Cellular Biology of Engulfment in *Drosophila melanogaster* hemocytes

Amara Saha and Dr. Adam Williamson

Biology Department , Bryn Mawr College

Abstract

Engulfment is the process of removal of cells that are dead, injured or invasive by a group of cells specialized to do so. However, many of the mechanisms of this complex and vast process are unknown. In my research, I studied, through primary literature review, the core biological workings of engulfment in the model organism *Drosophila melanogaster* or the fruit fly. Phagocytosis in *Drosophila* is mediated by an engulfment receptor called Draper, which is expressed by engulfing cells like hemocytes to detect targets to be eaten. This receptor is the fly homolog to other important receptors such as Ced-1 in worms and Megf10 in mice that also phagocytose cells. This research, in collaboration with my lab peers, shows that engulfment processes in these organisms in conserved, as many similar mechanisms relating to these receptors were observed. However, certain dissimilarities were also noticed, which account for the species-specific workings of the receptors. The learnings from this research are being used to develop newer strategies to program cells to force eat specific targets, thereby advancing therapeutics for various diseases.

Introduction

Engulfment is a process vital for survival of organisms and this can be achieved by both professional phagocytes like hemocytes that are programed to do so, or non-professional phagocytes like glia or epithelial cells when the need arises. The Draper receptor in fly is known to detect target cells for phagocytosis. It has two sites where tyrosine residues can get phosphorylated, performed by a kinase called Src42A. This phosphorylation of Draper then allows it to be recognized by molecules such as Shark that activate it for further downstream signaling processes.

Draper recognizes and interacts with ligands presented by target cells, after which cytoskeletal remodeling takes places to internalize the targets. There are a number of accessory molecules involved in Calcium homeostasis, actin dynamics, etc. that have also proven to play roles in Draper-mediated phagocytosis. There is also growing evidence towards similarities of Draper engulfment with the Ced pathway in worms. Our collective findings indicate that the cellular workings if engulfment in worm, fly and mice may be conserved.

Methods

To begin researching, a simple PubMed search was conducted by inserting “draper AND drosophila” into the search bar to obtain papers relating to the receptor draper. After a list of these papers were acquired, all the abstracts were thoroughly read, and an informed decision about which papers to read was made. Thus, the total list of papers was narrowed down to only the most relevant ones. The focus here was to read more primary research articles. While reading these papers, extensive notes were taken to highlight key results from the experiments conducted.

In addition, to gather more papers, another tool called Web of Science was used. An important paper that I found interesting could be inserted into the text bar and a list of papers that cited it would be seen. This was used to fill in information and see newer work regarding the same topic.

Once several principal articles were read and their fundamental results noted, a draft on the summary of engulfment on the fly was written up. This will later be used in the review article.

Results

Draper is shown to be vital for engulfment as its expression levels increase in the presence of a target such as apoptotic cells[1]. Its phagocytotic activity is also seen in glia to clear Aβ plaques that cause Alzheimer's disease[2].

The Draper receptor has been shown to recognize certain molecules acting as ‘eat me’ signals called ligands that are presented on the surface of target cells that may be pathogens, injured or dead. For example, the extracellular region of Draper binds to Phosphatidylserine[3], Pretaporter[4], DmCaBP1[5] on apoptotic cells and Lipoteichoic Acid[6] on invading bacteria. This helps in bridging the target to the engulfing hemocytes. Its intracellular region contains NPxY and YXXL domains[7] with the two tyrosine residues that are subject to phosphorylation by kinases. One such kinase is Src42A[8], considered to be a ‘writer’ capable of activating Draper. This phosphorylated version of Draper is subject to recognition by Shark, a non receptor kinase containing an SH2 domain that can ‘read’ such a modification, allowing for further downstream signaling processes to take place.

To prevent a continuous activation of Draper, phosphatases such as Corkscrew[9] dephosphorylate the Tyrosine residues to return it to its resting state, thereby seemingly ‘erasing’ the phosphorylation modification. Thus, an established network of kinases, SH2 proteins and phosphatases aids in regulating Draper activity. Such a network can also be called a network of writers, readers and erasers[10] respectively. In addition, there are several accessory molecules that act in relation to Draper to regulate phagocytosis. For example, D-SCAR and D-WASp[11] are proteins that overlook actin dynamics and their absence lead to defective Draper-mediated engulfment.

Figures

Engulfment signaling modules used by phagocytes across phyla

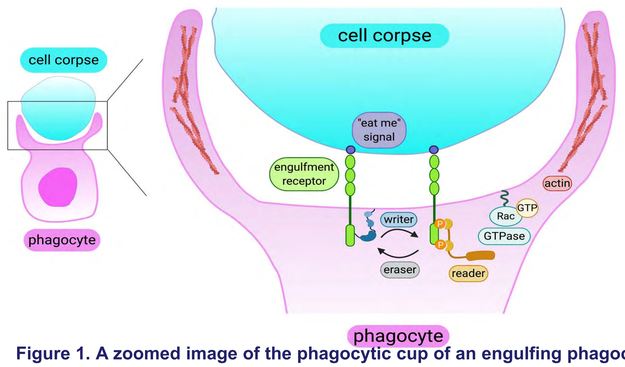

Figure 1. A zoomed image of the phagocytic cup of an engulfing phagocyte surrounding a target cell. The dead cell presents an eat me signal or ligand on its surface which is recognized and bound to the external region of receptor (Draper). The writer adds two Phosphate groups to the intracellular region, which is recognized by a reader. The eraser removes the phosphate groups to retrieve the inactivated form. Additional downstream processes lead to Rac GTPase activation that influences actin dynamics. Actin remodeling takes place in order to internalize the cell corpse for degradation in the phagolysosome. Figure by Dr. Adam Williamson

Element	Molecule	Function
Reader	Shark	SH2 domain recognizes phosphorylated tyrosine
Writer	Src42A Kinase	Phosphorylates Tyrosine residues
Eraser	Corkscrew Phosphatase	Dephosphorylates Tyrosine residues

Table 1. The Reader-Writer-Eraser framework within the fly system and its functions.

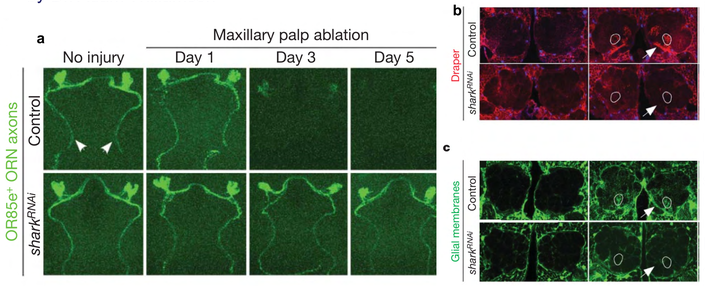

Figure 2. Significance of Shark in engulfment. (a) On injury, debris are cleared within 5 days, however, in flies lacking Shark, debris remain in the CNS. (b) Site of injury sees increased levels of Draper expression in control, but no Draper is recruited in flies lacking Shark. (c) Injured site also shows increased levels of Shark in control flies but not Shark mutants, indicating its role in Draper-mediated clearance. From Ziegenfuss, Jennifer S., et al. (8 in references)

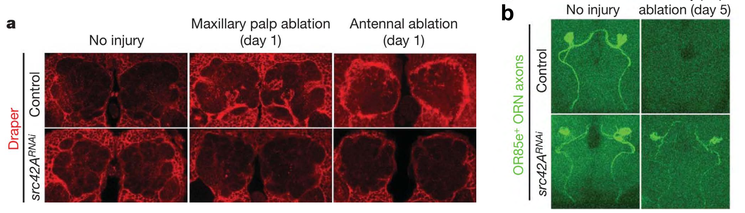

Figure 3. (a) Draper levels in injured axons in control vs Src42A lacking flies and (d) debris clearance control vs Src42A lacking flies. (a) On injury to the maxillary palp, control axons recruit Draper to engulf the debris. However, in flies lacking Src42A kinase, Draper is not extensively recruited. (b) In control flies, injured axons are cleared after 5 days but in those lacking Src42A, damaged axons remain in the CNS. Both these data imply the importance of Src42A in effective engulfment of damaged axons in relation to Draper. From Ziegenfuss, Jennifer S., et al. “Draper-Dependent Glial Phagocytic Activity Is Mediated by Src and Syk Family Kinase Signaling.”

Discussion

From discussions with my lab peers, it seems as though there are similarities in the way engulfment receptors work in worm, fly and mouse. Phosphatidylserine is known to bind to all three receptors. The reader-writer-eraser framework is believed to exists in all three organisms to regulate receptor activity. For example, MegF10 in mice is phosphorylated by Src kinases and recognized by shark as in fly. In addition, there is growing evidence of Draper following the Ced pathway in worms. It can be suggested that although these receptors have their own respective signaling pathways, they all converge at cytoskeletal remodeling and follow identical actin dynamics for internalization.

Conclusions

The existence of a complete reader-writer-eraser framework in Drosophila allows for mediation of Draper-driven engulfment where these key molecules work closely. This results in initiation of signaling pathways that further navigate engulfment. Learnings from this project will be utilized to write a review article on engulfment processes across different phyla.

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