SRF VACATION SCHOLARSHIP REPORT 2016

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Degree Title and year of study:	Natural Sciences, BA Degree (Honours, 3 years) Second Year		
Supervisor's Name:	Dr. Tim Weil	**Supervisor's Department and Institution:**	Department of Zoology, University of Cambridge
Project Title:	Investigating the Requirements for Egg Activation in *Drosophila*		

Briefly outline the background and aims of the project *(max 200 words)*

Egg activation is the release of an egg from arrest in meiosis and the beginning of a host of developmental events such as cytoskeleton rearrangement and mRNA translation, leading to the onset of embryogenesis. In *Drosophila*, it has previously been shown that a calcium wave accompanies egg activation which has been linked to the translation of *bicoid (bcd)* mRNA.

My work primarily focused on testing the required ionic composition of a solution for egg activation. I examined two solutions: Activation Buffer (AB), shown to activate oocytes *ex vivo*, and Schneider's insect medium, a standard medium that does not lead to egg activation.

My second aim was to explore the role of intracellular calcium in the translation of *bcd* mRNA. I used the novel Translating RNA Imaging by Coat protein Knock-off (TRICK) system to image the pioneer round of translation. Flies with the *bcd*-TRICK construct were crossed to those expressing a genetically-encoded calcium sensor (GCaMP) to image the calcium wave and translation together.

Understanding how egg activation is coupled to downstream events such as mRNA translation will develop our understanding of events in embryogenesis applicable to a wide range of organisms, including humans.

Did the project change from that proposed in the application? If so, what changes were made and why? *(max 100 words)*

The original aim was to test the mechanism(s) of mRNA translation at egg activation asking whether the calcium wave activates mRNA translation in a direct or an indirect manner. Due in part to technical challenges associated with TRICK imaging and new experimental interest, I spent the majority of my placement testing the required ionic composition of solutions for egg activation.

What were the main results/findings of the project? *(max 300 words)*

1. Determining the requirements for the calcium wave.
Conflicting results from previous work has (1) suggested that removing sodium or potassium ions from AB reduces the efficiency with which AB activates stage 14 *Drosophila* oocytes, (2) shown that a solution consisting of only sucrose and water, between 250-400 mOsm, will activate oocytes. I aimed to resolve this discrepancy by building solutions of varying composition to test the requirement of sodium and potassium ions for activation. To observe activation, the calcium wave was monitored with a genetically-encoded calcium sensor (GCaMP).

I show that a solution of sucrose in water made up to the same osmolarity as AB (260mOsm) activated oocytes with great efficiency (257mOsm, 81.1% waves), as did a solution of 50mM KCl (246mOsm, 78.4% waves). However, a solution of 50mM NaCl activates oocytes with considerably lower efficiency (248mOsm, 36.4% waves). The difference between the KCl and NaCl solutions is statistically significant (Fishers exact test, p=0.0296), and warrants further investigation. Knowing the requirement of certain ions will point us towards the membrane channels involved in ion fluxes at activation, and goes towards building a detailed mechanism.

2. **Investigating the relationship between the calcium wave and translational activation.**
Using flies expressing both *bcd*-TRICK and GCaMP, I attempted to visualise the first round of translation post-activation in concert with the calcium wave. The imaging was technically challenging, as it involved real-time manipulation of a single oocyte at 100x magnification on a confocal microscope. The main difficulty was keeping the oocyte from moving out of the plane of focus after addition of a solution. Although there was not time to test this, for future work I suggest immobilising oocytes, perhaps by constructing a small glass well to sit them in.

What do you conclude from your findings? *(max 150 words)*

My results show that external sodium ions in a solution at an osmolarity that supports activation reduces the propensity of the calcium wave at activation. In contrast, external potassium ions in a solution added to the egg chamber show the same likeliness of a calcium wave as AB. It is interesting to compare the composition of the aforementioned solutions: AB and Schneider's. AB has a relatively high concentration of potassium compared to sodium, and the opposite is true of Schneider's, which fit with my results. This suggests the involvement of sodium and potassium ion channels at activation. Further investigation should include testing TRP channels and the sodium channel family DEG/ENaC (specifically *rippled pocket*).

My attempts to troubleshoot the TRICK protocol have demonstrated that a method of immobilising the oocytes during imaging is essential for the TRICK system to act successfully in *Drosophila*, and this will apply to other systems.

How has this experience influenced your thinking regarding your future/ongoing studies, and/or career choice? *(max 150 words)*

This summer studentship has been invaluable in preparing me for post-graduate eduction. I was able to independently pursue questions that interested me, and working around the challenges I encountered was extremely rewarding. Moreover, exposure to primary literature and genetics, as well as technical skills such as dissection, microscopy, immunostaining and fly management, have prepared me for further research aspirations. I can say with confidence that I will seriously consider continuing research as a masters or PhD student.

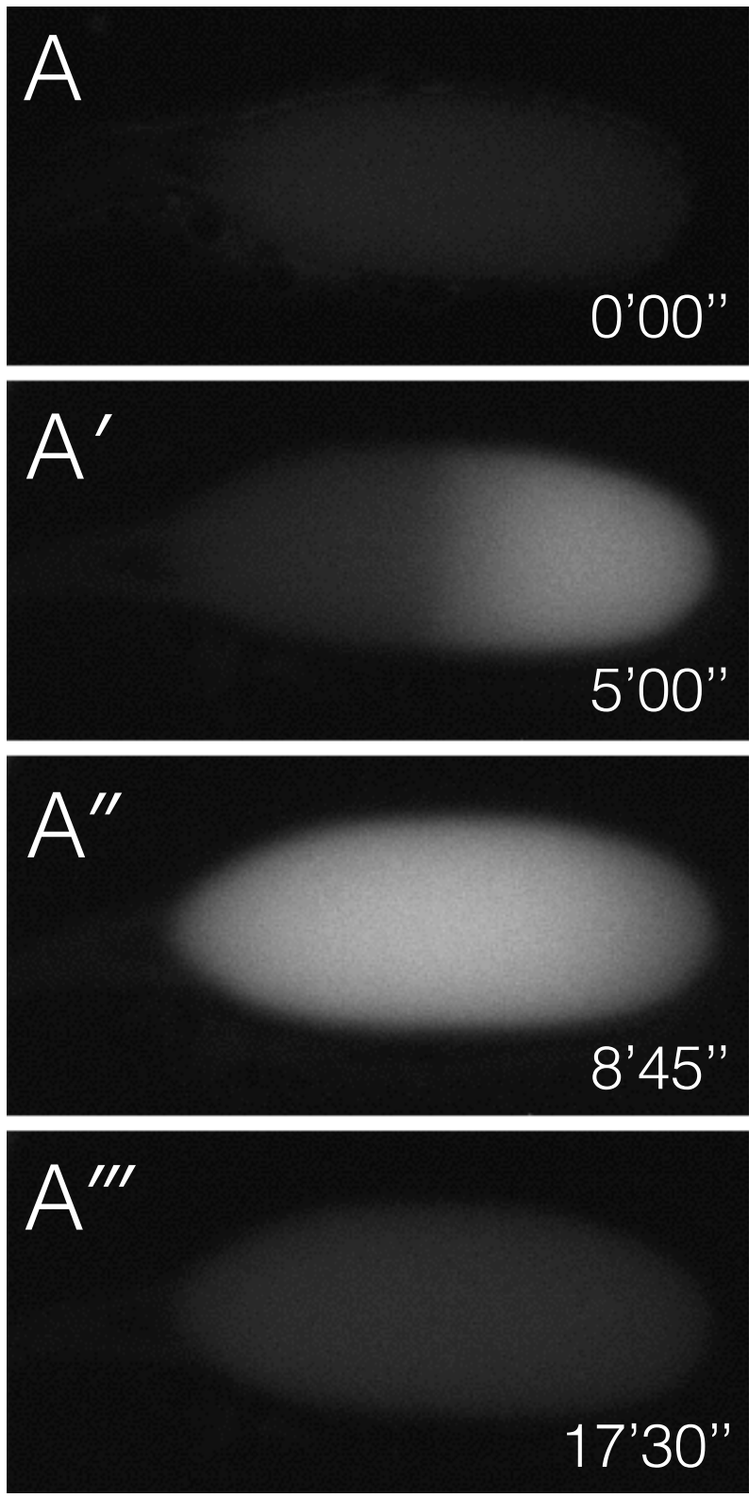

Figure 1. Calcium wave at *Drosophila* egg activation.
AB is added to an oocyte expressing the calcium sensor GCaMP. **[A]** 0’00’’ Prior to activation background levels of fluorescence are observed. **[A’]** 5’00’’ post addition of AB. Wave initiates from posterior pole. **[A”]** 8’45’’ post addition of AB. Wave has fully propagated across the oocyte. **[A”’]** 17’30’’ post addition of AB. Calcium has recovered to background levels. In all panels the posterior of the oocyte is on the left.