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Wing vein development in the sawfly *Athalia rosae* is regulated by spatial transcription of Dpp/BMP signaling components

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ABSTRACT

Wing venation among insects serves as an excellent model to address how diversified patterns are produced. Previous studies suggest that evolutionarily conserved Decapentaplegic (Dpp)/Bone Morphogenetic Protein (BMP) signal plays a critical role in wing vein development in the dipteran *Drosophila melanogaster* and the hymenopteran sawfly *Athalia rosae*. In sawfly, *dpp* is ubiquitously expressed in the wing during prepupal stages, but Dpp/BMP signal is localized in the future vein cells. Since localized BMP signaling involves BMP binding protein Crossveinless (Cv), redistribution of BMP ligands appears to be crucial for sawfly wing vein formation. However, how ubiquitously expressed ligands lead to a localized signal remains to be addressed. Here, we found that BMP binding protein short gastrulation (Sog) is highly expressed in the intervein cells. Our data also reveal that BMP type I receptors thickveins (Tkv) and saxophone (Sax) are highly expressed in intervein cells and at lower levels in the vein progenitor cells. RNAi knockdown of *Ar-tkv* or *Ar-sax* indicates that both receptors are required for localized BMP signaling in the wing vein progenitor cells. Taken together, our data suggest that spatial transcription of core- and co-factors of the BMP pathway sustain localized BMP signaling during sawfly wing vein development.

1. Introduction

Insect wings are highly diversified among species and provide a rich resource to address how morphological diversity manifests throughout evolution. Wing venations shows characteristic features in a species-specific manner due to the importance of aerodynamics associated with a specific flight system (Wootton, 1992; Wootton et al., 2003). Although morphological diversity has been historically described among many different species (Comstock and Needham, 1898), the molecular mechanisms of wing vein development have been mostly investigated in the dipteran *Drosophila melanogaster*. Therefore, very little is known about mechanisms underlying diversity in wing venation.

In *Drosophila*, wing vein development is established through two consecutive stages. The positional information of longitudinal veins (LVs) is determined by a morphogen gradient composed of Dpp and Hedgehog (Hh) signaling in the larval wing imaginal disc (Affolter and Basler, 2007; Shimmi et al., 2014). Formation of LVs is further established by the interactions of three growth factor signaling pathways: the Dpp, Epidermal Growth Factor (EGF) and Notch pathways (Blair, 2007). Then, during pupal stages, the positional information of crossveins appears to be established by inducing Dpp/BMP signaling (O'Connor et al., 2006; Shimmi et al., 2005; Ralston and Blair, 2005; Matsuda and Shimmi, 2012). Intriguingly, *dpp* expression is only observed in the LV progenitor cells during the early pupal stage, but Dpp/BMP signal is detected in all the future vein cells, including the crossveins (Matsuda and Shimmi, 2012; Ralston and Blair, 2005). Therefore, a combination of a short-range signaling in LVs and long-range signaling in crossveins is crucial for spatial regulation of BMP signaling during *Drosophila* pupal wing vein development.

The core components of BMP signaling pathway are highly conserved, including BMP ligands, BMP receptors, and downstream factor Smad proteins. In *Drosophila*, the following genes were identified as core components: the three ligands Dpp, Glass bottom boat (Gbb), and Screw (Scw); the two type I receptors Thickveins

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(Tkv) and Saxophone (Sax); the two type II receptors Punt (Put) and Wishful thinking (Wit); and the three Smad proteins Mothers against Dpp (Mad), Medea, and Daughters against Dpp (Dad) (Kahlem and Newfeld, 2009). Furthermore, co-factors are often utilized for spatial regulation of BMP signaling in a context-dependent manner. For example, two BMP binding proteins, Sog and Cv, are required for BMP ligand trafficking from LVs into crossveins to sustain long-range signaling (Matsuda and Shimmi, 2012). Interestingly, a similar mechanism comprising BMP and Sog (or Chordin in vertebrates) is widely utilized among species ranging from Cnidaria to all phyla of Bilateria to establish dorsal-ventral patterning during early embryogenesis (De Robertis, 2008, Bier and De Robertis, 2015). Therefore, gene sets involved in this network may have been co-opted for insect wing vein formation (Shimmi et al., 2014).

The sawfly *Athalia rosae* is a primitive member of Hymenoptera that is the most basal group of the Holometabola (Misof et al., 2014). The sawfly has two pairs of wings with different patterns of venation (Fig. 1A and B). Previous observations suggest that vein formation largely takes place in both fore- and hindwings during prepupal stages (Matsuda et al., 2013b). Furthermore, conserved Dpp/BMP signaling is needed for wing vein development in the sawfly. Intriguingly, in contrast to the localized expression of *Dm-dpp* in the *Drosophila* pupal wing, *Ar-dpp* is ubiquitously expressed in the sawfly prepupal wing to produce localized BMP signaling in the wing vein progenitor cells. The BMP binding protein Ar-Cv is

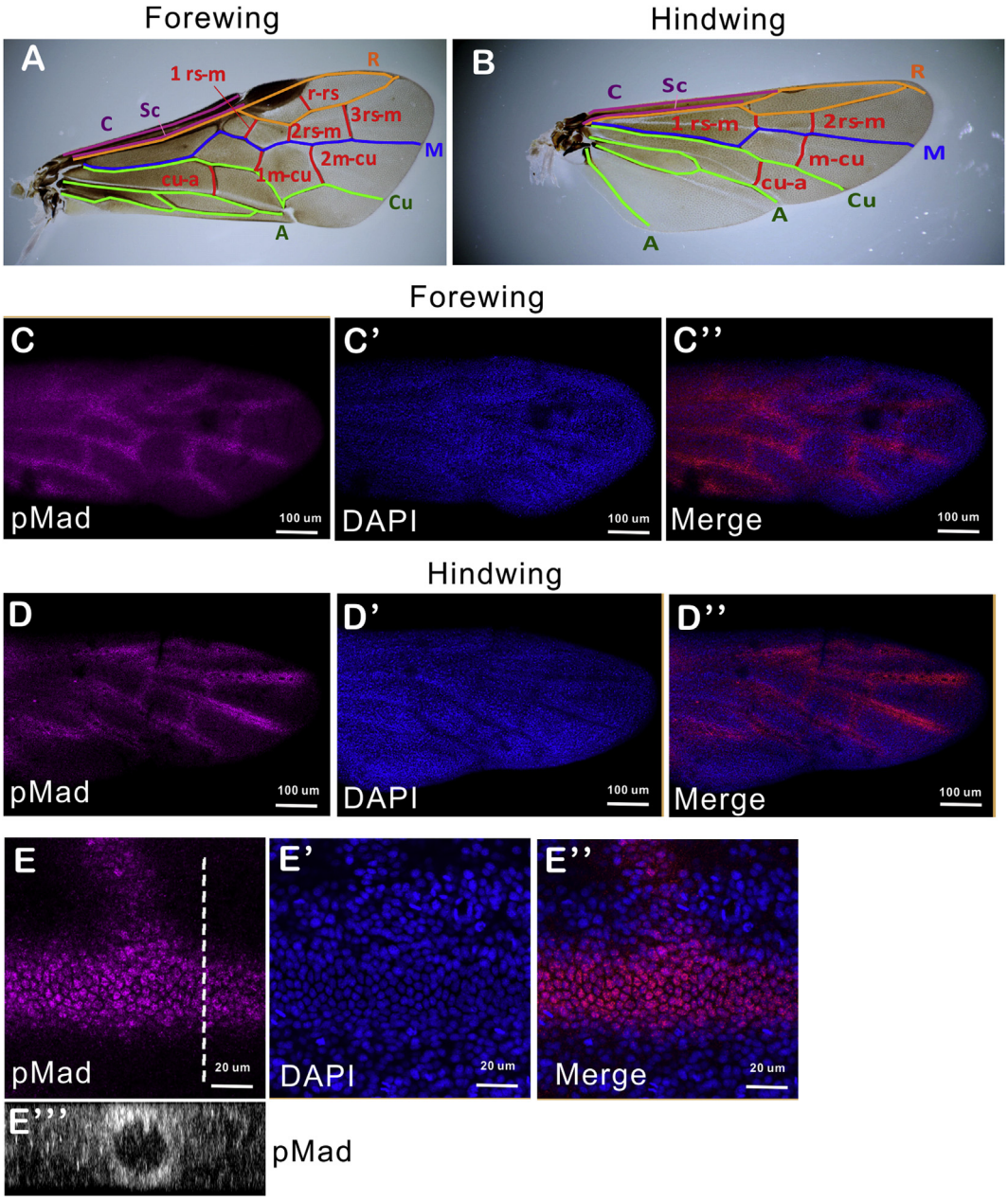

Fig. 1. BMP signal reflects wing vein formation in the sawfly. (A, B) Fore- and hindwings of adult sawfly. LVs are marked in capital letters and crossveins in small letters. LVs: Anal (A), Costa (C), Cubitus (Cu), Media (M), Radius (R), Subcosta (Sc). Name of crossveins reflect the connected LVs and are numbered sequentially from proximal to distal. (C, D) pMad accumulation (C, D), DAPI (C′, D′) and merged image (C″, D″) of forewing (C–C″) and hindwing (D–D″) at prepupal stage PCF4. (E) Higher magnification image of pMad (E), DAPI (E′) and merged image (E″) on Medial vein (M) of forewing at prepupal stage PCF4. Optical cross section of pMad accumulation on the dashed line is shown in the lower panel (B/W image) (E‴).

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