

 <p>ISSN NO. 2320-5407</p>	<p>Journal Homepage: -<a href="http://www.journalijar.com">www.journalijar.com</a></p> <h2>INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)</h2> <p>Article DOI:10.21474/IJAR01/8832 DOI URL: <a href="http://dx.doi.org/10.21474/IJAR01/8832">http://dx.doi.org/10.21474/IJAR01/8832</a></p>	 <p>INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR) ISSN 2320-5407 Journal Homepage: <a href="http://www.journalijar.com">http://www.journalijar.com</a> Journal DOI:10.21474/IJAR01</p>
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### RESEARCH ARTICLE

#### MECHANISMS OF PATTERN FORMATION OF FBP17 IN MAST CELLS.

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#### Manuscript Info

##### Manuscript History

Received: 06 February 2019

Final Accepted: 08 March 2019

Published: April 2019

#### Abstract

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#### Introduction:-

The distribution of FBP17 within the cell is known to exhibit time-varying behaviour such as spatiotemporal patterns or waves. FBP17 waves are linked to the waves of other proteins and lipid-derivatives, e.g. actin, N-WASP, Cdc42, PI(3)P etc. In fact, perturbing FBP17 waves causes changes in the waves of the proteins listed above. Thus, understanding the mechanisms behind FBP17 waves and manipulating FBP17 waves could be an effective way to simultaneously affect many subcellular systems. The binding of FBP17 to the cell membrane is affected by membrane curvature, and thus a natural question would be whether FBP17 waves are affected by the cell curvature. This hypothesis is not well investigated in literature, hence, the goal of this research will be to evaluate the truth of this hypothesis.

#### Experimental Set-up

Firstly, a variable dose of Cytochalasin D (CytoD) was introduced to change the peripheral curvature of cells. CytoD is an inhibitor of actin polymerisation, and it weakens cellular cytoskeleton causing adoption of a spherical liposomal shape. The resulting cell shape was characterised using Spinning-Disk Confocal Microscopy, and Surface Reflection Interference Contrast Microscopy (SRIC). This procedure was optimised for the dose of CytoD.

Total Internal Reflection Fluorescence Microscopy (TIRF) was used to obtain videos of the waves, and they were analysed using kymographs made in ImageJ to determine wave properties such as frequency, wavelength and speed. Finally, Mathematica was used to plot spatial intensity distribution graphs of each point within each cell. This was used to determine if FBP17 waves are curvature-sensitive.

#### Results:-

Firstly, Confocal and TIRF Microscopy confirmed that increasing the concentration of CytoD causes the cells to adopt a more spherical shape. However, cells treated with 20uM of CytoD did not exhibit waves. Thus, intermediate concentrations of CytoD (2uM and 4uM) were chosen for further experimentation.

Before the addition of CytoD, the cell had numerous, chaotic, waves that travelled in many directions without a clear wavefront. However, the addition of 2uM and 4uM of CytoD caused the waves to be less chaotic, with only 1 or 2 waves per cell. A clear wavefront was present. CytoD reduced the number of wave initiation sites, and thus acted like a global inhibitor of FBP17 waves. This supports the activator-inhibitor model for wave initiation.

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The addition of CytoD also affected the period of the FBP17 wave. Cells that were not treated with CytoD had a period of 26.4s (SD = 7.58s), while the period of the wave for cells treated with 2uM of CytoD was 46.2s (SD = 9.87s). Interestingly, it was observed that actin waves act as a refractory period between 2 consecutive FBP17 waves. Since CytoD is an inhibitor of actin polymerization, it causes the period of actin waves to increase and thus causes the period of the FBP17 waves to increase as well.

A Mathematica code was written to process the TIRF videos and plot graphs of the normalised intensity of each point within the cell against the normalised distance from the edge of the cell. The data for each cell was fitted to a line. For cells treated with 0uM, 2uM and 4uM, the mean gradient of the lines significantly exceeds zero, showing that the intensity of the wave is brighter at the centre of the cell, where cell curvature is lowest. However, there is no statistically significant difference in the mean gradient of the lines when the graphs for cells treated with 0uM, 2uM and 4uM CytoD were compared, indicating that the change in curvature when 2uM and 4uM CytoD was added was not sufficient to cause observable change in FBP17 waves.

### **Conclusion:-**

Firstly, Cytochalasin D causes cells to adopt the shape of a hemispherical cap, and functions as a global inhibitor of FBP17 waves. This supports the activator-inhibitor mechanism to explain the initiation of the wave. CytoD increases the mean period of waves, providing evidence that actin and FBP17 waves are coupled. The results also demonstrate the FBP17 waves are affected by cell curvature. Finally, the use of CytoD can be coupled with other methods to further probe spatiotemporal waves in cells.