Cytoplasmic streaming in cells | experiment

Cytoplasmic streaming is the cells transport system which moves a cells content around as required . This occurs in the cytoplasm of the cell, the fluid which fills the space between organelles and contains cell solutes. Movement in the cytoplasm is thought to be facilitated by actin-myosin motors (Britannica, 2012). These are molecules made up of the two proteins actin and myosin which interact to move solutes and even organelles around the cell. Long actin filaments line the cell and myosin molecules run along these filaments via active transport and attach to organelles such as endoplasmic reticulum (ribosomes) and mitochondria, transporting them around the cell along with the surrounding solutes in the cytoplasm (Kachar and Reece, 1988). The actin filaments run parallel to the direction of streaming in the cell.

Nitella is a genus consisting of various species of freshwater pond alga. These algae are weed like in appearance and have large rectangular cells observable by the naked eye. Each cell begins and ends at a node. Nitella species are thought to rely heavily on the actin-myosin protein motors to facilitate cytoplasmic streaming (Palevitz et al, 1974).

Cytochalasin are class of drugs derived from fungi (Turner, 1971). These drugs interfere with the interaction between actin and myosin by binding to the actin filaments that line the cell and reducing the capacity for the myosin molecules to bind (BIOL1004 Lab Manual, 2012). This then is thought to affect the rate of cytoplasmic streaming in the cell. This report will analyse the affect of two different types of Cytochalasin drugs (C and D) which are similar in structure but differ in the strength of the bonds they form with the actin filaments.

Aim

To observe cytoplasmic streaming in cells under the microscope and compare the effects of cytochalasin C and D on the speed of cytoplasmic streaming in Nitella cells via statistical analysis.

Methods and Materials

The method and materials used in this experiment is outlined in the: BIOL1004: Molecular and Cell Biology â€" Practical Manual (2012) on pp. 54-55 written by the Research School of Biology for the Australian National University.

The following deviations from the aforementioned method are noted:

The width of each cell was measured for comparison rather than the length as the cells were far too long to fit within the microscope field of view.

Results and Statistical Analysis 500

The average width of the cells was calculated at 183. 9um.

The table above summaries the important statistics calculated from the experimental data. Each data set appears to be comparative to each other, however it is noted that cells 1. 2, 3. 2 and 4. 2 have higher than average variances which indicates a possible deviation from normal distribution.

Figure 1 presents graphically the speed of streaming against the width of the cell. The linear trend line indicates a slight negative relationship between cell size and streaming speed.

Table 2 summarises the important statistics of each of the test groups. High variance values for the test groups other than the control group indicate a deviation from normal distribution, however the numbers are similar and thus the data sets are comparable. The results indicate that upon addition of both cytochalasin drugs the rate of cytoplasmic streaming fell from that observed in the control test. The recovery test taken after flushing the Cytochalasin from the slide with pond water indicates the rate is increasing, but has not quite reached the rate as observed in the control test.

Table 3 summarises the values calculated for a number of t tests performed to assist in analysis of the data. From this it can be concluded that we can have no less than 98% confidence that the rate of steaming with the addition of both cytochalasin drugs is significantly different from the normal or control streaming rate. These t tests also give an indication that the size of the cell influences the rate of streaming as the difference in speed between cells 3. 2 and 4. 2 which have the same recorded width has been determined not to be significant, while the difference in speed between the biggest and smallest cells is significant. However, these results are not consistent when comparing different data sets both of similar widths and of different widths.

Discussion

As summarised in the results the change in the rate or speed of cytoplasmic streaming in the Nitella cells upon the addition of the drugs Cytochalasin C and D was significantly different to that of the control rate, with 98% and 99. 9% confidence respectively. This is as expected. However as noted in Table 2 this change in speed was observed as a deceleration on the addition of both drugs. A deceleration of cytoplasmic streaming is expected with the addition

of cytochalasin D, which is a well documented actin inhibitor. The drug binds to the actin filaments and changing the secondary structure and inhibiting the actin-myosin interactions (Binder and Tamm, 2003). However, even though cytochalasin C has a similar shape, it does not bind to actin as tightly as cytochalasin D (BIOL1004: Lab Manual, 2012) and does not have the same affect on actin-myosin communication. A study in the Plant Cell Physiology journal documented that cytochalasin C had no real affect on the rate of cytoplasmic streaming even at very high concentrations (Foissner and Wasteneys, 2007). Thus the deceleration with the addition of cytochalasin C, as change that has been confirmed as significant via statistical analysis is not the expected result. It is possible that the deceleration of cytoplasmic streaming in this case could be due to the differing salt concentrations between the pond water and the cytochalasin C in solution. As Nitella is normally found in freshwater that is hypotonic, the replacement of the normal pond water on the slide with the drug in a hypertonic solution (with respect to the pond water) may have inadvertently caused the cells to change their osmolarity to compensate (Ladgies et al, 2010). A study has shown that transcellular osmosis in Nitella species can cause inhibition of cytoplasmic streaming (Tazawa et al, 1993). Further support for this explanation comes from the increase in streaming rate on recovery from treatment with the drug, where the hypertonic solution was flushed away and replaced with isotonic pond water (with respect to the cells). It should be noted that the statistics show there is 90% confidence that the difference between the rate of streaming with the addition of each drug respectively was significant, thus it follows that the cytochalasin D was

a much stronger inhibitor to the rate of streaming than that which caused the deceleration in the cytochalasin C test.

The relationship between width of the cell and rate of streaming is not quite clear. Figure 1 appears to indicate that there is a negative relationship between the two, thus as cell width increases the rate of streaming would decrease. However, the statistics provided by the t tests were inconclusive as to whether the differences between streaming rate for various sized cells were significant or not. If it were true that large size indicated a slower rate of streaming, then it should follow that all t tests between data sets of cells with similar widths would indicate a non-significant difference, however only one of the three tests yields this result. Similarly, the t tests between the data sets of different sized cells should indicate a significant difference; yet again only one out of the three tests yields this result. Further analysis with a larger data set could be required to confirm the trend observed in figure 1.

Therefore this experiment has demonstrated the decelerating effect of cytochalasin D on the rate of cytoplasmic streaming in Nitella cells due to its inhibition of the actin-myosin motor and has indicated a possible link between changing osmolarity and relative rate of cytoplasmic streaming in Nitella cells.