Observing mitosis in the cells of garlic biology essay

Aim: To observe the stages of the cell cycle in living tissue, generally and specifically in the meristematic cells of the root of garlic and hence consider the duration of the stages of mitosis in relation to the whole cell cycle.

Introduction:

The cell cycle, or cell-division cycle, is the series of events that takes place in a cell leading to its division and duplication (replication). In cells without a nucleus (prokaryotes), the cell cycle occurs via a process termed binary fission. In cells with a nucleus (eukaryotes), the cell cycle can be divided in two brief periods: interphase-during which the cell grows, accumulating nutrients needed for mitosis andduplicating its DNA-and the mitosis (M) phase, during which the cell splits itself into two distinct cells, often called “ daughter cells”. The cell-division cycle is a vital process by which a single-celled fertilized egg develops into a mature organism, as well as the process by which hair, skin, blood cells, and some internal organs are renewed. [1]

Figure 1: The cell cycle

http://adasilva. glogster. com/chapter-10-bio/

Mitosis is the process that facilitates the equal partitioning of replicated chromosomes into two identical groups. Before partitioning can occur, the chromosomes must become aligned so that the separation process can occur in an orderly fashion. The alignment of replicated chromosomes and their separation into two groups is a process that can be observed in virtually all eukaryotic cells. [2]

Both the alignment and separation processes are the consequence of the chromosomes interacting with filamentous proteinaceous structures, known as microtubules. The microtubules become organized into a biconical array known as a spindle, which forms early in mitosis, and then disassembles as mitosis nears completion. Mitotic spindles are visible in living cells with the polarizing light microscope. Some of the spindle microtubules become attached to the chromosomes at sites known as kinetochores. The kinetochores cannot be seen with the light microscope, but they reside near the place on the chromosome known as its centromere, which can be observed with the light microscope. There are two kinetochores on each replicated chromosome (one on each chromatid), and when the replicated chromosome splits apart at its centromere at the onset of anaphase, each daughter chromosome possesses one centromere and one kinetochore. The linkages between kinetochores and microtubules are thought to be central in controlling both the positioning of the replicated chromosome at the central portion of the spindle during the alignment phase, and in moving the daughter chromosomes apart after they split at their centromeres. The separation of daughter cells from each other is a process known as cytokinesis, and is separate from mitosis. In cytokinesis, animal and plant cells differ considerably from each other. These differences are the consequence of having or not having a cell wall. Cytokinesis in fungi reveals some similarities with plant cells, and exhibits other features unique to the group.[2]

Figure 1: Illustration of the process by which somatic cells multiply and divide.

http://www. accessexcellence. org/RC/VL/GG/mitosis. php

The specimen used in this experiment is the root of garlic where the meristematic cells are actively dividing. Cell division in flowering plants takes place in particular regions of the plant called meristems. Cells in meristems are not specialized for any particular function and divide repeatedly by mitosis. Some of the daughter cells remain meristematic; others cease dividing and become differentiated into appropriate cell types depending on their position. The root tip meristem is usually a denser white and more rounded than the cut end. Chromosomes in root tip tissue are made visible with the stain. Dividing cells (if present) will show up clearly with chromosomes in different forms according to the stage of mitosis. Individual chromosomes (as tightly-coiled threads) are visible during anaphase. The links between the cellulose walls of plant cells are broken down by the treatment with hydrochloric acid. This ensures that the stain can penetrate the cells and allows the tissue to be squashed out one cell thick. [3]

References:

[1] http://en. wikipedia. org/wiki/Cell_cycle

[2] http://www. life. umd. edu/CBMG/faculty/wolniak/wolniakmitosis. html

[3] http://www. practicalbiology. org/areas/advanced/cells-to-systems/cell-division/investigating-mitosis-in-allium-root-tip-squash, 121, EXP. html

Objective: Mitosis occurs in four phases as a continuous process without break in between the phases. Before mitosis, the cells are in rest during interphase stage. The activity of chromosomes is significant in determining

the stages of cell division. Therefore, to observe the chromosomes, the root cells are stained. There are two types of stains used; the toluidine blue stain. The toluidine blue stain causes the cytoplasm to appear light blue in colour while the chromosomes appear dark blue. In this experiment, the cells in the root tip of garlic is squashed, stained and observed under compound microscope to observe the stages mitosis.

Apparatus: Compound microscope, 2 Watch glasses, dropper, tissue paper, microscope slide, cover slip, forceps, mounted needles, stopwatch, scalpel or sharp knife, eye protection, small sample tube, filter paper, eye goggles.

Materials: Toluidine blue stain, 1M hydrochloric acid, 1 clove of garlic with roots, cold distilled water

Procedure:

1-2 cm of root tips of growing garlic roots are cut off. The root tips are ensured to be chosen from those which are white and have a firm rounded end.

The root tips are put into watch glass containing 2 cm3 of 1 M hydrochloric acid for exactly 5 minutes.

The root tips are then transferred into watch glass containing approximately 5 cm3 cold water, and left for 4-5 minutes. The root tips are then dried on filter papers.

Figure 3 : The root tips are transferred into glass with cold water

The root tips are then transferred onto a clean microscope slide. 4-5 mm of the root tips are cut and kept, while the rest are discarded.

The root tips are gently broken up with a mounted needle by a process called maceration.

Figure 4 : The root tips are break up

A small drop of toluidine blue stain is added to the root tips and left for 2 minutes.

The tips are covered with a coverslip, and blotted firmly with several layers of tissue. The coverslip is pressed gently to spread the root tip.

The samples are then viewed under the microscope (x40 magnifications) and the nuclei of the cells are located.

The microscope lens is switched to higher power (x100, x400 and x1000 magnifications and look for cells with chromosomes.

Figure 5 : The cells are observed under light microscope

The regularly shaped, actively dividing cells are focused and counted for every phases of mitosis.

The data obtained are recorded and tabulated.

Results:

Stages Of Mitosis

Interphase

Prophase

Metaphase

Anaphase

Telophase

Total

Cell counted

22

15

3

3

2

35

Percentage

62. 9

14. 3

8. 6

8. 6

5. 7

100

Table 1 : The stages of mitosis and the respective cell count and percentage

Calculation of the mitotic index :

Mitotic index = number of cell containing visible chromosome

total number of cells in the field of view

Mitotic index =

Interphase

Interphase is the period where the cell prepares itself before cell division. It is a phase before the mitosis. The chromosomes are uncoiled and it fills the nucleus. DNA replication occurs during this stage. The nuclear envelope is visible the centrioles appear and so do the nucleolus.

Prophase

During prophase, the replicated chromosomes undergo extensive condensation or coiling. The chromosomes are greatly thickened and shortened but are still contained within the nuclear envelope. Prophase ends with the sudden dispersion of the nuclear envelope. The chromosomes start to coil, shorten, and become distinct. The mitotic spindle or polar fibers begin to form from the poles of the cell towards the equator.

Metaphase

Chromosomes line up along the equator of the cell or what is called the metaphase plate. Polar fibers have reached the centromeres of the chromosomes and have begun interacting with them. Each kinetochore, plate like structure of the replicated chromosome is pointed toward one side of the spindle. The replicated chromosomes converge toward the center of the spindle, and once they get there, significant movements cease. On either sides of each centromere are sites for kinetochore microtubule.

Anaphase

Anaphase commences with the initial splitting of sister chromatids at their centromeres. These daughter chromosomes then begin to separate from each other, each moving away from the metaphase plate and toward one of the two spindle pole regions. Anaphase ends when the daughter chromosomes reach the end of the cell. Since the sister chromatids are identical copies of the original chromosomes, each pole of the cell will have a set of complete and identical chromosomes as in the parent cell.

Telophase

In telophase, the daughter chromosomes arrive at the spindle poles and are eventually redistributed into chromatin. After complete separation of the chromosomes and their extrusion to the spindle poles, the nuclear membrane begins to reform around each group of chromosomes at the opposite ends of the cell. The nucleoli also reappear in what will eventually become the two new cell nuclei. A new nuclear membrane forms. The new cell walls grow to form the two new, identical daughter cells. The cytokinesis

process starts to happen in order to complete the division of the two new cells.

Discussion:

After observing the mitosis process, we knew that percentages of the cells in each stage are different. In the experiment using toluidine blue stain, we observed that 22 cells out of 35 cells undergone interphase which covered 62. 9% of the cells. There are 15 cells undergone prophase covering 14. 3% of the cells. Metaphase and anaphase have the same percentage which is 8. 6%. While 2 cells undergone telophase which covered 5. 7%. The mitotic index using toluldine blue stain is which suggest that only 37. 14% of the cells in our view were undergoing mitosis and the rest are still in interphase. The percentage of cells in each stage is related to the length of time a cell spends in each stage of mitosis. The greater the percentage of cells in a stage, the longer the time spent by each cell in that stage.

Based on the table 1, it is clearly shown that the largest percentage of cells is found to be at interphase. Interphase is often referred as the resting phase. Interphase is the stage in which the cells prepare themselves to undergo mitosis. Since interphase has the largest percentage of cells, therefore each cell spends the longest time being at interphase stage. Metaphase and anaphase occupies the same length of time and same percentage of cells. This means cells spend almost equal length of time being at both phases. Telophase on the other hand, has the lowest percentage which means the shortest period of time a cell spends is during telophase. Prophase, being the first stage of mitosis has the second highest percentage of cells. It means compared to metaphase, anaphase and

telophase, prophase is the stage of mitosis in which each cell spends the most time. From the results, we knew that most of the cells spent longer time in interphase because cell growth, replication of the chromosomes, and many other activities are taking place during this time. The cells need to prepare first for the mitotic division to occur. On the other hand, based on results on table 2, is it clearly shown that, the size of cell during interphase is the smallest among all followed by the size of cell during prophase, metaphase, anaphase and finally the size of cell is the biggest during telophase. This is because, during interphase the cell will never grow in size exactly as the cell prepares itself for cell division. Meanwhile, during telophase the cell actually splitting into two daughter cells and it is obvious that the cell would have grown in size.

This experiment use the garlic root tips tissue for observing the mitosis process because the stages of development in plant growth can be distinctly observed at this part which is known as meristem. This meristem is actively divided by mitosis. So, every stage of mitosis can be observed clearly. We ensure that our results are reliable because we use 3 samples of root tip. This could minimize the error during this experiment and we can compare the results for each sample. Besides that, we stained the samples using toluidine blue first so that we can observe the chromosomes clearly. We believed that our results are valid because the outcomes from the experiment are the same with what we expect based on the theory and our results are quite similar with the other group’s results. Not only that, the usage of toluidine blue is reliable, cheaper and easy to handle with. In a way,

it is simple and it helps produce results quicker compared to the usage of other staining.

Conclusion:

Mitosis can be divided into four different stages; prophase, metaphase, anaphase and telophase. In conclusion, the mitosis process is a random process. So, different types of sample will have different length of time for mitosis to complete. Other than that, the longest stage of cell cycle is interphase with the highest percentage followed by metaphase and anaphase while the shortest stage is telophase with the lowest percentage. The greater the percentage of cells in a stage, the longer the time spent by each cell in that stage. Hypothesis is accepted.