Assignment 1: Research Protocol

Name of student: ##### ###
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Title of Project: Do *C. elegans* experience rigor mortis?

Background and rationale for the project:

Much has been learned about the biology of ageing in the nematode worm *Caenorhabditis elegans* yet very little is known about the mechanism of the terminal stage in this process; organismal death. It is hoped that a greater understanding of organismal death will allow identification of mechanisms of ageing that precede it. Far from being a chaotic and uncontrolled process, recent findings suggests that death in *C. elegans* is regulated and programmed. The work of Coburn et al (2010) has shown that death is driven by a wave of cellular necrosis in the intestine and is accompanied by a burst of blue fluorescence termed death fluorescence. This wave is propagated by calcium signalling.

Rigor mortis is a death related process known to occur in mammals that is also dependent on calcium. In skeletal muscle, a reduction in ATP concentration causes calcium pumps in the sarcoplasmic reticulum to cease functioning. Consequently calcium floods into the sarcoplasm resulting in muscle contraction due the formation of rigid non-covalent bonds between the myosin heads of the thick filaments and the actin subunits of the thin filaments of the sarcomere (Jeacocke, 1993). It is unknown what triggers the necrotic cascade in intestinal cells of *C. elegans* however it has been observed that death fluorescence originates in anterior cells adjacent to the pharynx, a large muscular organ. This raises the interesting possibility that *C.elegans* actually experience rigor mortis before death and the associated calcium release in the pharynx is what drives cellular necrosis in the intestine. An alternative theory is that rigor mortis in the pharynx results in a rise in pressure which places the intestinal cells under osmotic stress. It is already known that osmotic stress can induce cellular necrosis in the intestine of *C. elegans* (Luke et al, 2007).

Ultimately, the key question under consideration in this project is whether *C. elegans* does experience rigor mortis. If so, rigor mortis may be a promising candidate in the search for the trigger of cellular necrosis in the intestinal cells.

Experimental aims:

1. To examine whether worms exhibit a reduction in size immediately prior to death fluorescence.

2. To test whether death associated shrinkage can be enhanced or supressed by drugs that increase or decrease sarcoplasmic free calcium levels.

3. To identify any genetic mutations that cause rigor mortis to be supressed in *C. elegans.*

Experimental Design:

The model organism used in this project will obviously be *C. elegans*. Stocks of wild type *C. elegans* are readily available whilst specific mutant strains can be ordered from the Caenorhabditis Genetics Centre at the University of Minnesota.

This project will mainly involve microscopy work. In order to ascertain whether worms shrink prior to death, young adult wild-type worms will be killed by acute exposure to tert-butyl hydroperoxide (TBH). Time lapse photography of worms will be carried out as they die and will then be analysed by manually tracing the length of individual worms using a graphics tablet. Measurements will be

taken at regular intervals before and after the point of death which is indicated by a peak in brightness of death fluorescence. All measurements will be made using the Velocity Quantitation software package. Data from individual worms will be normalised and combined allowing the identification of any general trend. To show that any shrinkage is associated with death, rather than simply TBH exposure, a control will be carried out with a group of worms that are incubated in a non-lethal dose of TBH. If shrinkage is linked to death it should not be observed in this group.

Assuming that death-related shrinkage is observed, the same experimental procedure will be repeated for a different group of wild-type worms who have been incubated in the ionophore ionomycin. Another group will be incubated in ethylene glycol tetraacetic acid (EGTA). For these experiments negative controls will be carried out consisting of wild-type worms incubated in buffer solution only. Finally the experimental procedure will be repeated on strains of worms with specific mutations known to affect sarcomere assembly. Here, a negative control will be performed using wild-type worms. Ideally the sample sizes used will be as large as is possible given the time available to ensure that any statistical analysis is valid. For each experiment, an absolute minimum of 40 worms will be sampled.

Justification:

If worms are found to shrink before they die this would be the first indication that rigor mortis may be occurring. A reduction in whole body length would suggest a contraction of the longitudinal body-wall muscle. If this easily observable muscle is contracting it is possible that the pharynx is also contracting. The subsequent experiments are designed to test whether this death-related shrinkage is actually rigor mortis. Ionomycin is a substance that increases free calcium levels in the sarcoplasm whereas EGTA is a calcium chelater that has the opposite effect. If shrinkage is enhanced in worms treated with ionomycin and inhibited in worms treated with EGTA it suggests that changes in intracellular calcium levels are implicated. Finally, if death-related shrinkage is impaired in worms that carry mutations preventing proper sarcomere assembly this would provide further evidence that the reduction in length is caused by muscle contraction rather than some alternative factor such as dehydration.

Home office and ethical approval: Neither are necessary for this project.

References:

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Jeacocke, R.E. (1993) The concentrations of free magnesium and free calcium ions both increase in skeletal muscle fibres entering Rigor mortis. Meat Sci 35, 27-45.

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