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Signaling through the G-protein-coupled receptor Rickets is important for polarity, detachment, and migration of the border cells in *Drosophila*

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A B S T R A C T

Cell migration plays crucial roles during development. An excellent model to study coordinated cell movements is provided by the migration of border cell clusters within a developing *Drosophila* egg chamber. In a mutagenesis screen, we isolated two alleles of the gene *rickets* (*rk*) encoding a G-protein-coupled receptor. The *rk* alleles result in border cell migration defects in a significant fraction of egg chambers. In *rk* mutants, border cells are properly specified and express the marker Slbo. Yet, analysis of both fixed as well as live samples revealed that some single border cells lag behind the main border cell cluster during migration, or, in other cases, the entire border cell cluster can remain tethered to the anterior epithelium as it migrates. These defects are observed significantly more often in mosaic border cell clusters, than in full mutant clusters. Reduction of the Rk ligand, Bursicon, in the border cell cluster also resulted in migration defects, strongly suggesting that Rk signaling is utilized for communication within the border cell cluster itself. The mutant border cell clusters show defects in localization of the adhesion protein E-cadherin, and apical polarity proteins during migration. E-cadherin mislocalization occurs in mosaic clusters, but not in full mutant clusters, correlating well with the *rk* border cell migration phenotype. Our work has identified a receptor with a previously unknown role in border cell migration that appears to regulate detachment and polarity of the border cell cluster coordinating processes within the cells of the cluster themselves.

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1. Introduction

Collective cell migration is an important mechanism employed in critical developmental processes, including vertebrate neural crest migration (reviewed in Gammill and Bronner-Fraser (2003), Sauka-Spengler and Bronner-Fraser (2008)) and the wound healing response (reviewed in Valluru et al. (2011)). Misregulation of cell motility is also associated with human disease such as heart malformation and cancer metastasis (reviewed in Balzer and Konstantopoulos (2012), Castano et al. (2011) and Savagner (2010)). Here, we focus on border cell migration during *Drosophila* oogenesis, a well-established model system to study regulation of collective cellular migration (Montell, 2003; Montell et al., 2012; Rorth, 2002).

Border cell migration occurs during Stage 9 of oogenesis. At this stage, the *Drosophila* egg chamber consists of 16 germline cells – the oocyte and 15 nurse cells – surrounded by a monolayer of somatically derived follicle epithelial cells (Spradling, 1993). The

border cells initiate from within this somatic epithelium at the anterior of the egg chamber (Montell et al., 1992). Two specialized polar cells located within the epithelium at the anterior tip of the egg chamber secrete the ligand Unpaired (Upd), which activates the JAK/STAT pathway in neighboring epithelial cells (Ghiglione et al., 2002; Silver and Montell, 2001). This results in expression of the border cell specification marker Slow border cells (Slbo) and subsequent formation of the outer border cells (Silver and Montell, 2001). Once the border cell cluster is formed, its migration is then guided from the anterior epithelium posteriorly to the border between the oocyte and the nurse cells by guidance cues released from the oocyte (Duchek and Rorth, 2001; Duchek et al., 2001; McDonald et al., 2003). Border cell migration is typically complete by Stage 10 of oogenesis, and the biological function of the border cells once they complete their migration is to contribute to formation of the micropyle (Montell et al., 1992).

In order for the border cells to properly detach from the follicular epithelium, polarity and adhesion of the border cell cluster must be tightly regulated. While the border cells are still within the epithelium, they exhibit typical epithelial polarity with apical

markers such as Bazooka (Baz) and Par-6 on the apical side, and lateral markers such as Par-1 present basolaterally. The border cells undergo a shift in polarity when they detach and migrate. Disruption of apical polarity proteins interferes with the ability of the border cell cluster to migrate appropriately (McDonald et al., 2008; Pinheiro and Montell, 2004). Polarity proteins including Par-6 and Baz are imperative for the proper localization of the adhesion proteins E-cadherin and beta-Integrin (Pinheiro and Montell, 2004). E-cadherin allows border cells to remain together as a cluster, and E-cadherin and Integrin together provide traction to migrate across the nurse cells (Fox et al., 1999; Llense and Martin-Blanco, 2008; Niewiadomska et al., 1999).

Rickets (Rk) is a G-protein-coupled receptor that is known to play a role in *Drosophila* cuticle tanning and wing expansion (Baker and Truman, 2002; Natzle et al., 2008). Prior to expansion, the *Drosophila* wing consists of a sheet of epithelial cells. These cells undergo an epithelial-to-mesenchymal transition (EMT) and migrate out of the wing, allowing the wing membrane to unfold and flatten (Kiger et al., 2007; Natzle et al., 2008). Rk, and its heterodimeric ligand Bursicon consisting of Bursicon alpha (Burs alpha) and Partner of Bursicon (Pburs) (Baker and Truman, 2002; Dewey et al., 2004; Mendive et al., 2005; Luo et al., 2005), are important for EMT exhibited by the epithelial cells within the initially folded wing (Natzle et al., 2008), although its precise downstream effects have not been fully elucidated.

Here, we show a novel role for the G-protein-coupled receptor, Rickets (Rk), in border cell migration. We find that Rk is important for allowing the border cells to properly organize their polarity during migration. When Rk activity in the border cells is compromised, the adhesion protein E-cadherin, and apical polarity markers such as atypical Protein Kinase C (aPKC) and Par-6 become mislocalized within the border cells. In addition, individual cells often lag behind the main migrating border cell cluster, and in some cases, remain tethered to the anterior epithelium. Interestingly, we find that mosaic border cell clusters exhibit more significant migration defects than border cell clusters in which all of the cells are mutant. These findings demonstrate that a G-protein-coupled receptor previously implicated in an EMT-like process in the wing can also regulate collective cell movement in oogenesis. This receptor appears to be involved in intercellular communication, resulting in correct E-cadherin and polarity protein distribution among a group of migrating cells.

2. Materials and methods

2.1. Fly strains and genetics

The rk^{JL78} and rk^{KB61} alleles were generated in an EMS mutagenesis screen performed on chromosome 2L. This screen utilized mosaic egg chambers and sought to identify genes affecting general ovarian follicle cell development, similarly to the screen performed on the X chromosome (Denef et al., 2008). The rk^{JL78} allele was sequenced and found to contain a small deletion affecting the 4th intron and 5th exon of the *rk* gene. The rk^{w11p} allele (Bloomington *Drosophila* Stock Center) was recombined onto an *FRT40A* chromosome. Mosaic egg chambers containing homozygous *rk* mutant border cells were generated with the FRT/UAS-Flp/Gal4 system (Duffy et al., 1998), using *y w hsflp; ubi-GFP FRT40A* flies and heat shocking larvae and pupae at 37 °C for 1 h, 2–3 times. *rk* RNAi (Vienna *Drosophila* Resource Center, VDRC; line v29931) was driven by *upd*-Gal4 and *slbo*-Gal4 drivers in the *upd*-Gal4; *slbo*-Gal4, *UAS-GFP/ CyO* line (gift from S. Noselli). For live imaging experiments, *rk* RNAi was driven with the *slbo*-Gal4 driver. A line expressing *rk*-Gal4 consisting of a T2A-Gal4 in frame fusion with the *rk* coding sequence was obtained from B. H. White (Diao and

White, 2012). *Bursicon alpha* allele *burs alpha^{Z5569}* was a gift from J. Kiger. RNAi against *burs alpha* was induced by the JF02260 line from the TRiP collection, which has been used in the field as a tool to study *burs alpha* (Loveall and Deitcher, 2010) and has only one predicted amplicon with no off-target effects as per the TRiP web page. RNAi against Par-6 and aPKC was induced by lines HMS01410 and GL00007 respectively, from the TRiP collection. These lines as well as FRT and Flp lines were obtained from the Bloomington *Drosophila* Stock Center.

2.2. Complementation tests

Complementation tests with *rk* alleles rk^1, rk^4, and rk^{w11p} confirmed that *rk* was the affected gene in the line *JL78* from our screen. We also performed complementation tests with the *KB61* line and either *JL78* or one of the deficiency lines *Df(2L)BSC252* and *Df(2L)ED793*. Both *JL78* and *KB61* yield some homozygous escapers in trans to deficiencies that delete *rk*, as well as in trans to each other. Those escapers show the folded wing phenotype originally described for *rk* mutants (Baker and Truman, 2002). The rk^1 and rk^4 alleles as well as deficiency lines were obtained from the Bloomington *Drosophila* Stock Center.

2.3. Immunofluorescence

Ovaries were dissected in phosphate buffered saline (PBS) and kept on ice before being fixed in 4% paraformaldehyde (PFA) for 15 min. Standard staining procedures were then utilized according to Ashburner (1989). Primary antibodies used were rat anti-Slbo (1:500, gift from P. Rorth) (Borghese et al., 2006), rabbit anti-Baz (1:500, gift from J. Zallen), rabbit anti-Par-6 (1:500, gift from D. J. Montell) (Pinheiro and Montell, 2004), rabbit anti-aPKC (1:1000, DSHB), rabbit anti-GFP (1:500, Millipore), mouse anti-Sn (1:20, DSHB) and rat anti-DECadherin (1:20, DSHB, DCAD2). Secondary antibodies were conjugated to AlexaFluor568 (1:1000, Molecular Probes). Phalloidin conjugates and Hoechst were from Molecular Probes and used at 1:1000, with the exception of Phalloidin conjugated to AlexaFluor 647 which was used at 1:100. Images were taken with a Nikon A1 confocal microscope, a Nikon A1-RS spectral confocal microscope, and a Zeiss LSM510 confocal microscope.

2.4. Live imaging

Border cell migration was observed in egg chambers expressing *rk* RNAi (VDRC, line v29931) driven by *slbo*-Gal4. Cell membranes were visualized with a Gap43-mCherry marker (Martin et al., 2010), which labels the membranes of all cells, with especially strong signal surrounding the polar cells. Females were fed yeast for 40 h prior to dissection. Individual ovarioles were dissected out of the ovary, and the germaria and older egg chambers were removed from these ovarioles. Dissection was performed in media previously described (Prasad et al., 2007; Domanitskaya et al., 2014). Droplets of media containing dissected ovarioles were imaged with a Nikon Ti-E spinning disc confocal microscope equipped with a Perfect Focus System, a Yokogawa spinning disc, and a Hamamatsu detector. We imaged border cell cluster detachment and early migration for approximately 4–6 h in *rk* RNAi expressing egg chambers, and wild type egg chambers expressing the Gap43-mCherry membrane marker as a control. A 561 nm laser was utilized to detect the mCherry signal. 10–20 μm Z stacks were imaged to capture the whole border cell cluster, with Z stacks containing 1 μm steps taken every 3 min.

2.5. Statistical analysis

Statistical significance of differences between mutant and control phenotypes such as migration distance, detachment