Single cell wound healing in Drosophila melanogaster embryos

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Abstract

The role of contractile actin and myosin filaments in wound healing is a topic of great importance and studies in vertebrate systems has been valueable (Razzel, et al. 2011). For identifying specific proteins involved recent studies of the fruit fly, Drosophila melanogaster, suggest embryos are a valuable model for what is occurring at the unicellular level during wound-healing (Kiehart, et al. 2000). Early in development they are referred to as syncytial embryos, characterized by the presence of multiple nuclei from the division of nuclei in a common cytoplasm. Both skeletal muscle and the fruit fly embryo are examples of a syncytial structure. Much of what is known about single cell wound healing has been determined from the morphogenetic process known as dorsal closure. Dorsal closure is a valid model for wound healing because a comparable actin-myosin network is active in both the epidermal cells of Drosophila during dorsal closure and human epidermis during wound healing (Razzel, et al. 2011). Different organisms likely occur throughout different phases of dorsal closure, and may correlate to different cellular events regulated by cytoskeletal proteins. Determining orientations of the proteins in the actin-myosin ring structure at its connections along the wound’s edge will offer a better understanding of the exact involvement of various proteins in wound healing.

Introduction

Wounds are a part of life for any organism. Disruptions of cell structure make the ability for single cells to heal various wounds and injuries is integral to maintaining health. Wound healing is a topic of great importance and studies in vertebrate systems has been valueable (Razzel, et al. 2011). For identifying specific proteins involved recent studies of the fruit fly, Drosophila melanogaster, suggest embryos are a valuable model for what is occurring at the unicellular level during wound-healing (Kiehart, et al. 2000). Early in development they are referred to as syncytial embryos, characterized by the presence of multiple nuclei from the division of nuclei in a common cytoplasm. Both skeletal muscle and the fruit fly embryo are examples of a syncytial structure. Much of what is known about single cell wound healing has been determined from the morphogenetic process known as dorsal closure. Dorsal closure is a valid model for wound healing because a comparable actin-myosin network is active in both the epidermal cells of Drosophila during dorsal closure and human epidermis during wound healing (Razzel, et al. 2011). Different organisms likely occur throughout different phases of dorsal closure, and may correlate to different cellular events regulated by cytoskeletal proteins. Determining orientations of the proteins in the actin-myosin ring structure at its connections along the wound’s edge will offer a better understanding of the exact involvement of various proteins in wound healing.

Materials and Methods

Embryos underwent laser microsurgical wounding, followed by a high pressure rapid freezing in halocarbon oil and freeze substitution. Wounding and freezing were done in the lab of our collaborator, Susan Parkhurst of the Fred Hutchinson Cancer Research center in Seattle, WA. Embryos were embedded in Epon-Araldite resin in our laboratory at the University of Puget Sound. After polymerization embedded embryos underwent a trimming procedure before sectioning for light and transmission electron microscopy.

Results

Subcellular structures had difficulty surviving the freezing process and remaining intact for analysis.

The hypothesized actin filaments were not discovered however other structural similarities to dorsal closure were found.

Embryos are well preserved. Although we are at the beginning of analysis, examined wounds are early in the healing process, denoted by the presence of vesicles.

Sequential sectioning should reveal more advanced stages of healing.

Conclusions

Additional examination is necessary to obtain consistent results and make conclusions.

Future Directions

Acquisition of sapphire specimen holders may better sustain structural integrity during HPRF. The Parkhurst lab is now inserting a genetic miniSOG tag into various proteins such as actin, myosin and e-cadherin. Analysis using the insertion allows protein fluorescence at the light microscopy level and is also resolvable using TEM. Better analysis of a wider variety of proteins could also be conducted through tandem antibody staining with attached colloidal gold particles.

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Works Cited


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Figure 2. A) Photo of unwounded Drosophila embryo preserved by HPRF/FS at the Fred Hutchinson Research Center, taken for proposed research project using TEM. Arrow indicates the integrity of subcellular structures after the freezing process, in this case microtubules (2/25/12) B) Photo of Drosophila embryo during dorsal closure taken by Professor Rickoll using TEM. The arrow indicates actin arranged perpendicular to the wounds leading edge. C) Cross section of Drosophila embryo taken by Professor Rickoll using TEM. The arrow indicates a bundle of actin arranged parallel to the wounds leading edge. D) Schematic diagram of the single cell wound repair process. Upon plasma membrane disruption, a Ca influx triggers internal vesicles to fuse with each other and form a membrane patch. This “patch” fuses with the plasma membrane at specific sites along the periphery of the disruption. Membrane resealing is followed by a process of plasma membrane and cortical cytoskeleton remodeling (adapted from Minciel, et al).

Figure 4. A) Photo of unwounded early embryo showing structural damage from freezing process. (100x) B) Photo of single wounded embryo showing structural damage and expansion of wound from freezing (100x) C) Transmission electron micrograph of early single wounded embryo at wound site(3000x). D) Transmission electron micrograph of early single wounded embryo showing multivesicular bodies at wound site (2000x). E) Transmission electron micrograph of early single wounded embryo showing filopodial bodies, which are commonly found in the cytoskeleton (12000x).

Figure 3. A) Photo of early embedded Drosophila embryo with two wounds, one posterior, one anterior (40x). B) Photo of control early unwounded embryo post embedding (40x).