CONCLUSIONS AND FUTURE DIRECTIONS

The LIG family of proteins is emerging as mediators and modulators of various signaling pathways and cellular processes. In *Drosophila,* for example, Kek1 inhibits EGFR signaling, Kek5 is a modulator of BMP signaling and cellular adhesion, while Kek6 is excluded from tricellular junctions, possibly providing a paracellular barrier function (Alvarado et al., 2004; Evans et al., 2009; Arata and Duffy, 2011). Elucidating the mechanism of action of these and other LIGs in *Drosophila* will continue to shed further light on the overall function of this intriguing family of molecules, including vertebrate LIGs.

Kek5 and BMP signaling

Kek5 has been shown to be a modulator of BMP signaling. Both loss and gain of function of Kek5 causes crossvein defects, a phenotype associated with perturbations of BMP signaling. Prior work argued that Kek5 undertakes its role in BMP signaling primarily through its extracellular LRRs (Evans and Duffy, 2006). The work presented here demonstrates that conserved intracellular motifs are critical for Kek5's activity in BMP signaling as well. Thus, both the intracellular region and the extracellular region of Kek5 are important for its BMP related activity. Epistasis experiments with Kek5 and the ligands and receptors are consistent with a mechanism involving interactions on both sides of the membrane. Possible candidates for testing such association would be the BMP receptors, and is currently being pursued in the lab.

Kek5 and cell junction

The data presented here indicates Kek5 is capable of regulating the expression of adherens junction components. Altering the levels of Kek5 resulted in a striking upregulation of Arm, in addition to upregulation of p120 catenin. Two additional junction components, Pyd and Cno, were downregulated. Structure/function analysis of Kek5 demonstrated the Ig domain was essential, providing the first evidence for a Ig domain function among Kek family members. In contrast, the IC domain was mostly dispensable, providing only a localization component through IC 6 (PDZ domain binding site). Together with the fact that Arm upregulation is a post-transcriptional event, it is possible that Kek5 uses its Ig domain to associate with another Ig domain protein bound to Arm and thereby indirectly increases Arm levels at the membrane. To provide a complete picture of the role of Kek5 in this process, all the deletion variants should be tested for their ability to alter the levels of p120, Pyd and Cno. Although I would anticipate the results would mimic that seen for Arm, it will be important to confirm this. Ultimately however, to understand the *in vivo* role of Kek5, it will be important to correlate these gain-of-function effects with a loss-of-function phenotype, for Kek5, possibly wing blistering.

Functional relevance of the union of LRRs and Ig domain in LIGs

Despite the vast number of proteins containing either LRR or Ig domains, only a small number of metazoan proteins contain both of them together. What biological processes might require the two domains to function in concert or are these two domains always performing different roles in LIGs? The work presented here on Kek5 adds to our

understanding of how LIGs might function. While the LRR domain of Kek5 appears to be required for localization and stability (possibly additional functions as well), the Ig domain is specifically required in junction biology (i.e. upregulation of Arm) and is dispensable for BMP signaling. In this scenario the LRRs and Ig domains are performing distinct roles. However, since deletion of the LRRs resulted in mislocalization of the protein, it is difficult to assess the specific role of the LRRs in Kek5 activity. It is possible, for example, that the LRRs, in addition to being required for appropriate protein folding and stability, are also required in some way in concert with the Ig domain in causing Arm upregulation. In order to gain insight into the role of the structural elements in LIG proteins, generation of LRR and Ig swaps with other Kek family members will be helpful. Although, it is possible that even the swaps may show misdirected localization.

The work reported here has confirmed and revealed further details on the role of Kek5 in BMP signaling, including the implication of specific intracellular motifs in Kek5's activity. In addition, my work has implicated Kek5 and its' various sequence motifs in a variety of additional cellular processes, including regulation of adherens junctions, cell size, extrusion, and apoptosis. While my studies have provided important new insights, the complexity of these effects, their relationships to each other, and Kek5's role and mechanism of action in these processes remains to be fully elucidated. Likewise, although initial studies on Kek3 have revealed intriguing phenotypic similarities to Kek5 and confirmed a relationship to BMP signaling, the nature of its roles must be further clarified.

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