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Effect of Temperature on Cytoplasmic Streaming on Permeabilized Nitella Cell

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Abstract: ***Cells have evolved various ways of movement of materials and Structures within the cell and the common example of this is the rapid Cytoplasmic Streaming seen predominantly in some Algal Cells and other higher plant cells and also in many protozoans in which it is called as Cyclosis. Changesin the rate of Cytoplasmic Streaming of Permeabilized Nitella cell at different temperature was investigated by motion of small organenelles or vesicles passing from one arbitrary point to another. The time taken to pass the particular distance between the above two points was recorded through the stop watch. The organenelles selected were usually of diameter within 2 to 3 um which are dispersed in the endoplasm and considered to reflect bulk flow due to Cytoplasmic Streaming.***

Keywords: Cytoplasmic Streaming, Permeabilized, Nitella

1. Introduction

Movement of substances from one place to another in the cell is the basic feature of life. Active movement of Cytoplasm in rotational manner is known to occur in various kinds of Cells like green algae Nitella, Chara, Embryos of Caenorhabditiselegans, Amoeba, Ciliates, filamentous Fungi, Neurospora, Mesophyll cell of Vallisneria, Elodea and various others. This aids in delivery of nutrients, metabolites and genetic information to all parts of larger Plant cells. The transport of Mitochondria is vital for the development and maintenance of axon in the nervous System and for establishing the embryonic polarity in C.elegans. Giantmultinucleated algal cell of Nitella is especially favourable for the study of Cytoplasmic Streaming. The Cytoplasm immediately adjacent to the inner side of Plasma membrane is organised into a thin cortex or ectoplasmic gel. Most of the internal parts of the cell is occupied by a giant central Vacuole. Between the central Vacuole and the cortex is a fluid ENDOPLASMIC region which Undergoes continuous unidirectional streaming at the rate of up to 100 um per second. In Characeaninternodal cells it is a vivid and specialised phenomenon which can be observed even at considerable resolution as one internode (4to 12 cm in length) constitutes only one cell. The Cytoplasm keeps moving continuously as an unending process like rotating belt along a more or less helical path. The rate and flow pattern of Cytoplasmic Streaming are always constant and directional. It is now fully confirmed that actin and myosin occur in all Eukaryotic cell and these two Proteins are the machinery for the motility and contractiliy. Interaction of these two Proteins generate the required force for dragging the Cytoplasm. The example of giant Algal cell Such as Nitella, is very interesting in this respect as here only actin exists in filamentous form and the myosin exist in free molecular form and their interaction causes Cytoplasmic Streaming instead of contractile movement.

2. Materials and Methods

Nitella was procured from local seasonal pond. Both outdoor and indoor culture of Nitella is desirable for round the year requirement of experimental material. It can be grown outdoor in a small tank, 1 metre deep containing fine mud, rotting leaves, some compost of cow dung etc. The natural pond water is initially used to grow, which contains all the natural biota of the pond.

Several weeks prior to the experiment, Plants are removed and cultured indoor under constant fluorescent light for acclimatizing the plants to laboratory conditions. Intact internodes from apical as well as middle part were used in experiment. The interodal cells of diameter 1mm and 4 to 6 cm long were cut out from the plant and kept in Artificial Pond Water (APW) for a day or two before the experiment.

Permeabilization of Nitellainternodal Cell

In Nitella there is no cortical layer outside the plasmalemma of internodalcell. Hence it can be done by Simple method that is electrical pulse as adopted by Shimmen and Tazawa (1983). The present study on Cytoplasmic Streaming has been taken up in the internodal Cell of Nitella. The parameters selected here is the effect of temperature on the velocity of Cytoplasmic Streaming in both “intact cell” and the “permeabilizedcell”.

Procedure for the measurement of velocity of Cytoplasmic Streaming

Cytoplasmic particles of different sizes ranging from 0.5 micron to 10 micron were visible in the internodal Cell with a magnification (10X x 10X) while most of these move at roughly the same velocity. I chose to observe medium size particle for the determination of velocity of Cytoplasmic Streaming. For this purpose I used an ocular scale inserted in the place of one eye piece of microscope. With the help of a stage micrometer the scale of the ocular was calibrated for the magnification used. The time taken by selected Cytoplasmic particles for traversing along the scales of the ocular were recorded with the help of stop watch.

The temperature of the perfusion chamber was maintained by first insulating the chamber by thermocol cover around it and secondly by circulating water through a thermostatically controlled bath. The accuracy of temperature was kept around+- 1 degree centigrade.

3. Observation

According to my observations the cell was totally symmetric and homogenous in the distribution of Chloroplast and the movement of particles was not directional in the sense involving the Chloroplast. The situation may be other wise in rhizoids (Chloroplast free root Cells) and the nodal extremities of Characeancells. In these cases the activity of the actomyosin ATPase may indeed be coupled to active transport process at these locations. It is very interesting to mention some observations on the internodal cells where the countable number of Chloroplasts (10 to 100) are again without any rhyme and reason. Since the rotation is not around their own axis but along the periphery of the cell. F-actin bundles must be anchored at the only available fixed substratum i.e., the plasmalemma.

4. Result and Discussion

From the association of F-actin with the plasmalemma in several Eukaryotic cells one can come to the idea that ATP hydrolysis by actomyosin interaction in giant Characean cells could have a coupling with active transport process at their cell membrane. However, available evidence (Spudich et al 1985) of anatomical nature speak against this concept . F- actin fibres are known to be associated with Chloroplast towards the ENDOPLASMIC side. Hence, it is concluded that some useful process is being carried out by the actomyosin ATPase in the medium for which they offer no explanation. This proves that dispersed myosin some how participate in the movement of the beads. Naturally, the covalently bound myosin on the beads could also contribute to the same effect since their concentration on the beads is extremely high I;e, 10 to the 4 per beads (Spudich et al 1985).

The observation that Cytoplasmic particles do not tumble and rotate while they are moving speaks in favour of a global Streaming rather than the walking mechanism. In the latter model the motive force is thought to be localized at the surface of contact between the moving particle and the F-actin bundle. According to Nothangel et al (1982 b) the only conceivable way myosin coated Cytoplasmic bodies may cause Cytoplasmic Streaming is by the propulsion of ENDOPLASMIC by large number of these bodies imparting their momentum to the ENDOPLASMIC by viscous coupling .Sheetz and Spudich (1983) interpret their observations in terms of a walking of myosin coated beads along F- actin fibres.
Effect of temperature in intactNitella cell

The temperature of perfusion chamber was maintained and controlled by circulation of water through a thermostatically controlled bath.

A good correlation was found betweenrate of Cytoplasmic Streaming and the temperature. At 5 degree centigrade the velocity of streaming shows a fairly recordable level of about 8 to 10 um/ sec and by increasing the temperature up to 10 degree centigrade it shows a kind of of similar trend in the increase in velocity of Streaming. At the increase of further 5 degree centigrade I; e, at 15 degree centigrade temperature , the velocity however, rises to more than 40 um / sec. and shows the similar trend of increase in the velocity of streaming on the further increase of temperature .Thus the temperature dependece of velocity of Streaming is seen to be different above and below ~10 degree centigrade. The activation energy are estimated to be~ 10 k cal/ mol at more than 10 degree centigrade and 12 k cal/ mol at below 10 degree centigrade. Mustachich and Ware (1976) investigated the effect of temperature on Cytoplasmic Streaming by Laser Doppler Spectroscopy and found that the velocity varied linearly. It was also observed by optical microscopy by Kamiya(1959) and Tazawa(1968). Though my results differ from their's , the plot of my data as a function of temperature also nearly fit on a straight line with increasing rate of 2.76um/sec./ degree centigrade. This similarity is due to plotting the data in a narrow temperature range. Linearplots of velocity versues temperature predict that the flow should cease at about 2 to 6 degree centigrade but in my preliminary experiments, steady flow of protoplasm has been observed even at temperature below zero degree centigrade and it is supposed that the flow is able to continue as long as the protoplasm is not frozen. Thus, I believe that Arrhenius plots are preferable to linear plots for understanding Streaming mechanism.

Effect of temperature on Permeabilized Nitella Cell

The technique of permeabilization itself requires low temperature yet after resuming the Streaming it's velocity profile was recorded on further variation of temperature. There is not much appreciable change in the velocity of Streaming on shifting from 5 degree centigrade to 10 degree centigrade temperature. The maximum velocity remains around 20 to 25 um/sec. which is much lower than the normal velocity of Streaming. At temperature above 20 degree centigrade the Streaming ceases abruptly. This shows that due to increase of temperature the permeability of the membrane increaseswhich decreases the tolerance level of the cell and the Streaming therefore, is affected.

5. Conclusion

My observations support that the rotational Cytoplasmic Streaming in the internodal Cell of Nitella is prominent rather than walking of individual myosin coated particles. In intact cell of Nitella, increase in temperature appreciably increases the rate of Cytoplasmic Streaming. Thus the velocity of Cytoplasmic Streaming is seen to be temperature Dependent and different above and below 10 degree centigrade temperature. In Permeabilized Nitella Cell, the effect of temperature increases the permeability of plasma lemma which decreases the tolerance of the cell and that affects the Cytoplasmic Streaming.

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