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A cytoskeletal activator and inhibitor are downstream targets of the *frizzled/starry night* planar cell polarity pathway in the Drosophila epidermis

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ABSTRACT

The *frizzled* pathway regulates the planar polarity of epithelial cells. In insects this is manifested by the polarity of cuticular structures such as hairs (trichomes) and sensory bristles. A variety of evidence has established that this is achieved by regulating the subcellular location for activating the cytoskeleton in the epithelial cells. How this is accomplished is still poorly understood. In the best-studied tissue, the Drosophila pupal wing two important cytoskeletal regulators have been identified. One, *shavenoid* (*sha*), appears to be an activator while the second *multiple wing hairs* (*mwh*), appears to be an inhibitor. In vitro biochemistry has confirmed that the Multiple Wing Hairs protein inhibits the elongation of F-actin chains and surprisingly that it also bundles F-actin. These two activities can explain the multifaceted *mwh* mutant phenotype.

Contents

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1. Introduction

1.1. Planar cell polarity

When cell biologists talk about cell polarity they are usually referring to apical/basal polarity, which has been so extensively studied in epithelial cells. A second type of cellular polarity that is also often exhibited by epithelial cells is planar cell polarity (PCP). As the name suggests this is polarity within the plane of the epithelium (Goodrich and Strutt, 2011; Adler, 2012; Aw and Devenport, 2017; Butler and Wallingford, 2017). This is seen on the surface of many animals. For example, the hair on a mouse points distally on the limbs and posteriorly on the body (Wang and Nathans, 2007). Similarly, the scales on fish and the feathers on birds also display a similar PCP. PCP is not limited to vertebrates and is common in insects (Lawrence, 1972; Adler, 1992). For example, the sensory bristles and cuticular hairs that cover the surface of Drosophila all point in the same direction in any body region.

1.2. The Drosophila wing as a model system

PCP is exhibited by many regions on the fly epidermis but the fly wing has proven to be the most important model tissue (Wong and Adler, 1993). The wing has the advantages that it is flat making it favorable for microscopy, almost all wing cells differentiate in the same way and the developing pupal wing can easily be dissected from pupae in a pure form facilitating biochemical experiments (Wong and Adler, 1993; Ren et al., 2005; Sobala and Adler, 2016).

The adult wing contains few living cells. Most wing cells are lost soon after the eclosion of the animal from the pupal case leaving only the cuticular wing, which is essentially a cast of the apical surfaces of the wing cells. Each cell elaborates a single distally pointing hair that is formed in the pupal stage from a cytoplasmic outgrowth (sometimes called a prehair) that contains peripheral F-actin and central microtubules (Wong and Adler, 1993; Eaton et al., 1996; Turner and Adler, 1998). Disruptions in the function of either of these cytoskeletons results in morphologically abnormal hairs (see e.g. (Petersen et al., 1994) (Tilney et al., 1995) (Eaton et al., 1996; Turner and Adler, 1998; Wulfkuhle et al., 1998; Geng et al., 2000; Kiehart et al., 2004; Ren et al., 2007) (Bitan et al., 2012; Otani et al., 2015)). In the adult wing all of the cuticular hairs point distally. In the pupal wing all of the cytoplasmic prehairs also point distally from early in their growth (Wong and Adler, 1993). In a wild type wing the activation of the actin cytoskeleton that drives the outgrowth of the pre-hair is restricted to a small the region around the distal edge/vertex of the cell. This region represents 7–8% of the apical cell surface (Lu and Adler, 2015) (Fig. 1A). The pre-hair grows out away from the cell edge/vertex and hence points distally throughout its growth. Genetic studies indicated that this spatial restriction is a consequence of the action of the genes of the *fz/stan* PCP pathway (Wong and Adler, 1993) (Lu et al., 2015). Interestingly all of the proteins encoded by pathway genes accumulate asymmetrically in pupal wing cells prior to hair initiation (see e.g (Usui et al., 1999; Axelrod, 2001) (Strutt, 2001; Bastock et al., 2003) (Das et al., 2004) (Strutt and Warrington, 2008) (Yan et al., 2008) (Wang et al., 2017)). Some proteins accumulate on the distal side of wing cells (Frizzled (Fz), Dishevelled (Dsh) and Diego (Dgo), some on the proximal side (Van Gogh (Vang), Prickle (Pk), Inturned (In), Fuzzy (Fy), Fritz (Frtz) and Multiple Wing Hairs (Mwh)), and one on both sides (Starry Night (Stan)). Mutations that inactivate one or more genes in the pathway lead to the cytoskeleton being activated in an abnormal location and to the prehair growing in an abnormal direction (Wong and Adler, 1993).

In thinking about the pathway in the fly epidermis it useful to consider the pathway as a hierarchy consisting of three tiers (one upstream, one middle tier and one downstream). Mutants in all three tiers have similar polarity phenotypes but differ in how frequently mutant cells form more than the normal one hair. This is infrequent for mutations in upstream genes (*fz, dsh, dgo, Vang, pk and stan)* in the pathway but common for mutations in the middle (*in, fy, frtz*) and downstream (*mwh*) genes (Wong and Adler, 1993). For this phenotype, the downstream gene *mwh* has the strongest phenotype. *in, fy* and *frtz* wing cells average about 1.7 hairs per cell while *mwh* cells average almost 4 hairs per cell (Wong and Adler, 1993). The middle and downstream genes are epistatic to the upstream genes so that *frtz; fz* and *mwh fz* double mutants display the *frtz* and *mwh* phenotypes respectively. Further, *mwh* is epistatic to the middle tier genes so that a *frtz; mwh* double mutant resembles *mwh* (Wong and Adler, 1993). In addition, the middle and downstream proteins proximal accumulation is dependent on and instructed by the upstream proteins (Lu et al., 2010) (Strutt and Warrington, 2008). The upstream tier regulates cell-cell communication and the specification of the distal and proximal sides of cells. The middle tier (*in, fy* and *frtz*) serves to transduce the polarity signal from the upstream components to the downstream component *mwh* by localizing Mwh to the proximal side of the cell (Lu et al., 2010). It is worth noting that under some unusual circumstances the middle tier can influence the function and asymmetric localization of the upstream components (Wang et al., 2017). As will be discussed further below Mwh acts as a key regulator of the cytoskeleton.

1.3. Hair morphogenesis is more complicated than originally imagined

Following the process of hair formation in living pupal wing cells showed the process was more complicated than originally thought (Lu et al., 2015). As noted above an early sign of hair initiation is the accumulation of F-actin in a relatively small region (~7–8% of apical surface) around the distal apical edge of the cell (Fig. 1A). Several thin actin filled proto-hairs form in this region and grow in the distal direction away from the edge of the cell (Lu et al., 2015). These proto hairs soon fuse and form a single distally pointing prehair. This coalescence was usually completed by the

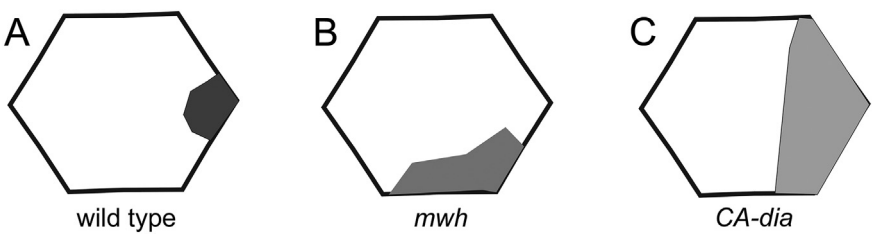

Fig. 1. Localized activation of cytoskeleton. Shown are examples of wing cells with regionsof increased F-actin shown in grey. A is a wild type cell, B a *mwh* cell and C a cell where CA-Dia is expressed. Similar results were obtained both by staining fixed cells with a fluorescent phalloidin or by in vivo imaging of pupae expressing a fluorescent actin reporter.

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