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Drosophila KASH-domain protein Klarsicht regulates microtubule stability and integrin receptor localization during collective cell migration

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ABSTRACT

During collective migration of the *Drosophila* embryonic salivary gland, cells rearrange to form a tube of a distinct shape and size. Here, we report a novel role for the *Drosophila* Klarsicht-Anc-Syne Homology (KASH) domain protein Klarsicht (Klar) in the regulation of microtubule (MT) stability and integrin receptor localization during salivary gland migration. In wild-type salivary glands, MTs became progressively stabilized as gland migration progressed. In embryos specifically lacking the KASH domain containing isoforms of Klar, salivary gland cells failed to rearrange and migrate, and these defects were accompanied by decreased MT stability and altered integrin receptor localization. In muscles and photoreceptors, KASH isoforms of Klar work together with Klaroid (Koi), a SUN domain protein, to position nuclei; however, loss of Koi had no effect on salivary gland migration, suggesting that Klar controls gland migration through novel interactors. The disrupted cell rearrangement and integrin localization observed in *klar* mutants could be mimicked by overexpressing Spastin (Spas), a MT severing protein, in otherwise wild-type salivary glands. In turn, promoting MT stability by reducing *spas* gene dosage in *klar* mutant embryos rescued the integrin localization, cell rearrangement and gland migration defects. Klar genetically interacts with the Rho1 small GTPase in salivary gland migration and is required for the subcellular localization of Rho1. We also show that Klar binds tubulin directly *in vitro*. Our studies provide the first evidence that a KASH-domain protein regulates the MT cytoskeleton and integrin localization during collective cell migration.

1. Introduction

Collective cell migration is a fundamental process during embryogenesis as well as in pathological conditions, such as cancer. During morphogenesis, cells migrate collectively to form and shape tissues and organs. For cells to migrate collectively as an intact group, multiple cellular processes, such as cell shape changes, cell rearrangements and modulation of cellular adhesions, have to be coordinated spatially and temporally.

The mechanisms by which cells migrate collectively are still poorly understood. Studies in genetically tractable model organisms have made important contributions to dissecting these complex processes. In particular, the *Drosophila* embryonic salivary gland is an excellent system for studying how cells migrate collectively as an intact tubular structure. The salivary glands consist of a pair of elongated secretory tubes that are connected to the larval mouth by the duct tubes (Chung et al., 2014; Maruyama and Andrew, 2012; Pirraglia and Myat, 2010). After salivary gland cells invaginate from the ventral surface of the embryo, the tube is initially oriented dorsally. The entire salivary gland then turns posteriorly and migrates beginning with cells at the distal end. Collective migration of the salivary gland is dependent on distinct activities at the distal and proximal ends that are coordinated temporally and spatially. Proximal gland cells change shape from columnar to cuboidal, rearrange and migrate in a manner dependent on Rho and Rac GTPases (Pirraglia et al., 2013; Xu et al., 2011, 2008). In contrast, the distal gland cells elongate and extend basal membrane protrusions by forming integrin mediated contacts

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with surrounding mesoderm-derived tissues (Bradley et al., 2003; Pirraglia et al., 2013). During salivary gland migration, two classes of integrin adhesion receptors become enriched at gland–mesoderm contact sites: αPS1βPS (expressed in the salivary gland) and αPS2βPS (expressed in the surrounding mesoderm) (Jattani et al., 2009). This integrin accumulation is functionally important as the salivary gland fails to turn and migrate posteriorly in embryos mutant for integrin subunits, such as *myospheroid* (encoding the βPS subunit), *multiple edamatous wings* (encoding αPS1) or *inflated* (encoding αPS2) (Bradley et al., 2003). αPS1βPS integrin controls salivary gland migration by downregulating E-cadherin and promoting basal membrane protrusions through Rac1 in the distal gland cells (Pirraglia et al., 2013).

Nuclear envelope spectrin-repeat proteins (Nesprins) are well characterized as nuclear-cytoplasmic linker proteins. They localize to the outer nuclear membrane of the nuclear envelope (NE) via their Klarsicht/ANC-1/Syne Homology (KASH) domains. KASH proteins connect the nucleoskeleton and the cytoskeleton through their interactions with SUN domain proteins that reside in the inner nuclear membrane (Starr and Fischer, 2005; Yu et al., 2011). They interact either directly with F-actin or indirectly with intermediate filaments and microtubules (MT), the latter through associations with the MT motor proteins kinesin and dynein (Mellad et al., 2011; Starr and Fridolfsson, 2010). In *Drosophila*, the KASH-domain protein Klarsicht (Klar) mediates nuclear migration in photoreceptor cells (Fischer et al., 2004; Starr and Fischer, 2005; Welte et al., 1998) and regulates nuclear positioning in striated muscle (Elhanany-Tamir et al., 2012).

It is becoming increasingly clear that in addition to their established roles in linking the nucleoskeleton and cytoskeleton, nesprins and their relatives play critical functions in the cytoplasm. Numerous isoforms of the mammalian KASH proteins have been shown to localize to structures other than the nuclear envelope, such as the plasma membrane and Golgi (Raigor and Shanahan, 2013) and link, for example, P bodies to microtubules (Raigor et al., 2014). *Drosophila* Klar also performs cytoplasmic functions: we previously showed that Klar controls salivary gland lumen size, possibly through the targeted transport of the apical transmembrane protein Crumbs (Myat and Andrew, 2002). Klar also regulates the transport of lipid droplets in early embryos (Guo et al., 2005; Welte et al., 1998) and of *oskar* mRNA in the oocyte (Gaspar et al., 2014). For lipid-droplet and RNA transport, these cytoplasmic roles of Klar are mediated by distinct isoforms that lack the KASH domain and arise by alternative splicing (Gaspar et al., 2014; Guo et al., 2005; Kim et al., 2013). Here, we report a novel role for KASH-containing forms of Klar in salivary gland migration that is mediated by regulation of MT stabilization and integrin receptor localization.

2. Results

2.1. Klarsicht is required for salivary gland migration

There are five isoforms of klar with klar α, γ and δ containing the conserved KASH domain (Supplementary Fig. 1) (Kim et al., 2013). We previously showed that in *Df(3L)emc^{E12}* homozygous embryos that completely lack *klar* salivary gland morphology was grossly disrupted (Myat and Andrew, 2002). To determine a role for Klar in salivary gland migration, we analyzed embryos with lesions in *klar*. In particular, we focused on the function of KASH-domain containing isoforms and employed alleles that specifically lack the KASH domain. *klar^{mCD4}* contains a nonsense mutation just before the KASH domain whereas *klar^{mBX13}* has chromosomal breaks before the KASH domain (Fischer et al., 2004; Guo et al., 2005). Loss of *klar* resulted in glands that failed to migrate where the distal cells initiated the posterior turn but the proximal cells did not turn (Fig. 1B). This is in contrast to glands of *klar* heterozygous embryos where both the distal and proximal gland cells turned and migrated posteriorly (Fig. 1A). Quantification of the migration defect showed that 65% of *klar^{mCD4}* mutant glands failed to migrate completely by stage 14 as opposed to only 10% of glands of heterozygous siblings (Fig. 1C). The gland migration defects of *klar* mutant embryos were accompanied by defects in the rearrangement of proximal gland cells as manifested by an increased number of cells surrounding the lumen of the gland (Fig. 1D–G). In wild-type glands, eight cells on average surround the lumen whereas in *klar^{mCD4}* and *klar^{mBX13}* mutant glands 12 and 11 cells surround the lumen, respectively (Fig. 1D–G). In wild-type salivary gland cells, endogenous Klar was enriched in the apical domain and also localized to discrete puncta in the basolateral and apical domains (Supplementary Fig. 2A). Expression of wild-type Klar α, the longest KASH-domain containing isoform, specifically in the salivary glands of *klar* mutant embryos rescued the migration and cell rearrangement defects, demonstrating that *klar* is required cell-autonomously for gland migration (Fig. 1C and G).

2.2. Integrin localization is altered in klar mutant salivary gland cells

The salivary gland migration defect of *klar* mutant embryos resembled that of embryos with mutations in integrin subunits where distal gland cells turn posteriorly but proximal gland cells do not (Bradley et al., 2003; Pirraglia et al., 2013). For example, in embryos homozygous for *mew^{M6}*, a mutation in the αPS1 integrin subunit, 100% of salivary glands fail to complete posterior turning (see also Fig, 1C). To test if *klar* and integrins act in the same pathway, we analyzed embryos *trans*-heterozygous for *klar^{mBX13}* and *mew^{M6}*: 55% of glands failed to complete migration (Fig. 1C). Because of this genetic interaction, we tested if *klar* mutations affect the subcellular localization of the βPS integrin receptor in migrating salivary glands. We previously showed that the βPS and αPS2 integrin subunits become enriched at sites of contact between the migrating salivary gland and the overlying circular visceral mesoderm and underlying fat body (Jattani et al., 2009). In salivary glands of *klar^{mBX13}* heterozygous embryos, as in wild-type embryos, βPS integrin was localized predominantly at the basal membranes in contact with surrounding tissues and only at low levels at the apical membrane (Fig. 2A and data and shown). By contrast, in glands of *klar^{mBX13}* homozygous embryos, βPS integrin was not enriched at gland–mesoderm contact sites and instead was enriched in the apical domain (Fig. 2B). Quantification of βPS integrin levels based on measurements of fluorescent intensity ratio between the basal gland–mesoderm contact site and the apical membrane revealed significant enrichment in the apical membrane and reduction at the gland–mesoderm contact site in *klar^{mBX13}* homozygous embryos compared to heterozygous siblings (Fig. 2C). In contrast, apical membrane markers, such as aPKC and Crumbs and the adherens junction protein E-cadherin, which marks apical–lateral membranes, showed no difference in salivary gland cells of *klar* homozygous and heterozygous embryos (Supplementary Fig. 3 and data not shown). Thus, the altered localization of βPS integrin in *klar* mutant salivary glands does not reflect loss of apical–basal polarity and, rather, represents a specific defect. Klar regulation of βPS integrin likely occurs post-transcriptionally since RNA *in situ* hybridization for *myospheroid* encoding βPS integrin showed no differences between *klar* heterozygous and homozygous embryos (data not shown).

2.3. Klaroid is not required for salivary gland migration

In photoreceptors and muscle cells, KASH-domain isoforms of Klar have critical roles in positioning of nuclei (Elhanany-Tamir