of the chromosomes to get attached to the spindle fibre and to move to either of the two poles. The induction of lagging chromosomal aberration also called laggard/s.

Stickiness of chromosomes: It resulted from the improper folding (clumping or clustering) of chromosome fibers which makes the chromatids connected by subchromatid bridges or increased chromosomal contraction and condensation or might from the depolymerization of DNA and partial dissolution of nucleoproteins. Sticky chromosomes may also results from the defective functioning of one or two types of specific non-histone proteins involving chromosome organization which are needed for chromatid separation and segregation. This reflects toxic effects, usually of an irreversible type and probably leading to cell death. It may appear in any of phases.



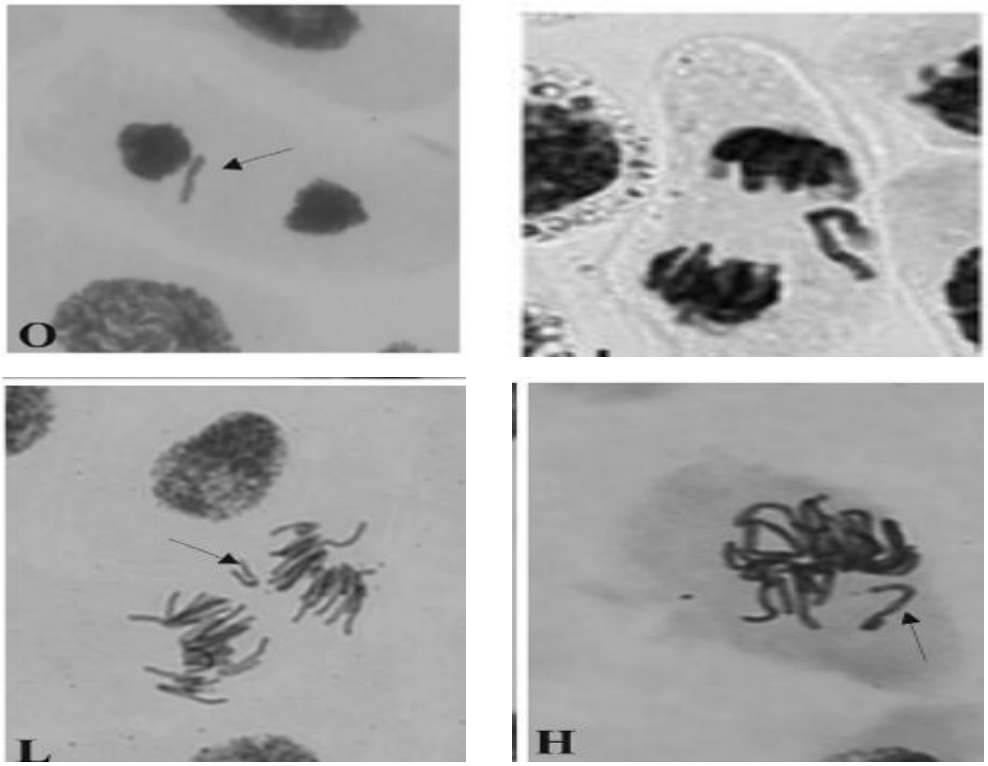






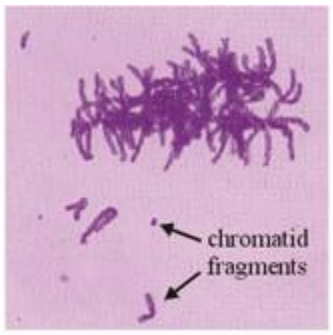
In vagrant chromosome/s: chromosome moves ahead from its chromosomal group toward poles and leads to the unequal separation of number of chromosomes in the daughter cells. This aberration may be due to the failure of the spindle apparatus to organize and function in a normal way.

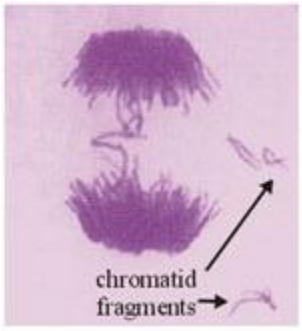
Loss of chromosome:

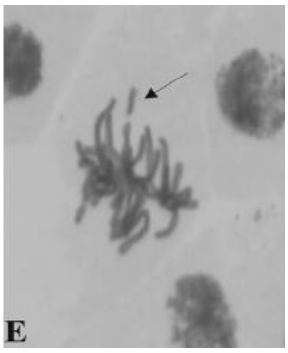


Clastogenic aberrations:

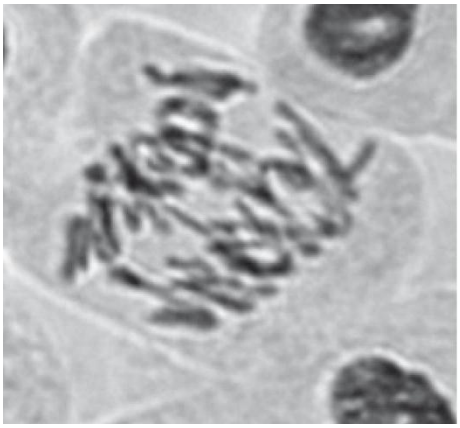
Chromosome break/fragmentation: Most abundant abnormality is fragmentation of chromosome which reflects probable activation of the endogenous nuclease generating small inter-nucleosomal fragments. **Ring chromosomes** are type of breaks as the result of loss of chromosomes from the telomeric side.

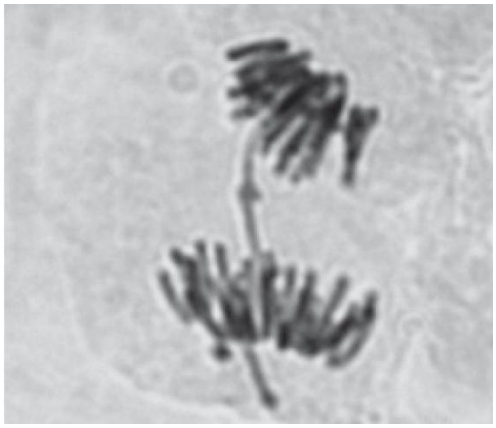




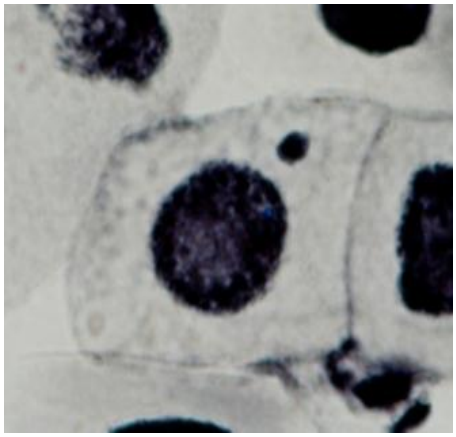


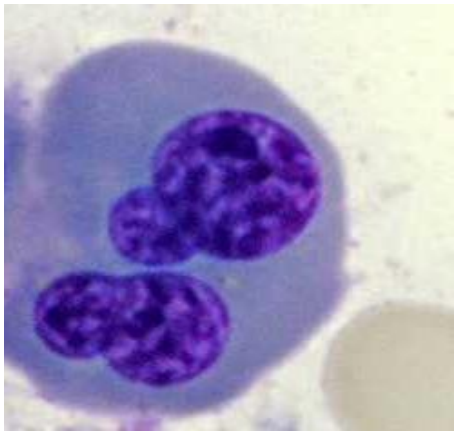
Chromatin/chromosome bridges: It is commonly observed during anaphase and telophase. The bridges were probably formed by breakage and fusion (reunion) between broken ends of sister chromatids or subchromatids or between different chromosomes (unequal exchange).

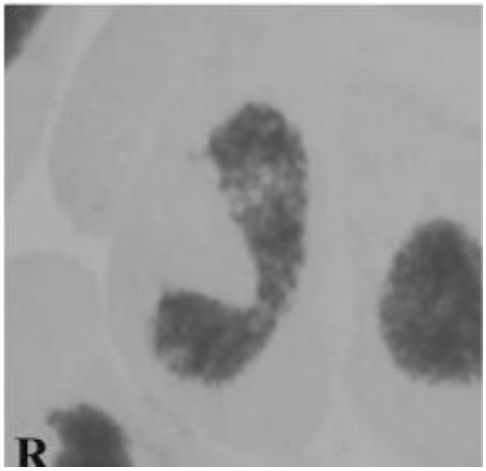




The exceeding DNA of a cell may originate a bud **(nuclear budding)** and which gives rise to a micronucleus and it is subsequently expulsed as a mini cell (small cytoplasmatic portions bearing a small nuclear content). **Micronuclei (MN)**: MN can be spontaneously originated due to the development of the isolated chromosome that results from an unequal distribution of genetic material caused by malformation of spindle fibers. MN can be a formed as a result of acentric fragments or entire chromosomes not incorporated to the main nucleus during the cell cycle. Their induction is commonly used to detect genetic damages derived from exposure to mutagenic chemicals. Therefore, any substance that is able to promote micronuclei formation is said to be clastogenic or aneugenic. Cells bearing micronuclei were observed at interphase and prophase stages.

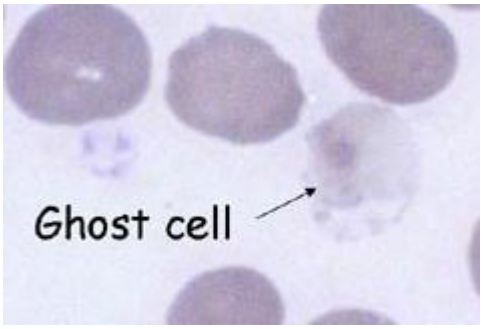




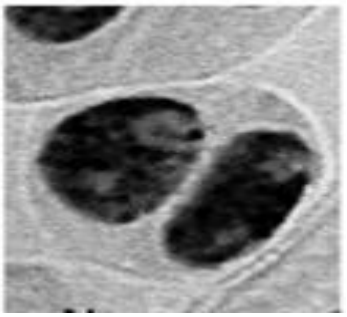


Other Abnormalities:

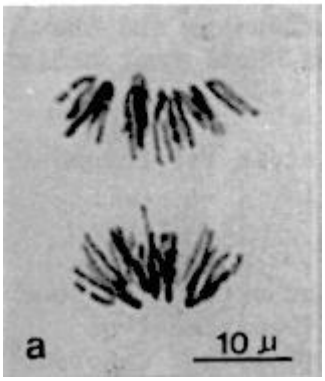
Ghost cell is a dead cell in which the outline is visible but nucleus and cytoplasmic structure is not stainable. Apoptosis is an energy-dependent biological process of living organisms which is genetically controlled by which unnecessary or damaged cells die. The cell death was induced by high concentrations of toxic chemicals.

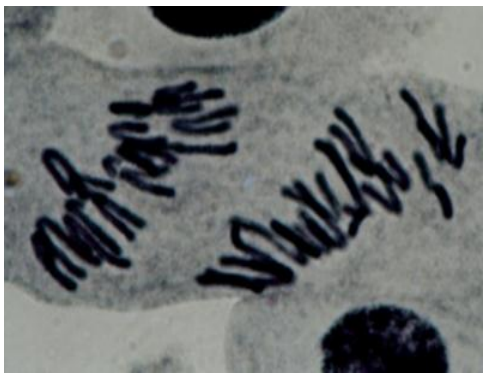


Binucleated/multinucleated cells occur when the nucleus divided normally but cytokinesis process of cell division was inhibited. Such cells look geant and have 2 to several nuclei.



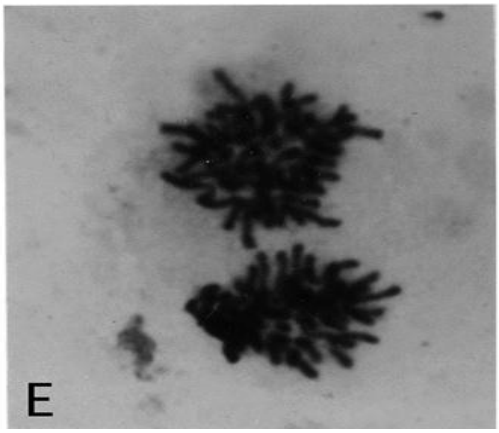
Multipolarity of anaphase: it happens due to the presence of multipolar spindle in anaphase.



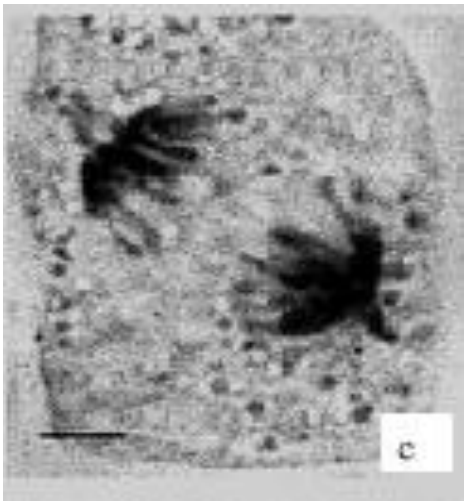


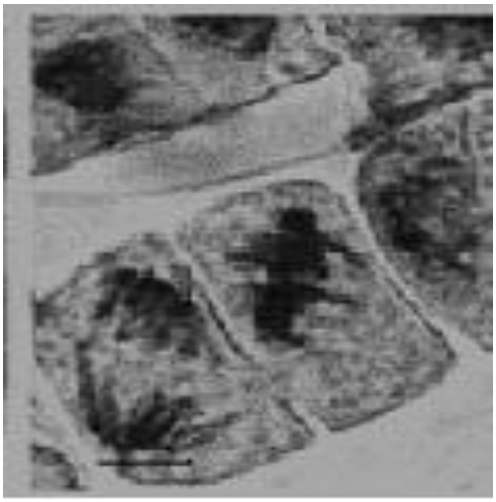
Star shaped anaphase:

Star shaped telophase:



Diagonal anaphase and metaphase: orientation fault of equatorial plate





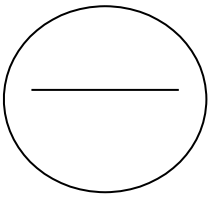
WORK SHEET

1. The provided root tip of Onion was soaked in nano-silver solution with concentration 50 ppm for 4 h.
2. The root tips treated in distilled water was used as control.
3. After treatment, the root tips of control and experimental samples were thoroughly washed in distilled water and fixed in acetic-alcohol (1:3).
4. Prepare your own root tip squashing technique (same as lab 2) to observe the different cell cycle aberrations.
5. Sketch each aberration
6. Photograph the Phases of the Mitotic cycle aberrations.
7. Calculate: mitotic index, % cells in mitosis, % cells in phase and the frequency of chromosomal aberration.

Student name: **Code number:**

STUDENT'S ASSIGNMENT

Give it to your laboratory instructor



1. Search in the Internet or the Library for the mode of action (mechanism) of silver-nano on the Mitotic cycle in root tips of onion (*Allium cepa*). Add references.

……

……

……

……

……

……

……

……

……

……

……

……

……

……

……

……

……

……

……

……

……

……

2. Search for photos showing the effect of silver-nano on the Phases of the Mitotic cycle in root tips of onion (*Allium cepa*). Labels the photos.