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In *Drosophila melanogaster*, the Rolling pebbles isoform 6 (Rols6) is essential for proper Malpighian tubule morphology

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Abstract

During myoblast fusion, cell–cell recognition along with cell migration and adhesion are essential biological processes. The factors involved in these processes include members of the immunoglobulin superfamily like Sticks and stones (Sns), Dumbfounded (Duf) and Hibris (Hbs), SH3 domain-containing adaptor molecules like Myoblast city (Mbc) and multidomain proteins like Rolling pebbles (Rols). For *rolling pebbles*, two differentially expressed transcripts have been defined (*rols7* and *rols6*). However, to date, only a muscle fusion phenotype has been described and assigned to the lack of the mesoderm-specific expressed *rols7* transcript. Here, we show that a loss of the second *rolling pebbles* transcript, *rols6*, which is expressed from the early bud to later embryonic stages during Malpighian tubule (MpT) development, leads to an abnormal MpT morphology that is not due to defects in cell determination or proliferation but to aberrant morphogenesis. In addition, when Myoblast city or Rac are knocked out, a similar phenotype is observed. Myoblast city and Rac are essentially involved in the development of the somatic muscles and were proposed to be interaction partners of Rols7. Because of the predicted structural similarities of the Rols7 and Rols6 proteins, we argue that genetic interaction of *rols6*, *mbc* and *rac* might lead to proper MpT morphology. We also propose that these interactions result in stable cell connections due to rearrangement of the cytoskeleton.

Keywords: Malpighian tubules; Stellate cells; Myoblast fusion; Endoderm; Hibris; Nephrin; DOCK180; Rac; Cytoskeleton; Cell migration

1. Introduction

The mesoderm of *Drosophila* is subdivided into distinct parts each giving rise to the dorsal vessel, the fat body, the visceral muscles and the body wall musculature (reviewed in Bate, 1993). The cell fate decisions leading to this subdivision depend on intrinsic factors as well as ectodermal signalling (Riechmann et al., 1997). The visceral mesoderm gives rise to the circular and longitudinal musculature of the gut and arises by fusion of founder cells and fusion competent myoblasts (Klapper et al., 2001, 2002; San Martin and Bate, 2001; Stute et al., 2004). Founder cells and fusion competent cells of the circular muscles arise from the visceral trunk mesoderm. The founder cells of the longitudinal visceral muscles, however, arise from a different primordium (Georgias et al., 1997; Kusch and Reuter, 1999).

Recently, Denholm et al. (2003) have shown that this primordium not only contains the future founder cells of the longitudinal muscles but also a fraction of cells that migrate to the posterior ectodermal/endodermal border where the Malpighian tubules (MpTs) are formed (reviewed in Skaer, 1993). These mesodermal cells form the stellate cells (SCs) within the MpTs. The SCs are functionally distinct from the ectodermally derived principal MpT cells (PCs) and have a function in controlling the movement of water and anions such as chloride. The PCs make up the majority of the tubule epithelium and via active cation transport they control the flow of hydrogen and potassium ions (O'Donnell et al., 1996, 1998; Dow et al., 1997; Denholm et al., 2003; reviewed in Cagan, 2003). In MpTs of embryos lacking the cell adhesion-mediating protein Hibris, the number of SCs is significantly reduced (Denholm et al., 2003). Hibris belongs to the group of immunoglobulin-like proteins that

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are involved in cell–cell recognition and adhesion in myoblast fusion processes (Artero et al., 2001; Dworak et al., 2001; reviewed in Dworak and Sink, 2002).

Since *rols6* is expressed early on during MpT development (Rau et al., 2001), we asked whether *rols6* expression persists during this process. To this end, we analysed *rolling pebbles* mutants for phenotypes in MpTs. The *rols* genomic region harbours two distinct promoters leading to two mRNAs (*rols6* and *rols7*), which differ only in the N-terminal region (Fig. 1; Rau et al., 2001). Here, we show that the formation of the MpTs is affected in *rols6*-specific mutant embryos leading to defective cell arrangement, misguided outgrowth and mislocalisation of the anterior tubules. However, myoblast fusion, which solely depends on *rols7*, is not affected in *rols6*-specific mutants.

2. Results

2.1. Malpighian tubules are malformed in rolling pebbles mutants

The Malpighian tubules (MpTs) of *Drosophila melanogaster* arise as four buds from the hindgut anlage close to its boundary with the posterior midgut primordium. The cells of the four buds are characterised by the expression of the transcription factor Cut (Ct; Liu and Jack, 1992) at stage 10 of embryogenesis. During germ band extension at stage 11, the cells of the four tubule primordia undergo cell proliferation, and the tubules begin to bud out. By stage 13, proliferation is complete and short tubules have formed (Fig. 2A). From stage 13 onwards, cells from the caudal mesoderm join the MpT primordia and later the stellate cells (SCs). From the end of germ band retraction, the tubules begin to elongate due to cell rearrangement. In stage 15 and 16 embryos, the characteristic stereotypic course of the four renal tubules through the embryonic body is clearly visible. The paired posterior tubules span the posterior abdominal and terminal segments of the embryo. The anterior tubules extend forwards into abdominal segments 2/3 (Fig. 2B,C, arrows) where the tubule loops back on itself so that the tips of both anterior tubules lie more posteriorly within the abdomen (reviewed in Skaer, 1993; Ainsworth et al., 2000; Denholm et al., 2003; Jung et al., 2005).

We have analysed the *rols* deficiency Df(3L)BK9 and the EMS-induced allele rols^{xx117} for aberrations in MpT development and have identified a new allele of *rolling*

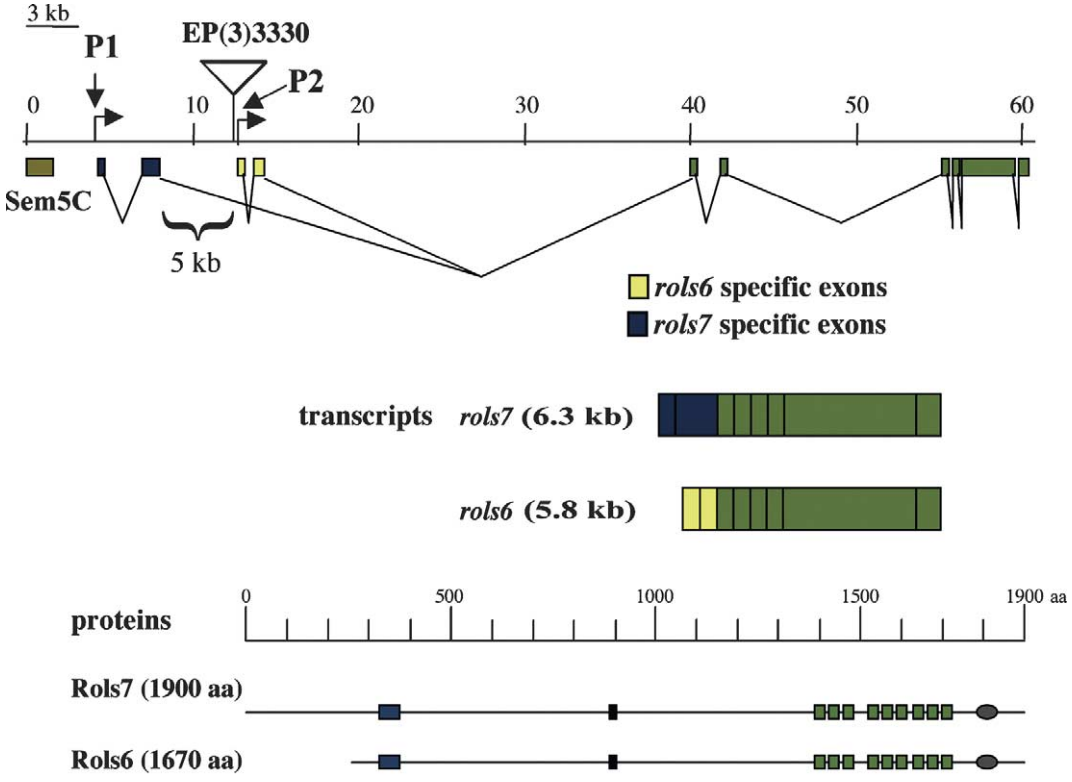

Fig. 1. Genomic organisation of the *rolling pebbles* gene locus (modified from Rau et al., 2001). The genomic size of the *rolling pebbles* gene is about 60 kb and gives rise to two individual transcripts of different length, *rols7* and *rols6*. Transcript-specific exons are indicated and marked by different colours (blue: *rols7* exons 1+2; yellow: *rols6* exons 1+2). Green coloured exons are shared by both transcripts. Rols proteins and common predicted protein domains are indicated by coloured boxes (blue, RING finger; black, P-Loop; green, Ankyrin-repeats; grey, TPR-repeat). The proteins differ from each other through specific N-terminal aa: Rols7: 303 aa (encoding a lipolytic domain) (Menon and Chia, 2001); Rols6: 79 aa. All other predicted protein domains are shared by both isoforms. The intronic region between *rols7* Exon2 and the transcription start site of *rols6* (which was determined by primer extension) comprises 5 kb (curly bracket). This region contains the putative *rols6* promoter (P2). Within this region, the P[EP]3330 is inserted about 60 bp upstream of *rols6* exon 1. P1: *rols7*-specific promoter.