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ScienceDirect
Developmental Biology 313 (2008) 519–532
DEVELOPMENTAL BIOLOGY
www.elsevier.com/developmentalbiology

The *detached* locus encodes *Drosophila* Dystrophin, which acts with other components of the Dystrophin Associated Protein Complex to influence intercellular signalling in developing wing veins

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Received for publication 3 June 2007; revised 24 September 2007; accepted 24 September 2007
Available online 29 September 2007

Abstract

Dystrophin and Dystroglycan are the two central components of the multimeric Dystrophin Associated Protein Complex, or DAPC, that is thought to provide a mechanical link between the extracellular matrix and the actin cytoskeleton, disruption of which leads to muscular dystrophy in humans. We present the characterization of the *Drosophila* 'crossveinless' mutation *detached* (*det*), and show that the gene encodes the fly ortholog of Dystrophin. Our genetic analysis shows that, in flies, Dystrophin is a non-essential gene, and the sole overt morphological defect associated with null mutations in the locus is the variable loss of the posterior crossvein that has been described for alleles of *det*. Null mutations in *Drosophila Dystroglycan* (*Dg*) are similarly viable and exhibit this crossvein defect, indicating that both of the central DAPC components have been co-opted for this atypical function of the complex. In the developing wing, the *Drosophila* DAPC affects the intercellular signalling pathways involved in vein specification. In *det* and *Dg* mutant wings, the early BMP signalling that initiates crossvein specification is not maintained, particularly in the pro-vein territories adjacent to the longitudinal veins, and this results in the production of a crossvein fragment in the intervein between the two longitudinal veins. Genetic interaction studies suggest that the DAPC may exert this effect indirectly by down-regulating Notch signalling in pro-vein territories, leading to enhanced BMP signalling in the intervein by diffusion of BMP ligands from the longitudinal veins.

Keywords: *Drosophila*; Dystrophin; Dystroglycan; Wing venation; Signalling

Introduction

Studies on the patterning and morphogenesis of the veins in the *Drosophila* wing have shown this process to be an ideal system for studying interactions between intercellular signalling pathways in development. To date, four different signalling pathways have been implicated in the process: the Epidermal Growth Factor Receptor (EGFR) pathway, the Bone Morphogenetic Protein (BMP) pathway, the Notch pathway, and, more recently, a pathway involving Rho GTPases (Denholm et al., 2005). These different pathways interact and feed back on one another, initially to define vein and intervein territories in the wing imaginal disc, and later to regulate and refine the vein pattern as the wing undergoes differentiation during pupal development (reviewed in Bier, 2000; Crozatier et al., 2004; de Celis, 2003).

The *Drosophila* wing is characterized by a stereotypical pattern of five longitudinal veins (L1–L5) and two crossveins, the anterior crossvein (acv) and the posterior crossvein (pcv) (Fig. 1A). The pattern of longitudinal veins is laid down in the late third instar with respect to territories that are defined by the morphogens Decapentaplegic (Dpp) and Hedgehog (Crozatier et al., 2004; de Celis, 2003). This pattern is first evidenced by the localized expression of *rhomboid* (*rho*), a protease required for activation of EGFR ligands (Urban et al., 2002), in a series of stripes corresponding to each of the longitudinal veins

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0012-1606/$ - see front matter.
doi:10.1016/j.ydbio.2007.09.044

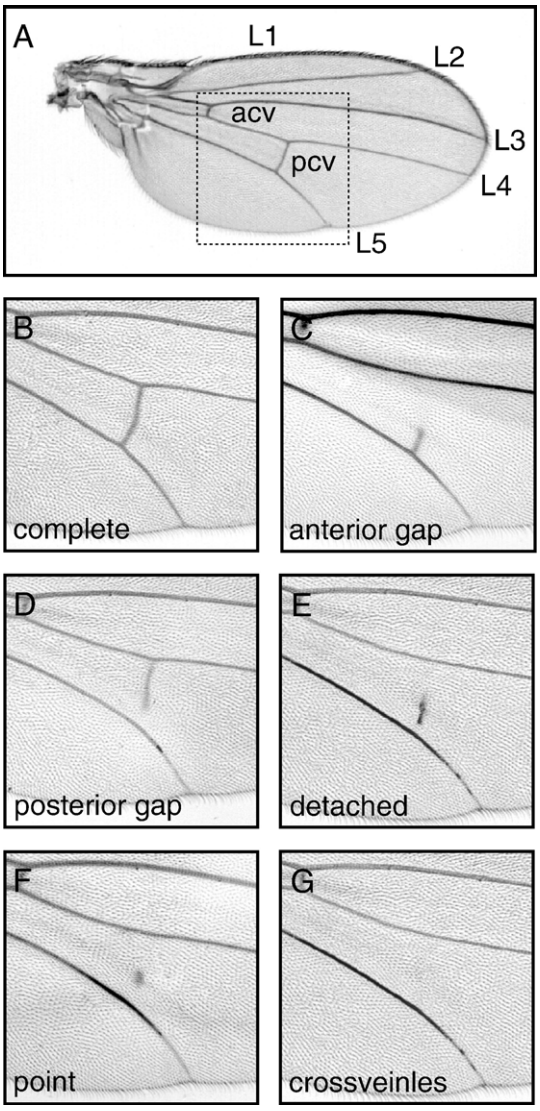

Fig. 1. Wing phenotypes produced by *detached* mutations. (A) A wild type wing showing the positions of the two crossveins, the anterior crossvein (acv) between longitudinal veins L3 and L4, and the posterior crossvein (pcv) between longitudinal veins L4 and L5. The region of the wing shown in panels B to G is boxed. Representative pcv defects observed in flies homozygous for det^{1}. In these flies, the vein may be: "complete" (CO), (B); gapped (GP), either from L4 as an anterior gap (C); or from L5 as a posterior gap (D); "detached" (DE), (E); "point" (PT), (F); or "crossveinless" (CV), (G). In all *det* mutations we have studied, the acv is not consistently affected, so we have not considered it in this report.

(Sturtevant et al., 1993). Activation of the EGFR pathway in these cells results in an initial "ON–OFF" pattern of vein and intervein cells, the former expressing *rho* and the Notch ligand Delta, and the latter expressing the *Drosophila* ortholog of the Serum Response Factor, or Blistered (Bs), and Notch itself (de Celis et al., 1997; Montagne et al., 1996). The establishment of these expression patterns initiates the basic regulatory loop that governs vein development, with *rho*-dependent EGFR signalling activating expression of Delta and repressing expression of Bs and Notch, and, in return, both Notch signalling and Bs repressing the activity of the EGFR pathway and the expression of *rho*.

This basic signalling network is elaborated during pupal development at the time of the final apposition of dorsal and ventral wing surfaces. At this stage, the wing blade is subdivided into the so-called 'vein', 'pro-vein', and 'intervein' territories. *rho* expression prefigures the longitudinal vein pattern and is expressed in all vein territories, Delta is expressed in a broader domain including both vein and pro-vein territories, Notch is expressed in pro-vein and intervein territories and up-regulated in pro-vein cells, and Bs is expressed uniformly in pro-vein and intervein cells (see Fig. 8A; Sotillos and De Celis, 2005). Notch activity, as evidenced by the expression of the Notch target gene *Enhancer of split* (*E(spl)*), is confined to the pro-veins (de Celis, 1997). Expression of the BMP ligand *dpp* is first detectable in vein cells at this time, and overlaps the *rho* pattern, while its receptor, *thickveins* (*tkv*), is expressed in a pattern similar to Notch (de Celis, 1997). The BMP and EGFR signalling systems form a positive feedback loop, with expression of *rho* depending on the activity of *dpp* and vice versa (de Celis, 1997), and both systems are independently repressed by the activity of the Notch pathway in pro-vein cells (de Celis et al., 1997). In the case of *rho*, Notch signalling results in repression of *rho* and down-regulation of EGFR activity, and for *dpp*, Notch signalling up-regulates *tkv*, which limits Dpp diffusion and confines BMP signalling to the vein territory (Fig. 8A; de Celis, 1997; de Celis et al., 1997).

Crossvein specification begins after the two surfaces of the wing have apposed, and after the regulatory network involving EGFR, Notch, and BMP signalling has been established in the longitudinal veins. In this regard, the crossvein pattern is superimposed on the pre-existing pattern of longitudinal veins. This process is initiated by BMP signalling, as evidenced by early expression of the phosphorylated form of the BMP signal transducer Mothers Against Dpp (P-Mad), which first appears in broad domains at the sites of the presumptive acv and pcv, and then sharpens until the crossveins resemble the longitudinal veins (Conley et al., 2000). Two BMP ligands are required for crossvein development, Dpp and the BMP5,6,7,8 ortholog Gbb (Ray and Wharton, 2001). The early broad expression of P-Mad is dependent on Gbb and precedes the localized expression of *dpp* that first appears once the crossvein domains have refined (Ralston and Blair, 2005). Expression of *dpp* is coincident with the expression of *rho* and Delta, and once all three genes are expressed, the regulatory circuit operating in the longitudinal veins is recapitulated in the crossveins.

Crossvein specification appears to be quite sensitive to perturbations in BMP signalling, and a number of 'crossveinless' genes, including the BMP-1 ortholog *tolkin*, *crossveinless* (*cv*), and *crossveinless-2* (*cv-2*) have been shown to be components of the BMP signalling pathway (Finelli et al., 1995, Conley et al., 2000; Shimmi et al., 2005; Vilmos et al., 2005). The recent characterization of *crossveinless-c* (*cv-c*) proved to be an exception as it was shown to encode a RhoGAP, thus implicating a different signalling pathway in the mechanism of crossvein specification (Denholm et al., 2005). Notably, the crossvein defect associated with *cv-c* is weaker than that of the