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Time-specific regulation of airway clearance by the *Drosophila* J-domain transmembrane protein Wurst

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ABSTRACT

At the end of embryogenesis, *Drosophila* and mammalian airways convert from liquid-filled to air-filled tubes. This process is regulated by Clathrin-mediated endocytosis. However, these molecular mechanisms are poorly understood. In *Drosophila*, the DnaJ transmembrane protein Wurst interacts with Clathrin and Hsc70 to mediate early steps of endocytosis. Wurst is expressed in epithelial tissues from early stages onwards. Here we show time- and tissue-specific requirement of Wurst in airway liquid-clearance and air-filling. RNAi experiments demonstrate that Wurst activity is specifically required at the final stage 17 of embryogenesis. Furthermore, we show that the apical membrane organizer Crumbs regulates Wurst-mediated airway liquid–air-transition.

1. Introduction

Higher organisms develop respiratory systems for effective oxygen delivery. The human lung comprises a complex network of tubular branches that carries oxygen to the alveoli for diffusion into the blood system and distribution into tissues [1,2]. Throughout fetal development, the lung epithelium secretes fluids into tube lumina for proper lung size development. Later on during birth, the lung epithelium switches from net secretion to net absorption. The resulting airway liquid clearance is critical for respiration of newborns [3]. Defects in the clearance process can lead to clinical syndromes, such as the neonatal respiratory distress syndrome (nRDS) and the transient tachypnea of the newborn (TTN) [3,4]. Additionally, it has been suggested that airway liquid clearance and edema liquid clearance may involve similar mechanisms [5]. However, the molecular mechanisms that force airway and edema clearance are poorly understood.

Insects and mammals share several organizational and molecular features for branching morphogenesis, such as conserved growth factors and signaling cascades to regulate lung and tracheal development [1,6]. The *Drosophila* tracheal system arises from clusters of ectodermal cells that invaginate at mid-embryogenesis and subsequently form a ramified network of tubular branches [1]. During embryogenesis, tracheal cells secrete chitin and proteins into the developing tube lumina for tube size control [7,8]. At the end of embryogenesis, at stage 17, tube lumina undergo protein and liquid clearance [9,10]. The embryonic airway clearance is required for accompanied air-filling [9,10] and respiration to conduct oxygen from spiracular openings to the internal tissues.

Previously, we identified the transmembrane J-domain protein Wurst as an essential regulator of tube size and airway clearance in *Drosophila* [9]. *wurst* mutant embryos show elongated tracheal tube size. Later on airway clearance fails, leading to a loss of air-filling [9]. Wurst is involved in Clathrin-mediated endocytosis, which regulates airway clearance. Wurst interacts with Clathrin and Heat shock cognate protein (Hsc)70-4 as basic components of the Clathrin coated vesicles [9,11]. Evolutionarily, Wurst is highly conserved in sequence and structure and contains a single ortholog in humans (DNAJC22) [9,12]. Expression studies indicate that *Drosophila wurst* has a maternal component. Zygotic *wurst* expression starts from stage 13 onwards when the tracheal tubes grow out until the end of embryogenesis in the differentiated tracheal tube cells [9]. Furthermore, *wurst* is expressed in other epithelial organs such as the epidermis and gut. In order to elucidate the functional

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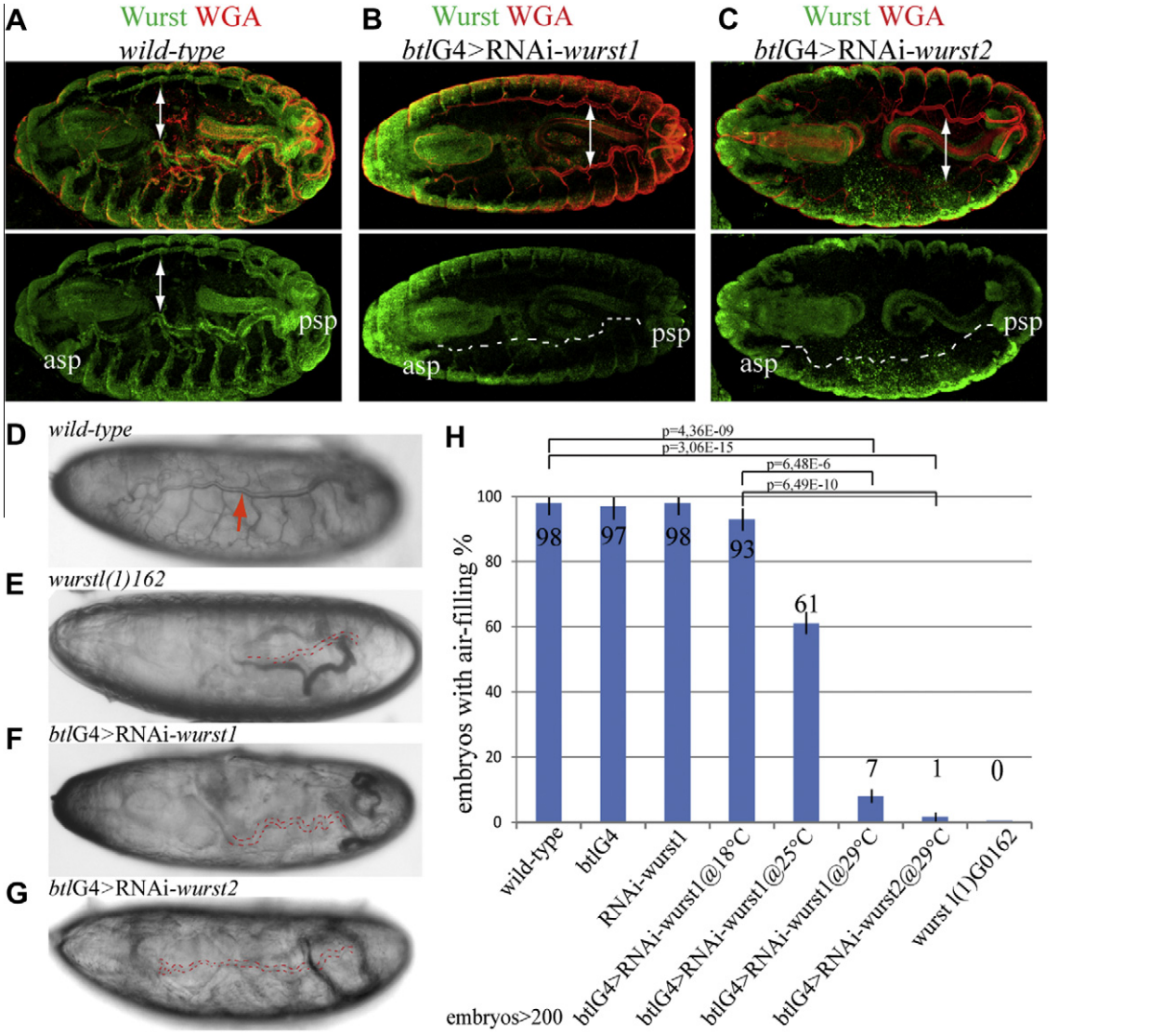

Fig. 1. Tracheal specific *wurst* knock-down inhibits air-filling. (A) Confocal projection of stage 17 embryo using anti-Wurst antibody shows tracheal (arrow) Wurst (green) expression in *wild-type*. Wheat germ agglutinin (WGA, red) indicates the airways. (B, C) In stage 17 embryos, *btl*G4-driven expression of independent UAS-RNAi-*wurst* constructs (referred as 1 and 2) strongly reduces Wurst in the tracheal cells. Main dorsal airways are indicated by double-headed arrows and white dashes between anterior spiracles (asp) and posterior spiracles (psp). (D) Stage 17 *wild-type* embryos undergo airway liquid clearance and accompanied air-filling (arrow). (E–G) Stage 17 *wurst* mutants and *btl*G4-driven UAS-RNAi-*wurst* knock-down embryos show air-filling defects; red dashes mark the main airway, the dorsal trunk. (H) The histogram shows quantifications of air-filling in percentage. For this and other histograms, the minimum amount of embryos (embryos $\geqslant$200) analyzed for each individual genetic experiment, *p*-values (*p*), and standard deviations (bars) are indicated; additional histograms reveal distribution within the datasets, which are presented in the Supplementary Figs. S1, S7.

requirement of *wurst* during airway development and physiology, we explore the time- and tissue-specific requirement of *wurst* in the developing tracheal system.

2. Materials and methods

Bloomington/Vienna stock centres (http://flystocks.bio.indiana.edu/): w^{1118} (referred as *wild-type*), *breathless*GAL4, crb^{2}, crb^{j1B5}, UAS-$crbRNAi^{TRiP.JF02777}$. UAS-$wurstRNAi1^{\#40966}$, UAS-$wurstRNAi2^{\#110270}$, UAS-$crbRNAi^{39178i}$; $wurst^{l(1)G0162}$ [13]. Methods and antibodies are described in the Supplementary data.

3. Results and discussion

In order to generate tracheal specific *wurst* knock-down, we expressed different UAS-RNAi-*wurst* constructs [14] under the control of the UAS-GAL4 system [15]. For our experiments we used the *breathless*GAL4 (*btl*G4) driver line, which specifically expresses GAL4 in tracheal cells throughout embryogenesis. For optimal conditions, we used high temperature (29 °C) and subsequently performed temperature shift experiments to induce time-specific *wurst* knock-down.

First, we tested the optimal knock-down conditions at 29 °C. Fluorescent immune-labeling using a specific anti-Wurst antibody [9] showed strong Wurst expression in tracheal cells of *wild-type* embryos at stage 17 (Fig. 1A). In contrast, the *btl*G4-driven UAS-RNAi-*wurst* expression resulted in severe Wurst reduction exclusively in tracheal cells, but not in other tissues (Fig. 1B, C). This indicates that we can induce strong tracheal specific *wurst* knock-down. Next, we tested the Wurst involvement in the last steps of airway clearance, the transition from luminal liquid-clearance to air-filled airways, here referred to as air-filling. Due to differences in light-diffraction this can be monitored in vivo at stage 17 by bright-field microscopy (Supplementary movie S1; [9,10]). In