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TEMPORAL THERMAL EFFECT ON POLYTENE CHROMOSOME OF *DROSOPHILA MELANOGSTER*

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ABSTRACT

Polytene chromosomes are the distinct inter-phase chromosome composed of heaps of DNA strand making them giant. These also exhibit a feature of band-inter band morphology. With the distinctive formation of these chromosomes these have been utilized in diverse research studies. The salivary glands of third in star larvae of *Drosophila melanogaster* were taken and was squashed and treated with various temperatures at different short time intervals. The study was done to monitor the changes that occurred in the arrangements of puffs in the chromosome when heat shock was induced at differing time intervals. The analysis showed that there was change in the chromosomal pattern by increase in number of puffs in the arms of polytene chromosome when compared to the normal squash. The gene functioning of the polytene chromosome can be directly viewed under microscope by the help of puffs formed. New puffs formed are the position of intense RNA-synthesis.

INTRODUCTION

The fly, *Drosophila melanogaster*, is an insect with complete metamorphism that is mainly divided into four stages of life cycle: embryo, larva, pupa and adult. In the larval stage, the organism is primarily interested in acquiring food for speedy increase in the characteristic size during this developmental stage. In the course of this time, the salivary glands must be of considerable size and developed to provide adequate mass of salivary enzymes for digestion. Usually, the species of Drosophila and other insects attain the growth of their salivary gland by expanding the cell mass and volume rather than multiplying the number of cells. In the early larval development, after a certain quantity of salivary gland cells are produced, there will be a halt of cell division. This leads to the increase in cell size and with the growth of the nuclei due to repeated duplication of the chromosome. The formed structures are gigantic and are called as polytene chromosomes. These chromosomes will have unique patterns of light and dark bands. These bands carry genes, which on active transcription, produces puffs that develops in the chromosomal region that accommodates that gene. The heat shock response studies have been performed mainly with the help of puffing patterns of polytene chromosomes. As these chromosomes helps in understanding the genes that control and letting the induction of these puffs. The puffs can be used to study the chief polypeptides formed during the response that are coded by their genes. The molecular arrangement of the

genes that induce heat shock has been studied (Ashburner and Bonner, 1979).When *Drosophila melanogaster* is exposed to different temperature above its optimum temperature (20-22°C), there is an activation of a set of specific genes, in which the other genes that were present are suppressed once there is an induction of heat shock. The induction of about 8-9 puffs occurs due to heat response. Such high temperature contact of *Drosophila melanogaster* larvae with short interval of period, outcomes the changes in puffing patterns of chromosomes isolated from salivary gland. Few puffs that are required for the development of normal puffing are also suppressed due to heat shock. The shock induced by temperature can be rapid and also very quick even with small increase in temperature. The temperature above 39°C are usually considered to be lethal and can start causing abnormal suppressions of genes.The data presented here is an extension of experiment done to analyse induction of puffs in polytene chromosome of the *Drosophila melanogaster* larvae. This was done by treating the larvae with different temperatures to induce heat shock at various time differences.

Materials and Methodology

The third instar larvae of *Drosophila melanogaster* have been used in the experiment. The polytene chromosomes present in the salivary glands of the larvae are particularly favorable for the experimental analysis. The culture media was prepared using jaggary [50g], Rava[50g], Agar - agar[5g], Distilled water [500ml] and Propionic acid [3.75ml], which is an odorless preservative, used against the growth of unwanted molds or other organisms. All these together were cooked with continues stirring avoiding formation of clumps. The media ones cooled was poured in to sterilized bottles in required quantity. The Drosophila melanogaster flies were then transferred into the bottles with media. They were maintained at an optimum temperature of 22 - 25°C for their survival and proper growth

Dissection of Salivary gland

A drop of saline solution was placed on a clean microscope slide. An appropriate larva was transferred to the slide and placed on the stage of a dissecting microscope. The posterior end of the larva was firmly grasped with forceps and another pair of dissecting needle piercing through the head, just behind the darkly pigmented mouth parts. Using a continuous motion, pull the needle (and attached head) away from the body. The salivary glands are paired and are identical in size and shape. They also have a glistening, translucent appearance. Each gland has an opaque fat body associated with it. Separate the salivary glands from any extraneous material without damaging it. Once cleaned, remove the used saline and debris from the slide.

The Squash Method

The dissected salivary glands of the third instar larvae were placed in 1N HCl for 5 minutes and later removed and the lobe was flushed with 45% acetic acid for 1 minute. This was then stained with aceto - orcein stain for 30-35 minutes. The excess stain was removed by using glacial Acetic acid for 1-2 minutes, this helps in the softening of the tissues which in-turn makes squashing easy and helps in getting a perfect spread. Carefully blot the excess stain away from the salivary gland. Add one drop of fresh stain and incubate for two minutes. Apply a cover slip to the stained salivary gland and place a paper towel or folded laboratory tissue over the cover slip. Using steady, moderate pressure, press down on the cover slip in a vertical direction using the thumb. To seal the edges of the cover slip use nail polish, this helps in avoiding the shearing of chromosome.

To Induce Heat shock to identify the puffs

Few live larvae, each was taken in to small vials with distilled water and was incubated for heat- treatment using water - baths set at different temperature: 30°C, 32°C, 35°C, 38°C. Each temperature was taken with three different time intervals as follows: 45 seconds, 1 minute, and 1minute: 15 seconds respectively. Each were taken into a new slide and labeled accordingly. The salivary glands were dissected out and the squash method was applied and observed under the compound microscope. Observe the salivary gland squash at high-dry magnification with a compound microscope. Observe for elongated

chromosomes with distinct banding patterns, and chromosomal puffing. If puffs are observed in the chromosome uses the oil immersion lens.

Results

Formation of puffs

The robustresponse to different temperatures leads to a phenomenon called the chromosomal puffing. Heat shock treatment at various temperature induced the formation of additional puffs on the chromosomes.Temperatures ranging from 30^0C-38^0C shows that there is an increase in the number of puffs as temperature is increased. The action of temperatures on tissue cell lines were not similar but shows the same result of increased number of puffs when the same procedure is performed twice. At 30^0C a small number of puffs were formed, and similar increased pattern were formed till 38^0C.When the third instar larvae were squashed without any heat shock treatment, we obtained a normal pattern of polytene chromosome as shown in the figure 1. This was then used as a control.

When salivary glands underwent heat shock at 30^0C at different short time intervals,there is evidence that, transcriptional activity took place by observing the formation of puff. The results from the figure 3 shows that larvae incubated under 32^0C at different time interval is in an active condition and capable in terms of its transcriptional activity by examining the production of puffs. Puff formations can be seen in proximal and distal regions of the chromosome. Dark puff as well as lighter puffs can be seen while examining the chromosome puff formation. Under the conditions of heat shock at 35^0C at different time intervals as shown in figure 4 there is a slightly higher increase of the puff formation.

The number of puffs in darker and lighter colour as shown in figure 5 is the result when the polytene chromosomes are treated at 38^0C. The induction of translational activity suddenly increases after 37^0C.Several prominent new bands and puffs are seen at the elevated temperature,when tissue-culture cells are shifted and incubated at a temperature of 38^0C leading to high-rate synthesis of the heat-shock proteins.

The number of puffs formed at different temperature was then plotted on the graph, as number of puffs verses temperature.

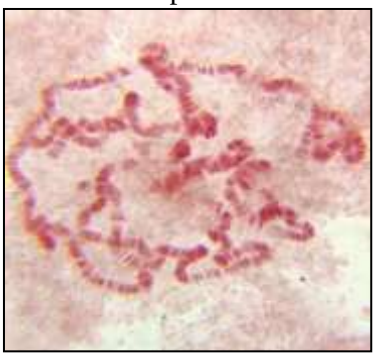

Fig 1: Normal polytene chromosome without any heat shock treatment

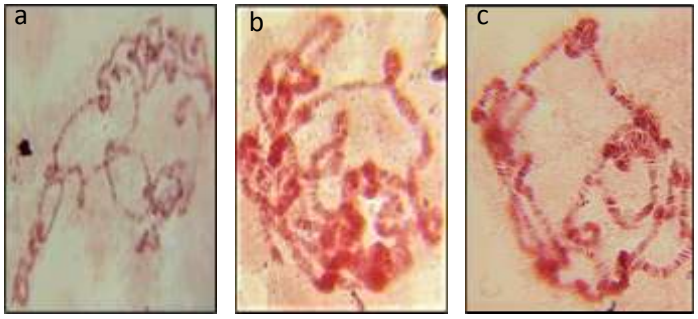

Fig- 2 Polytene chromosome treated at different time intervals at 30^0C. (a) Represents time interval of 45sec**. (b)** represents time interval of 1min. **(c)** represents time interval of 1min: 15sec.

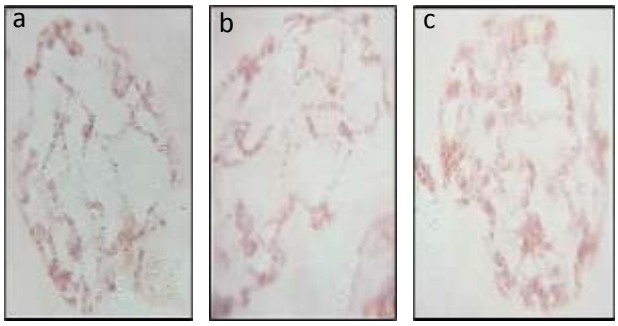

Fig- 3 Polytene chromosome treated at different time intervals at 32^0C. (a) represents time interval of 45sec**. (b)** represents time interval of 1min. **(c)** represents time interval of 1min: 15sec.

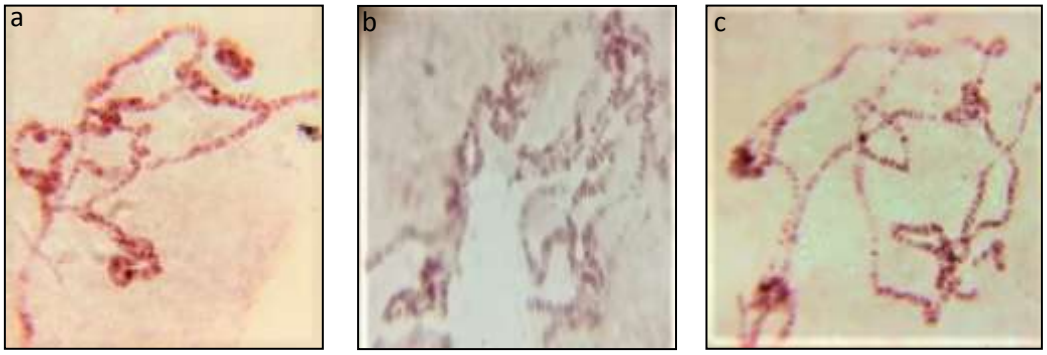

Fig- 4 Polytene chromosome treated at different time intervals at 35^{0}C. (a) represents time interval of 45sec**. (b)** represents time interval of 1min. **(c)** represents time interval of 1min: 15sec

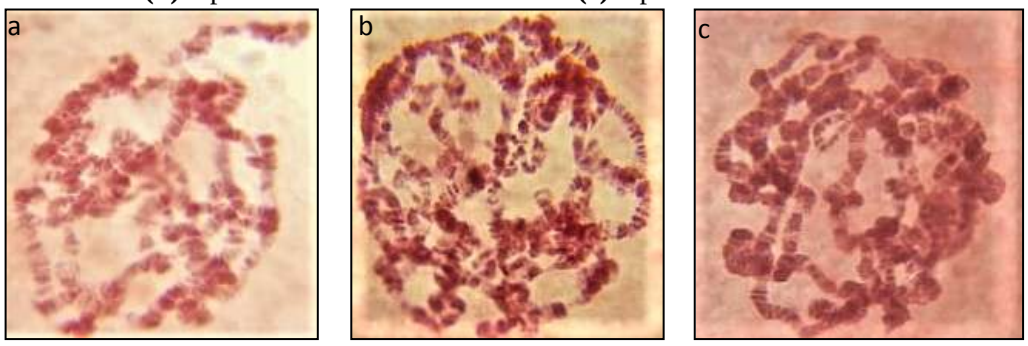

Fig- 5 Polytene chromosome treated at different time intervals at 38^{0}C. (a) represents time interval of 45sec**. (b)** represents time interval of 1min. **(c)** represents time interval of 1min: 15sec

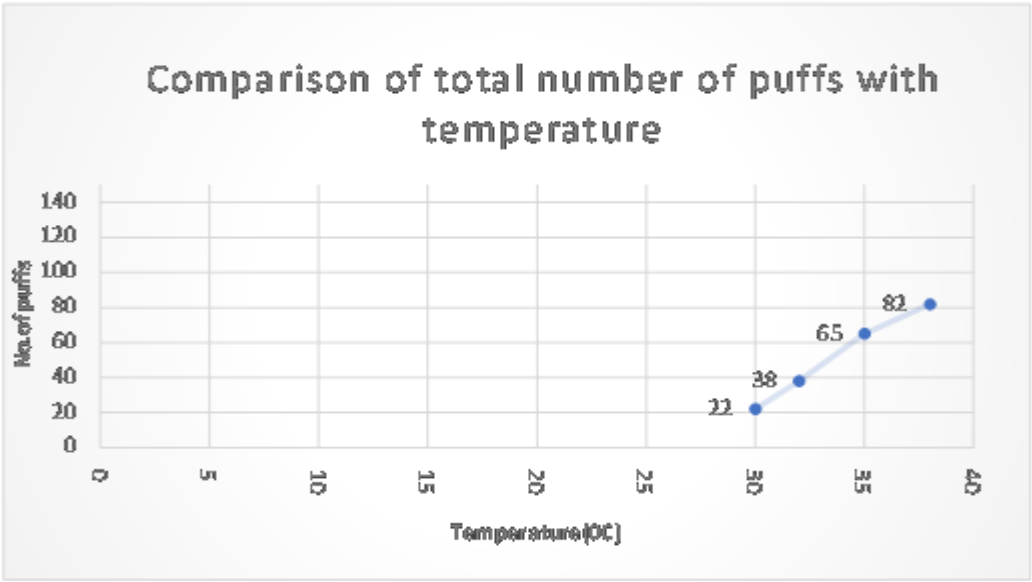

Table 1- Comparison between the temperatures and number of puffs formed.

DISCUSSION

The study used a method to observe the changes that occur in the puffing pattern of the polytene chromosome of the *Drosophila melanogaster*, when there is an induction of heat shock. There is activation of transcription of heat shock genes as a response to the temperature. The transcription activity was maximum when the temperature was at 35^{0}C, and a rapid uplift was detected after rise in temperature to 38^{0}C. The comparison between puffs at different temperature are as

shown in the table 1. Temperatures outside the lab were also considered which favours the larvae to be in active condition. Due to sudden elevation of the temperature, there is activation of the heat shock genes as response, which is observed both in prokaryotes and eukaryotes. These are proteins called heat shock proteins, which helps in survival of the cell from the stimulus for a period of time, by shielding all the cellular components that are essential from the harm caused by the heat. Incubation at various temperatures of *Drosophila melanogaster* salivary glands results in the appearance of several newly synthesized puffs and bands in the protein pattern. Few scientists (Mayrand and Pederson, 1983) suggested that, the transcriptional activity of genes of Drosophila sustains these damages, and that the stress on gene expression was caused at the RNA level. The heat shock gene transcription regulation is done by transcriptional activators (Parker and Topol, 1984; Wu et al., 1987). There is evidence that the heat shock proteins in Drosophila can be activated even with a small change in conformation (Westwood et al., 1991;Zimarino et al., 1990). The heat shock transcriptional factors are observed to be activated within 30sec of inducing the heat shock. It shows that there is direct correlation between transcription of genes and temperature.This study has helped to observe that the transcriptional activity of the heat shock genes of the Drosophila are repressed after a heat shock of above 38^0C. The heat shock studies has shown that the response to heat in all the organisms are universal and are vastly conserved. These acts as a mechanism of defence to varied ranger of stresses.

CONCLUSION

The puffing behaviour of polytene chromosomes, as the results reported in this paper, gives us an understanding of the characteristics response pattern due to heat shock. It is understood that there are few underlying causes that induces extra puffs as a response when there are external factors like heat shock. These changes are also related to the time of stress period upon the chromosome. These changes in the puff pattern can be studied directly from the squash method and microscopical analysis. However, for a final analysis the resulted puffs must be studied genetically using molecular analysis correlating it with the cell reaction and the change in their chromosomal level and the process of inducing the response. Only a combination such numerous analyses, can help in qualifying and describing the method involved in control and operating of puff formation at the chromosomal level in the salivary glands of *Drosophila melanogaster*.

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