Commentary & View

The Wurst protein

A novel endocytosis regulator involved in airway clearance and respiratory tube size control

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The mammalian lung and the Drosophila airways are composed of an intricate network of epithelial tubes that transports fluids or gases and converts during late embryogenesis from liquid- to air-filling. Conserved growth factor pathways have been characterized in model organisms such as Drosophila or the mouse that control patterning and branching of tubular networks. In contrast, knowledge of the coordination of respiratory tube size and physiology is still limited. Latest studies have shown that endocytosis plays a major role in size determination and liquid clearance of the respiratory tubes and a new key regulator of these processes was identified, the Drosophila Wurst protein. wurst encodes a J-domain transmembrane protein which is essential for Clathrin-mediated endocytosis. It is evolutionarily conserved and single Wurst orthologs are found in mammals (termed DNAJC22). In this commentary, we discuss the role of Wurst/DNAJC22 and address whether these proteins may be general regulators of Clathrin-mediated endocytosis.

Many organs of the body, including the lung, the cardiovascular system, the liver and the kidney, consist of ramified networks of epithelial tubes. The proper size and shape of these tubes are crucial for their transport function since they affect flow rates of transported materials and are therefore important determinants of organ function. Genetic pathways controlling some of the early steps in development of branched tubular networks, including branch budding and tube formation, have been identified in the fruit fly Drosophila melanogaster. In the last decade it has turned out that many of the key growth regulators and signaling cascades bear an evolutionary conserved function in tubular network formation.¹ In contrast, knowledge of molecular processes that regulate and maintain distinct sizes and shapes of epithelial tubes is still scarce. During development of the airways, morphological and physiological processes, such as tube size determination and the transition from liquid- to air-filled tubes, occur in later stages of embryogenesis in Drosophila²⁵ or fetal development in mammals.⁴ In a clinical context, residual lung liquid at birth impairs oxygenation of the blood and severe fluid retention is an important feature of the neonatal respiratory distress syndrome, the most common cause of death among premature and newborn infants.⁴ Nevertheless, respiratory tube regulators that coordinate both tube morphology and physiology are mostly unknown.

Using the Drosophila tracheal (respiratory) system as a model system, several genes that influence tube diameter and length have been identified. These include genes involved in the synthesis of a cylindrical chitin matrix secreted by tracheal cells and genes that encode chitin modifying enzymes.⁵¹⁰ Furthermore, regulators of septate junctions, the insect cognate of vertebrate tight junctions, are involved in determining tube morphogenesis.¹¹¹⁵ Latest findings have now shown that endocytosis is crucial for both size determination and liquid clearance of respiratory tubes. By genetic screening, a new evolutionary conserved key regulator has been identified, the Drosophila wurst gene.

**wurst Mutants show Tube Size and Liquid Clearance Defects**

Searching for genes controlling tube maturation in the Drosophila tracheal system, a novel genetic locus was identified, named wurst. wurst mutants show an increased tracheal length and diameter resulting in a curved dorsal trunk which is the main branch of the tracheal system.¹⁶

The wurst tube length phenotype is caused by defective extracellular matrix (ECM) organization in the tracheal tubes. In wild type embryos secretion and modification of ECM establish a central chitinous cable inside the tube lumen. In late embryogenesis, the cable is degraded and removed from the lumen together with the remaining liquid. This ensures gas filling of the respiratory tubes shortly before the larva hatches. In contrast to wild type, chitin matrix organization is defective and lumen clearance is absent in wurst mutants. As a consequence, gas filling completely fails to occur and the wurst mutants die as late embryos.
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Clathrin-mediated endocytosis is a key pathway of cellular endocytotic events and thereby necessary for specific cargo internalization. As an initial step of endocytosis, Clathrin triskelion molecules assemble at the inner surface of the plasma membrane forming a coated pit. The budding-in process is carried out by Clathrin assisted by a set of cytoplasmic proteins that include adaptors, such as AP-2, β-Arrestins and Epsins. Fission, whereby the pit is converted into a vesicle, is mediated by the large GTPase Dynamin, encoded by the shibire gene in Drosophila. Later on the vesicle fuses with endosomes and proceeds in the endocytic pathway. The Clathrin triskelions dissociate after vesicle fission and might be reused. This dynamic nature of Clathrin coat formation and dissociation is controlled by the ATPase activity of Hsc70 in conjunction with a J-domain protein cofactor, such as Auxilin or Rme-8. The J-domain interaction is important to stimulate the low intrinsic Hsc70 ATPase activity supporting Hsc70 function.

wurst encodes a transmembrane protein that contains a type 1 Clathrin binding motif (C1) at the C-terminus and a highly conserved J-domain. Wurst protein is localized in the apical plasma membrane and in cytoplasmic vesicles, such as early and late endosomes. It was shown that the Wurst C-terminus interacts with Clathrin and Hsc70-4 which is supported by co-localization. Genetic experiments suggest that Wurst and Clathrin localization is mutually dependent on each other. Furthermore, it was found that Wurst protein accumulates at the apical plasma membrane in dynamin mutants, which block vesicle endocytosis. A general reduction of internalization processes was observed in wurst mutant embryos and Drosophila S2 tissue culture cells, further providing evidence.

Figure 1. The putative role of Wurst in endocytosis of Clathrin-coated vesicles. (A) Wurst interacts via its C-terminal Clathrin binding motif type 1 (C1) and the J-domain with Clathrin and Hsc70-4, respectively, and may recruit both to the membrane at very early steps of endocytosis. After endocytosis Wurst remains inside the vesicle. In contrast Clathrin and Hsc70-4 are released and recycled. (B) Orthologs of the Wurst protein are found among metazoa and can be evolutionary grouped. As a major search engine we used NCBI protein blast with basic settings. (C) The Wurst protein domain arrangement is conserved among species including humans; the signal peptide is depicted in yellow, transmembrane domains in red, the C1 in black and the J-domain in grey. (D) The predicted folding of the C-terminal J-domain including the α-helices (red ribbons) and the essential Hsc70 interaction HPD tripeptide are highly conserved in Wurst orthologs.
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for an important role of \textit{wurst} in endocytosis.\textsuperscript{16} Together, these data suggest a model in which the transmembrane protein \textit{Wurst} may be involved in the early steps of Clathrin-mediated endocytosis (Fig. 1A). It may facilitate both Clathrin and Hsc70-4 binding at sites of vesicle formation. After vesicle fission and subsequent uncoating of the Clathrin triskelions, \textit{Wurst} is kept in vesicles that enter the endosomal pathway where it may have additional unknown functions. Consistently, mutants for \textit{clathrin}, \textit{hsc70-4} and \textit{dynamin} mimic the \textit{wurst}-like airway tube size and liquid clearance phenotypes. These data identified endocytosis as a major cellular process that is required for size regulation and liquid clearance in the Drosophila tracheal system. In a parallel study, endocytosis was also identified as essential for size regulation and liquid clearance in the Drosophila tracheal system.\textsuperscript{27}

**Table 1** The \textit{Wurst} protein orthologs

| species | length aa | size kDa | id / sim. % | signal | TM | Cl | J-domain |
|---------|-----------|----------|-------------|--------|----|----|---------|
| \textit{Drosophila melanogaster} | 406 | 46 | - | no | 6 | 1 | C-term |
| \textit{Trichoplax adhaerens} | 372 | 42 | 33 / 53 | 1 | 6 | 1 | C-term |
| \textit{C. elegans} | 409 | 47 | 27 / 48 | anchor | 6 | 1 | no |
| \textit{Nematostella vect} | 334 | 39 | 37 / 57 | anchor | 6 | 1 | no |
| \textit{Apis mellifera} | 373 | 43 | 45 / 65 | 1 | 6* | 1 | C-term |
| \textit{Anopheles gambiae} | 440 | 50 | 52 / 68 | 1 | 6 | 1 | C-term |
| \textit{Tribolium castaneum} | 360 | 42 | 53 / 70 | 1 | 6* | 1 | C-term |
| \textit{Culex pipiens} | 412 | 48 | 59 / 75 | no | 6 | no | C-term |
| \textit{Aedes aegypti} | 421 | 49 | 58 / 75 | no | 6 | 1 | C-term |
| \textit{Xenopus tropicalis} | 340 | 39 | 30 / 49 | 1 | 6* | 1 | C-term |
| \textit{Danio rerio} | 338 | 38 | 31 / 48 | no | 6* | 1 | C-term |
| \textit{Monodelphis domestica} | 338 | 38 | 29 / 47 | 1 | 6 | 1 | C-term |
| \textit{Rattus norvegicus} | 341 | 38 | 30 / 48 | 1 | 6* | 1 | C-term |
| \textit{Mus musculus} | 339 | 38 | 29 / 47 | 1 | 6* | 1 | C-term |
| \textit{Canis familiaris} | 678 | 73 | 29 / 46 | no | 7 | 1 | C-term |
| \textit{Equis caballus} | 341 | 38 | 29 / 47 | 1 | 6 | 1 | C-term |
| \textit{Bos Taurus} | 347 | 39 | 28 / 46 | 1 | 6 | 1 | C-term |
| \textit{Pongo abelii} | 341 | 38 | 28 / 45 | 1 | 6 | 1 | C-term |
| \textit{Pan troglodytes} | 341 | 38 | 29 / 45 | 1 | 6 | 1 | C-term |
| \textit{Macaca mulatta} | 341 | 38 | 29 / 46 | 1 | 6 | 1 | C-term |
| \textit{Homo sapiens} | 341 | 38 | 29 / 46 | 1 | 6 | 1 | C-term |

| species | \textit{wurst}/DNAJC22 expression profile |
|---------|-------------------------------------------------|
| \textit{Mus musculus} | lung, intestine, kidney, liver, pancreas, spleen, skin, brain |
| \textit{Homo sapiens} | lung, intestine, kidney, liver, pancreas, colon, vascular system, heart, mammary glands, uterus, testis, ovary, bone, brain |

The \textit{Wurst} protein family is highly conserved with similar size and significant sequence identities and similarities. The \textit{Drosophila melanogaster} \textit{Wurst} is highlighted in orange, other insects in blue and vertebrates in yellow. The common \textit{Wurst} protein contains an N-terminal signal peptide, six transmembrane domains and at the C-terminus one Clathrin binding motif type 1 (C1) and a single J-domain. The amount of TM predictions can differ (between 5 and 6) for some members (*), depending on the prediction program. The \textit{Wurst} protein of \textit{Caenorhabditis elegans} possesses no J-domain. For the protein domain analysis we used SMART, SignalP 3.0, TMHMM 2.0., TMPred and SOSUI. The mouse and human \textit{Wurst} is highlighted in orange, other insects in blue and vertebrates in yellow. The common \textit{Wurst} protein contains an N-terminal signal peptide, six transmembrane (TM) domains, and have been identified in nematodes, flies and vertebrates.\textsuperscript{28}

In Drosophila the ENaC/DEG members are encoded by \textit{pickpocket} (\textit{ppk}) genes, \textit{ppk 4} and \textit{11}, in particular, are involved in luminal liquid clearance of the tracheal tubes.\textsuperscript{29} Similar, ENaC activity is required for liquid clearance of the murine lung at birth. The amiloride-sensitive epithelial sodium channel is a heteromultimeric protein consisting of three homologous subunits, \(\alpha\), \(\beta\) and \(\gamma\).\textsuperscript{30} Whereas mutations in \(\beta\)- or \(\gamma\)-ENaC subunits cause a mild delay in liquid clearance of the lung.\textsuperscript{31,32} \(\alpha\)-ENaC knockout mice show respiratory distress syndrome (RDS) in which liquid is retained within the lung.\textsuperscript{33} It has been suggested that liquid clearance is mediated by the concerted action of apical ENaCs and basolateral Na-K-ATPase, facilitating sodium transport from the lumen into the lung tissue.\textsuperscript{34} Water passively follows into the adjacent tissue where it can be absorbed into the pulmonary circulation.\textsuperscript{3} One of the major mechanisms that seem to regulate ENaC activity in pulmonary cells is the alteration of channel density at the apical membrane of polarized epithelial cells by modulating membrane trafficking. The half-life of ENaCs is short and it has been shown that the presence of ENaC at the cell surface is negatively regulated by the E3 ubiquitin ligase \textit{Nedd4-2}.\textsuperscript{35} Internalization of ubiquitinated ENaCs occurs entirely via Clathrin-mediated endocytosis.\textsuperscript{36} Abnormalities in ENaC function have been directly linked to several human diseases including neonatal RDS,\textsuperscript{4,37,38} Liddle syndrome,\textsuperscript{39} and cystic fibrosis, and may be implicated in states as diverse as salt-sensitive hypertension, nephrosis and pulmonary edema.\textsuperscript{40}

**Wurst/DNAJC22 Proteins: Conserved Regulators of Endocytosis?**

Is \textit{Wurst} a tissue-specific regulator of Clathrin-mediated endocytosis required only for the airway system or a more general regulator essential for endocytosis? For the latter, one would expect evolutionary conservation of \textit{Wurst} since Clathrin-mediated endocytosis and its key regulators Clathrin, Adaptins and Dynamin are highly conserved. \textit{clathrin} mutants survive until end of embryogenesis due to the strong maternal contribution.\textsuperscript{41} Quite similar, maternally deposited \textit{wurst} mRNA is expressed ubiquitously from early stages onwards. First zygotic expression becomes abundant beyond stage 13 (second half of embryogenesis) when the first mutant phenotypes occur.\textsuperscript{11} tracer uptake assays further indicate that \textit{Wurst} function is also required in other cell types, including epidermal cells arguing for a more general function of \textit{Wurst}.\textsuperscript{16} Single copies of \textit{Wurst} orthologs exist in a broad spectrum of animals reaching from placozoa to primates as depicted in the evolutionary tree (Fig. 1B). Most of them show significant sequence similarities and a conserved protein domain arrangement: an N-terminal signal peptide, six transmembrane (TM) domains,
and the C-terminal C1 motif and J-domain (Fig. 1C, Table 1). The J-domain has been well characterized in *E. coli* comprising four α-helices and a tripeptide (HPD) motif between the second and third helix. The Wurst orthologous proteins reveal similar J-domain topology of α-helices (Fig. 1D, indicated in red as ribbons) and HPD motif, which is essential for interaction with Hsc70 proteins. Altogether this indicates that Drosophila Wurst represents the prototype of a novel class of conserved J-domain transmembrane proteins, leaving the question if it may have similar functions in other animals as in Drosophila.

Further hints for a potential role of *wurst* in addition to its involvement in airway clearance arise from the fact that Drosophila *wurst* as well as single mouse and human orthologs are expressed in tubular epithelia such as lung, kidney or intestine (Table 1). As a concluding remark, one can summarize that Wurst orthologs are highly conserved in sequence and protein domain arrangement leading to the speculation that Wurst proteins may have a conserved function in endocytosis.

**Accession number for Wurst orthologs**

EDV26866 (trichoplax); NP_492450 (caenorhabditis); XP_001635909 (nematostella); XP_394206 (apis); EAA04160 (anopheles); XP_971138 (trilobium); XP_001868697 (culex); XP_001661613 (aditis); NP_001107370 (xenopus); XP_001335380 (danio); XP_001519327 (ornithorynchus); XP_001374068 (monodelphis); AAH86949 (rattus); NP_789805 (mus); XP_543683 (canis); XP_001492076 (equus); NP_001069169 (bos); CAH90923 (pongo); XP_522378 (pan); XP_00110945 (macaca); NP_079178 (human)

**Links for programs and expression profiles**

http://smart.embl-heidelberg.de/

http://www.cbs.dtu.dk/services/SignalP/

http://www.cbs.dtu.dk/services/TMHMM/

http://blast.ncbi.nlm.nih.gov/Blast.cgi

http://searchlauncher bcm.tmc.edu/seq-search/struct-predict.html

http://bmm.cancerresearchuk.org/~3djigsaw/

http://www.ncbi.nlm.nih.gov/UniGene/ESTProfileViewer.cgi?list=Mm.30544

http://www.ncbi.nlm.nih.gov/UniGene/ESTProfileViewer.cgi?list=Hs.659300

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