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Trynity models a tube valve in the *Drosophila* larval airway system

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Abstract

Terminal differentiation of an organ is the last step in development that enables the organism to survive in the outside world after birth. Terminal differentiation of the insect tracheae that ends with filling the tubular network with gas is not fully understood at the tissue level. Here, we demonstrate that yet unidentified valves at the end of the tracheal system of the fruit fly *Drosophila melanogaster* embryo are important elements allowing terminal differentiation of this organ. Formation of these valves depends on the function of the zona pellucida protein Trynity (Tyn). The tracheae of *tyn* mutant embryos that lack these structures do not fill with gas. Additionally, external material penetrates into the tracheal tubes indicating that the *tyn* spiracles are permanently open. We conclude that the tracheal endings have to be closed to ensure gas-filling. We speculate that according to physical models closing of the tubular tracheal network provokes initial increase of the internal hydrostatic pressure necessary for gas generation through cavitation when the pressure is subsequently decreased.

Keywords: organ differentiation, organ communication, cuticle, trachea

Introduction

Organs or organ systems often consist of multiple structures that act in concert and are crucial for their function. Effective respiration, for instance, in vertebrates implies the coordinated function of the nose including nasal valves and the lung composed of muscles and lung epithelial cells. Likewise, gas-exchange in insects occurs in their breathing organ, the tracheal system that is a network of tubes ending at distinct sites in the epidermis called spiracles. To avoid unnecessary water loss, often the spiracles are closed (Quinlan and Gibbs, 2006). They open when the partial pressure of carbon dioxide rises to a threshold value. In the fruit fly *Drosophila melanogaster* larval spiracles consist of a chamber and the filzkörper that connects the tracheal tubes with the spiracular ending. Several genes have been shown to be needed for correct spiracle formation. Among others a cascade of transcription factors including Hox proteins, Empty spiracles (Ems) and Spalt (Sal) govern overall organ morphogenesis (Castelli Gair Hombria et al., 2009; Hu and Castelli-Gair, 1999). Despite the detailed description of this process, the mode of function of spiracles and their importance for fly survival are understudied.

Tracheal gas-filling in *D. melanogaster* involves coordinated function of the tracheal epithelial cells and muscles (Wang et al., 2015a). Initially, the tracheal lumen that is filled with chitin and proteins that are required for tube diameter regulation (Devine et al., 2005; Luschnig and Uv, 2014; Tonning et al., 2005; Wang et al., 2006). This