ABSTRACT AND SPECIFIC AIMS

During epithelial tissue morphogenesis, cells must undergo planar rearrangements while maintaining the ability to resume regular spatial distributions and packing formations. Throughout these planar rearrangements, adherens junctions are destabilized and shortened to facilitate intercalation and subsequent formation of new, elongated junctions [1]. One example of this process is *Drosophila* pupal wing development, in which planar rearrangements are necessary for tissue elongation [2]. While many of the mechanisms of junction shortening have been elucidated, junction elongation remains a largely unstudied phenomenon. The main objectives of the following proposal are to better characterize the protein-protein interactions as well as the processes underlying the membrane dynamics that occur during planar rearrangement. Understanding the mechanisms involved in junction stabilization will illuminate many other mechanisms responsible for epithelial organization during embryonic development.

Specific Aim 1: Determine whether PTEN and Rho-kinase directly interact at the cell membrane.

Specific Aim 2: Determine if PTEN-dependent removal of myosin II from the membrane is essential for junction elongation.

BACKGROUND AND SIGNIFICANCE

Previous studies have demonstrated a role for the tumor suppressor PTEN in cellular packing in the epithelia [3]. PTEN is a lipid phosphatase whose main function is to dephosphorylate phosphatidylinositol (3,4,5)-triphosphate (PIP_3) to generate phosphatidylinositol (4,5)-bisphosphate (PIP_2). PTEN is often mutated in a range of human cancers, with the highest incidence in endometrium, skin, central nervous system, and prostate cancers [4]. PTEN has also been shown to be an important regulator of junction stability in *Drosophila* pupal wing development [5]. In wild-type tissues, junction shortening is characterized by an uneven distribution of Myosin II at the membrane, with the shortest junctions having the highest amounts of myosin II [6-9]. The loss of PTEN activity is characterized by a prolonged increase of MyoII at the newly formed short junctions. These junctions take longer to stabilize, and never fully elongate. This localization is cell-autonomous: small WT tissue islands surrounded by *pten* cells show normal packing and MyoII distribution.

Rho-kinase (Rok) is known to regulate MyoII activity by phosphorylating its light chain [10]. Similar to MyoII, Rok is properly enriched but remains at newly formed junctions in *pten* mutants. These junctions are also destabilized and exhibit an elongation defect [5]. Interestingly, generation of loss-of-function *rok* clones in *pten* tissue rescued the short junction defect. This suggests that PTEN somehow regulates the Rok-dependent activation of MyoII during planar rearrangement. Further understanding of this process will shed light on the dynamics of junction elongation.

RESEARCH DESIGN AND METHODS

Specific Aim 1: Determine whether PTEN and Rho-kinase directly interact at the cell membrane.

I plan to use two different but complementary biochemical assays to determine whether PTEN and Rok directly interact with one another. A protein complex immunoprecipitation (Co-IP) is one method of searching for a direct interaction. I will generate a Rok-maltose binding protein fusion. Maltose binding protein (MBP) is a commonly used tag for protein purification because it improves the solubility of the protein of interest while providing an affinity tag for purification. Since Rok is often membrane-associated, this increase in solubility may be necessary

for efficient purification. I plan to dissect the wing tissues when planar rearrangement occurs from 24-30hAPF (hours after pupal formation) and perform a purification of Rok-MBP by applying the membrane fraction to an amylose column, which will bind the MBP while allowing other proteins to flow through. The Rok-MBP fusion protein can then be eluted from the column with maltose. I can assess the purity of this protein isolation by SDS-PAGE followed by Coomassie blue staining, which will stain any protein in the gel. A Western blot using an antibody to PTEN will probe for any specific interaction between the two proteins. One control for this experiment is to do the reverse: pull down an MBP-tagged PTEN and then probe for Rok. A positive result in both directions would rule out the possibility of a nonspecific association. As junction elongation is dynamic and reversible, any direct interaction between these proteins is likely to be transient. If this is the case I will utilize a common chemical crosslinking agent such as EDC to preserve the interaction prior to purification and Western blotting.

A second method to visualize a direct interaction between Rok and PTEN is bioluminescence resonance energy transfer (BRET), combined with a previously optimized live imaging approach [5]. BRET is very similar to FRET but takes advantage of a bioluminescent donor that intrinsically emits photons, and eliminates the need for fluorescent excitation [11]. This reduces effects of two of the main disadvantages of FRET: background noise and photobleaching. A Rok-luciferase fusion emits photons in the excitation spectrum of a PTEN-YFP fusion protein. During live imaging of the pupal wing tissue, any observed YFP signal at the remodeled junctions could be attributed to a direct interaction between Rok and PTEN. However, one limitation to this experiment is the possibility that the presence of fluorophores could inhibit the normal activity of these proteins.

Specific Aim 2: Determine if PTEN-dependent removal of myosin II from the membrane is essential for junction elongation.

Regardless of whether Rok and PTEN directly interact with one another, it has been shown that Rok and MyoII both linger at shortened junctions in *pten* mutants, which fail to properly elongate [5]. In WT tissues a decrease of MyoII at the junction immediately precedes the elongation of that junction, which suggests that reduction of MyoII is necessary for elongation. To test the hypothesis that depletion of MyoII from the membrane is both necessary and sufficient for junction elongation, I will examine the effects of an unstable MyoII protein.

Several methods have been developed for chemical induction of targeted protein degradation [12]. One such method that I would like to apply utilizes a destabilization domain called a degron, which when fused to a target protein confers instability to its fusion partner. A proven example of such a domain is the 107-amino acid FKBP L106P peptide [13]. FKBP L106P can confer instability when fused to the myosin light chain with 1-10% residual protein function. A small, cell-permeable ligand called Shld-1 can also be added in nM concentrations to stabilize the FKBP domain and restore function to the protein of interest. This effect is rapid and reversible [13]. I will express this unstable form of myosin in both WT and *pten* pupal wing tissues and assess its effect on adherens junction elongation. In WT tissues, I expect adherens junctions to elongate more quickly than usual. In *pten* tissues, a complete rescue of the shortened junction phenotype would suggest that removal of MyoII from the membrane is both necessary and sufficient for junction elongation. A partial rescue of the mutant phenotype, for example junctions that stabilize more quickly but don't achieve the length of wild-type junctions, would suggest that MyoII removal is necessary but not sufficient for proper junction elongation and stabilization during planar cell rearrangements.

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