LKB1 regulates polarity remodeling and adherens junction formation in the *Drosophila* eye

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The serine–threonine kinase LKB1 regulates cell polarity from *Caenorhabditis elegans* to man. Loss of *lkb1* leads to a cancer predisposition, known as Peutz–Jeghers Syndrome. Biochemical analysis indicates that LKB1 can phosphorylate and activate a family of AMPK- like kinases, however, the precise contribution of these kinases to the establishment and maintenance of cell polarity is still unclear. Recent studies propose that LKB1 acts primarily through the AMP kinase to establish and/or maintain cell polarity. To determine whether this simple model of how LKB1 regulates cell polarity has relevance to complex tissues, we examined *lkb1* mutants in the *Drosophila* eye. We show that adherens junctions expand and apical, junctional, and basolateral domains mix in *lkb1* mutants. Surprisingly, we find LKB1 does not act primarily through AMPK to regulate cell polarity in the retina. Unlike *lkb1* mutants, *ampk* retinas do not show elongated rhabdomeres or expansion of apical and junctional markers into the basolateral domain. In addition, nutrient deprivation does not reveal a more dramatic polarity phenotype in *lkb1* photoreceptors. These data suggest that AMPK is not the primary target of LKB1 during eye development. Instead, we find that a number of other AMPK-like kinase, such as SIK, NUAK, Par-1, KP78a, and KP78b show phenotypes similar to weak *lkb1* loss of function in the eye. These data suggest that in complex tissues, LKB1 acts on an array of targets to regulate cell polarity.

AMPK | SIK | NUAK | Par-1 | KP78

Mutations in *lkb1* result in Peutz–Jeghers syndrome (PJS), a disease characterized by benign gastrointestinal hamartomatous polyps. PJS patients are predisposed to develop malignant cancers of epithelial tissue origin throughout their lifetime. LKB1 (Par-4/XEEK1/STK11) is a serine/threonine kinase (1), and most of the identified mutations in PJS patients have inactivating mutations in the kinase domain (2).

LKB1 (Par-4) is essential for the correct distribution of polarity determinants during *Caenorhabditis elegans* (3, 4) and *Drosophila* (5) development. In mice, loss of LKB1 leads to embryonic lethality and neural tube defects (6), and *lbk1* heterozygous mice exhibit intestinal polyps (7). In mammalian cells, overexpression of LKB1 can induce polarization of membranes in the absence of cell contacts (8). It is thought that LKB1's role in cancer may be linked regulation of cell polarity.

In *Drosophila* epithelia, membranes are subdivided into 3 domains: the subapical region (SAR), the zonula adherens (ZA), and the septate junctions (SJ) (9). The SAR is located apical to the ZA and comprises 2 essential complexes: Crumbs (Crb)/Stardust (Sdt)/PatJ and Bazooka (Baz; Par3)/Par6/aPKC. These complexes interact to regulate ZA formation (9). ZA formation also depends on E-cadherin and Armadillo (Arm; β-catenin), which join the plasma membrane to the intracellular Actin cytoskeleton and mediate adhesive contacts between cells. Basal to the ZA is the SJ, composed of the Scribble/Lgl/Dlg complex, which regulate the Crb and Baz complexes (10, 11). The SJ functions as a barrier to paracellular diffusion (10).

LKB1 has been extensively examined in *Drosophila* (5, 12, 13). In *lkb1* embryos and larval wing discs, apical and basolateral markers are mislocalized (13). Notably, in follicle cells, severe defects in epithelial polarity were observed in large *lkb1* clones but not in smaller clones induced during later cell divisions (5). Polarity defects become fully penetrant under glucose starvation, suggesting a link between cell polarity and energy levels (12). LKB1 can phosphorylate and activate AMP kinase (AMPK) and the AMPK-like family of proteins (1). AMPK regulates tight junctions (14, 15) and *ampk*$\alpha^{-/-}$ mutants phenocopy *lkb1* polarity defects in embryos and follicle cells. Significantly, *lkb1* mutants can be rescued by the expression of a phosphomimetic version of AMPKα (AMPKα^{T184D}) (12, 13). These data have led to a model whereby LKB1 regulates polarity establishment via AMPK.

There may be tissue-specific differences in how LKB1 regulates polarity. In *lkb1* follicle cells, aPKC and Arm become diffuse or ectopically localized along lateral membranes (5). In low-energy conditions, polarity defects worsen. Dystroglycan extends laterally and occasionally mislocalizes to the apical domain and F-actin accumulates apically. aPKC, Coracle, Crb, Dlg, and E-Cadherin are lost, but Baz is not affected (12). In contrast, in *lkb1* embryos, aPKC, Baz, Arm, and Dlg lose their apical localization and become more basal (13). Although Par-1 appears to be a critical direct target of LKB1 in some tissues (1, 16), polarity establishment in the embryo is independent of Par-1 (13).

The *Drosophila* retina arises from the eye imaginal disc, a columnar epithelium that undergoes a dramatic remodeling of tissue structure during pupal development. Cells undergo a 90° rotation that turns the apices of the photoreceptor cells (PRCs) toward each other; a process that depends on the adherens junction (AJ) (17). Between 37% and 55% pupal development (pd), the PRC apical surfaces expand, dramatically increasing in depth perpendicular to the plane of the epithelium. At ~37% pd, PRCs apical surfaces begin to differentiate into rhabdomeres (16).

Here, we show that LKB1 regulates apical–basal polarity in the *Drosophila* eye. Loss of LKB1 does not affect the establishment of polarity but, rather, the dramatic remodeling of polarity that occurs in the pupal retina. LKB1 also is needed to restrict the length and placement of AJs. The effects of loss of LKB1 are independent of nutritional status, and loss of *ampk*α in PRCs does not lead to polarity defects in the retina, irrespective of nutrient conditions. Instead, we found that Par-1 and the hitherto uncharacterized *Drosophila* AMPK-like kinases NUAK, SIK, KP78a, and KP78b contribute to epithelial polarity in the retina and that loss of these genes yields phenotypes similar to (although weaker than) *lkb1*.

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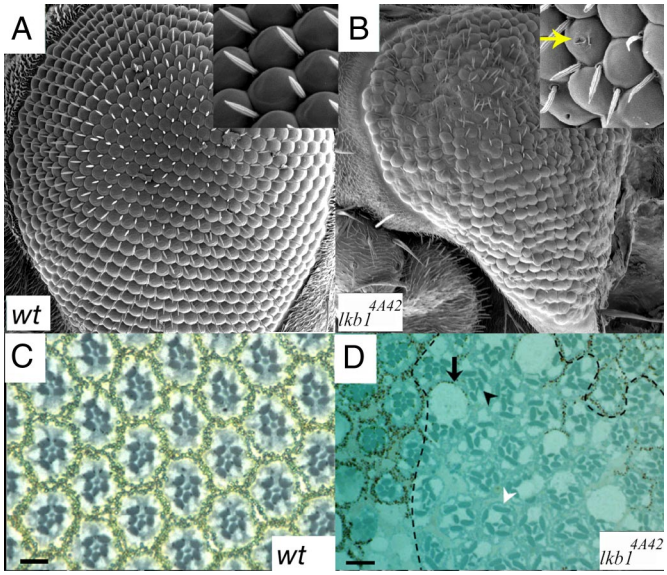

Fig. 1. Mutation of *lkb1* disrupts eye development. (*A*) SEMs of a wild-type eye show a regular array of ommatidia and bristles. (*B*) SEMs of the $lkb1^{4A4\text{-}2}$ eye; defects include fused ommatidia, missing and excess bristles, and disorganized bristles. The eye is also smaller, rougher, and misshapen with ''pitting'' of the surface (*Inset*). (*C*) Light micrograph of a 1-μm cross section through a wild-type retina reveals a stereotypical arrangement of photoreceptors and ommatidia. (*D*) *lkb1* clones are identified by the lack of pigment and are contained within dashed lines. Loss of *lkb1* leads to a loss of photoreceptors (black arrowhead), misshapen rhabdomeres (white arrowhead), and enlarged cell bodies (black arrow). (Scale bars, 10 μm.)

Thus, in contrast to recent studies that have proposed that AMPK is the major effector downstream of LKB1, we find that in the more elaborately polarized pupal retina, LKB1 acts on diverse targets to regulate polarity and morphogenesis.

Results

Mutation of *lkb1* Leads to the Disruption of Pupal Photoreceptor Development. To examine the role of LKB1 in eye development, we used 2 different *lkb1* alleles. $lkb1^{4A4\text{-}2}$ has a deletion removing the untranslated region, the start codon and the start of the ORF, and $lkb1^{4B1\text{-}11}$ contains a nonsense mutation at amino acid 98, disrupting the coding region for the kinase domain (5). We used the FLP/FRT system to analyze homozygous $lkb1^{4A4\text{-}2}$ and $lkb1^{4B1\text{-}11}$ tissue. FLP was driven by the *eyeless* promoter and a *Minute* mutation was included on the wild-type chromosome to slow proliferation of wild-type cells, so the eye was largely composed of *lkb1* tissue.

$lkb1^{4A4\text{-}2}$ and $lkb1^{4B1\text{-}11}$ mutant retinas were smaller than wild type, suggesting possible defects in growth and/or apoptosis (Fig. 1). Bristle and ommatidia organization was disrupted (Fig. 1*B*). We also observed pitting in *lkb1* mutant ommatidia (Fig. 1*B Inset*). Pitting indicates defects in the lens material secreted by cone cells, and suggests defects in cone cells structure or function. Mutant retinas occasionally had small black spots in the center of the eye, suggesting cell death (18). $lkb1^{4B1\text{-}11}$ eyes were intermediate in size between $lkb1^{4A4\text{-}2}$ and wild-type retinas, and defects in ommatidial and bristle organization were less severe than $lkb1^{4A4\text{-}2}$ retinas [compare Fig. 1*D* and supporting information (SI) Fig. S1*A*], suggesting that $lkb1^{4B1\text{-}11}$ possesses residual function. However, apart from the differences in severity between the alleles, all phenotypes described here were observed in both alleles.

To analyze phenotypes at a higher resolution, we examined 1-μm sections. Sections revealed severe disruption of photoreceptor morphology with ommatidia containing extra or missing photoreceptor cells (PRCs). R7 was frequently lost (Fig. 1*D*). PRCs were frequently enlarged, and rhabdomeres were elongated (white arrowhead). These phenotypes are reminiscent of $crb^{-/-}$ clones in the retina (19, 20). The $lkb1^{-/-}$ phenotype could be rescued by transgenic expression of LKB1 (Fig. S1*C*), confirming that these defects are due to loss of LKB1.

These phenotypes could be due to misspecification of PRCs, defects in epithelial polarity, and/or cell death. To distinguish between these possibilities, we looked earlier in development. We found in larval discs that cell fate was unaffected in *lkb1* mutant clones. Spalt was used to mark the R3 and R4 PRCs (21), Boss to highlight the R8 (22), Prospero to mark the R7 PRCs (23), Bar to mark PRCs 1 and 6 (24), and Rough to mark R2 and R5 (25). The full complement of correctly specified PRCs are present in larval $lkb1^{-/-}$ clones (Fig. S2). Bar staining in *lkb1* clones revealed mild defects in PCP patterning (see also Fig. S1*B*), consistent with a weak link between PCP and cell polarity (26). $lkb1^{-/-}$ larval tissue also maintains correct polarity, as assessed by staining of aPKC, Arm, and phalloidin (Fig. 2*A*).

During pupal development, the apical surfaces of cells in the

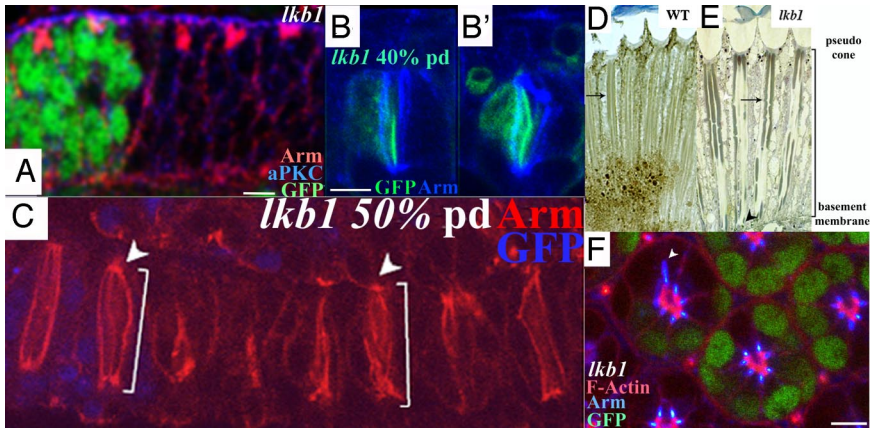

Fig. 2. *lkb1* affects polarity at pupal stages and PRCs extend properly. (*A*, *B*, and *F*) $lkb1^{4B1\text{-}11}$. (*C* and *D*) $lkb1^{4A42}$. (*A*) Epithelial polarity is maintained in *lkb1* third-instar clones; aPKC (blue) and Arm (red) are correctly localized in *lkb1* tissue (marked by loss of GFP). (*B*) Junctional membranes in 40% pd *lkb1* PRCs do not fragment, as shown by continuous Arm (blue) staining. (*C*) At 50% pd, PRCs undergo normal proximodistal extension (white scale bar), and rhabdomere feet remain attached to the basement membrane (white arrowhead). (*D* and *E*) Adult longtitudinal sections; *lkb1* rhabdomeres (*E*) show breaks throughout the proximal–distal length of the rhabdomere, but PRCs extend normally to the basement membrane (black arrowhead). *lkb1* rhabdomeres show ''waviness'' of the lateral membranes (arrow in *E*) compared with wild type (arrow in *D*). (*F*) Confocal section although a 40% pd, *lkb1* mosaic eye, showing a wild-type ommatidia (green) alongside a mutant ommatida. Arm staining (blue) extends in cells lacking *lkb1*. (Scale bars, 5 μm.)

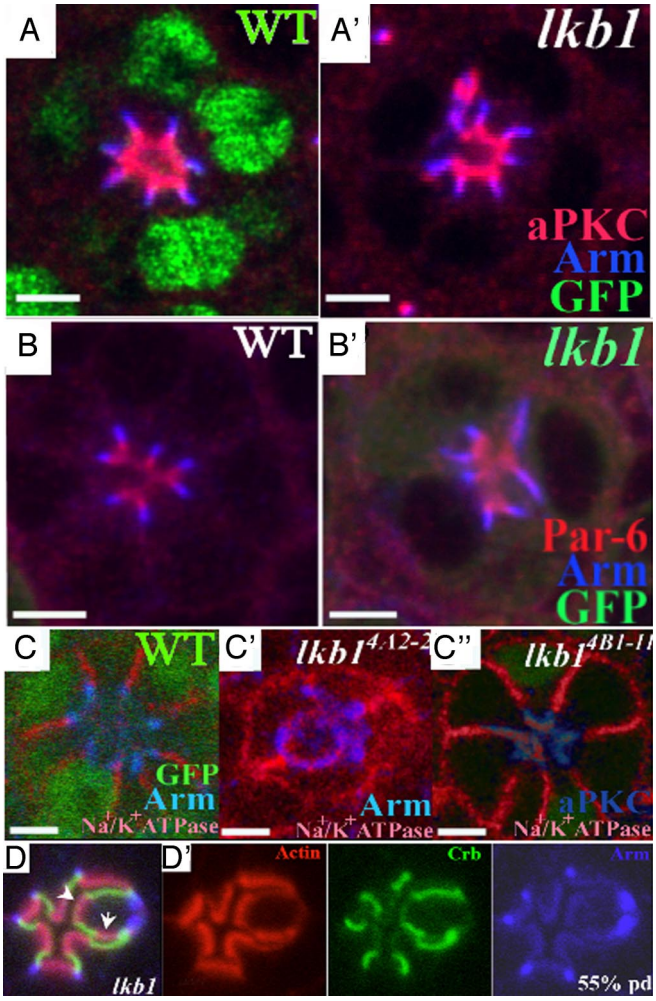

Fig. 3. *lkb1* loss of results in polarity defects in the pupal retina. GFP (green) marks wild-type tissue in *A* and *C* and mutant tissue in *B*. $lkb1^{4B1\text{-}11}$ (*A*–*C*) and $lkb1^{4A42}$ (*D* and *E*). Apical markers aPKC and Par-6 (red) show expansion into the basolateral domain in *lkb1* mutant clones. (*B*′) The junctional marker Arm (blue) also shows aberrant expansion into the basolateral domain. (*C*′) The basolateral marker Na^+/K^+ ATPase (red) also mislocalizes to the apical membrane in $lkb1^{4B1\text{-}11}$ mosaic PRCs. (*C*″) $lkb1^{4A42}$ mosaic retinas show a more severe phenotype, where Na^+/K^+ ATPase (red) can be found in a ring like structures overlapping Arm (blue). (*D*′) Extra membrane domains are sometimes observed, e.g., 3 subapical domains, 2 apical domains (white arrowheads). (Scale bars, 5 μm.)

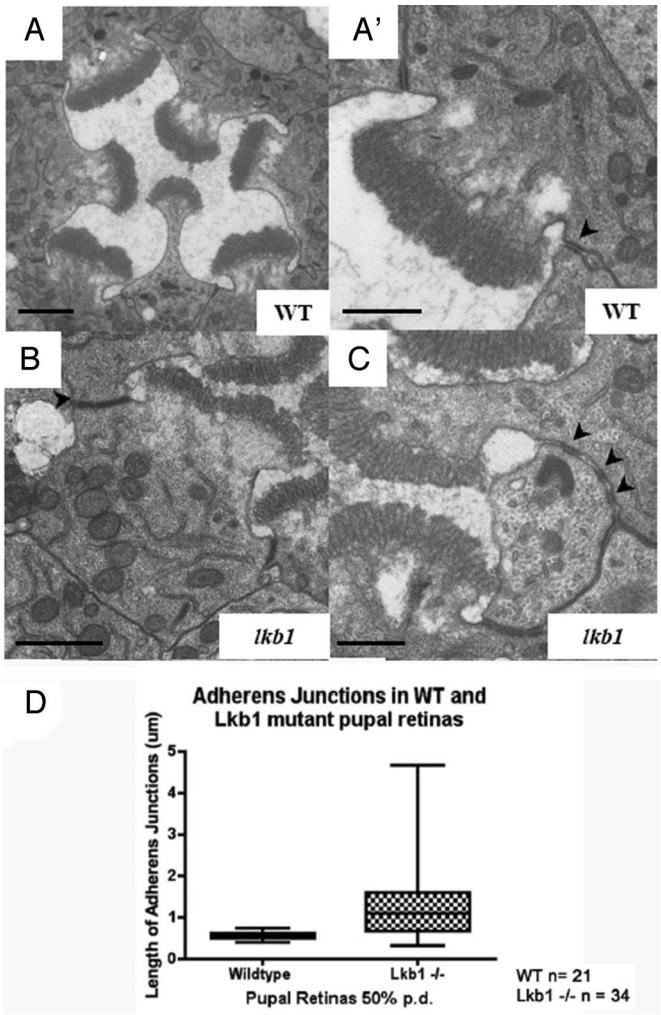

Fig. 4. Adherens junctions are longer, more numerous, and mislocalized in *lkb1* photoreceptor cells. (*A* and *A*′) Ultrathin sections (70 nm) of a wild-type ommatidium at 50% pd. AJ in wild-type PRCs occupy an apicolateral position in the cell, and each cell has 2 AJs (black arrowhead) of uniform length (0.5 μm). (*B*–*C*) *lkb1* AJs are frequently longer (white arrowhead in *B*) and sometimes disjointed (black arrowheads in *C*). (Scale bars, 1 μm in *A* and *B* and 0.5 μm in *A*′ and *C*.) (*D*) Box-plot analysis of *lkb1* AJ length in PRCs in 50% pd pupal retinas. The average length of AJs in *lkb1* PRC increased to 1.28 μm. AJs also exhibit an increased range in length. Smaller junction lengths may indicate fragmented AJs.

eye disc are remodeled and rotate 90°, and apical surfaces converge at the center of the ommatidium as early as 10% pd. Developing ommatidia mutant for LKB1 lacked the regular size and shape seen in wild-type ommatidia. Members of the SAR complex (Crb, Std, and Patj) and members of the Par complex (aPKC, Baz, and Par-6) have similar defects in rhabdomere formation, resulting in mutant rhabdomeres that are often elongated, split, bulky, or fused (19, 20, 27, 28).

Because *lkb1* adult PRC morphology resembled *crb* mutants, we tested whether PRC development was similarly affected. $crb^{-/-}$ pupal PRCs show fragmented ZA as early as 43% pd and fail to extend rhabdomeres (20, 29). Side views of the ZA (marked by Arm) show that *lkb1* rhabdomeres are not fragmented from 40% pd to eclosion (Fig. 2 *B*–*E*). Instead, there is a marked waviness of the lateral membranes (Fig. 2 *C* and *E*) compared with wild type (arrow in Fig. 2*D*), suggesting a weakening of structural integrity. Z-sections reveal that *lkb1* ZAs properly extend all of the way to the retinal floor in a manner identical to wild type (Fig. S3). Thus, defects observed in *lkb1* mutant PRCs are not due to fragmentation of junctions or a failure of extension.

***Lkb1* Mutants Lose Polarity at Pupal Stages.** Analysis of polarity markers revealed dramatic defects in *lkb1* mutant pupal PRCs. Apical markers such as aPKC and Par6 spread basal to their normal domain at 43% pd (Fig. 3 *A* and *B*). The stalk domain was similarly affected in *lkb1* mutant PRCs, as assessed by the stalk domain components, PatJ, Std, and Crb (Fig. S4) (19). The AJ marker Arm normally localizes just basal to the stalk membrane. In *lkb1* PRCs, Arm frequently expands, occasionally overlapping with stalk membrane markers but mostly spreading toward the basolateral membrane (Fig. 2*F* arrowhead, Fig. 3 *A*–*D*, and Fig. S5). In controls, there is clear separation of junctional and basolateral domains (Fig. 3*C*), but in *lkb1* PRCs, there is significant overlap of Arm and the basolateral marker Na^+/K^+–ATPase (Fig. 3*C*′) (30, 31), suggesting that PRCs have lost distinct lateral membrane identity. Extra membrane domains are also sometimes observed, e.g., 3 subapical

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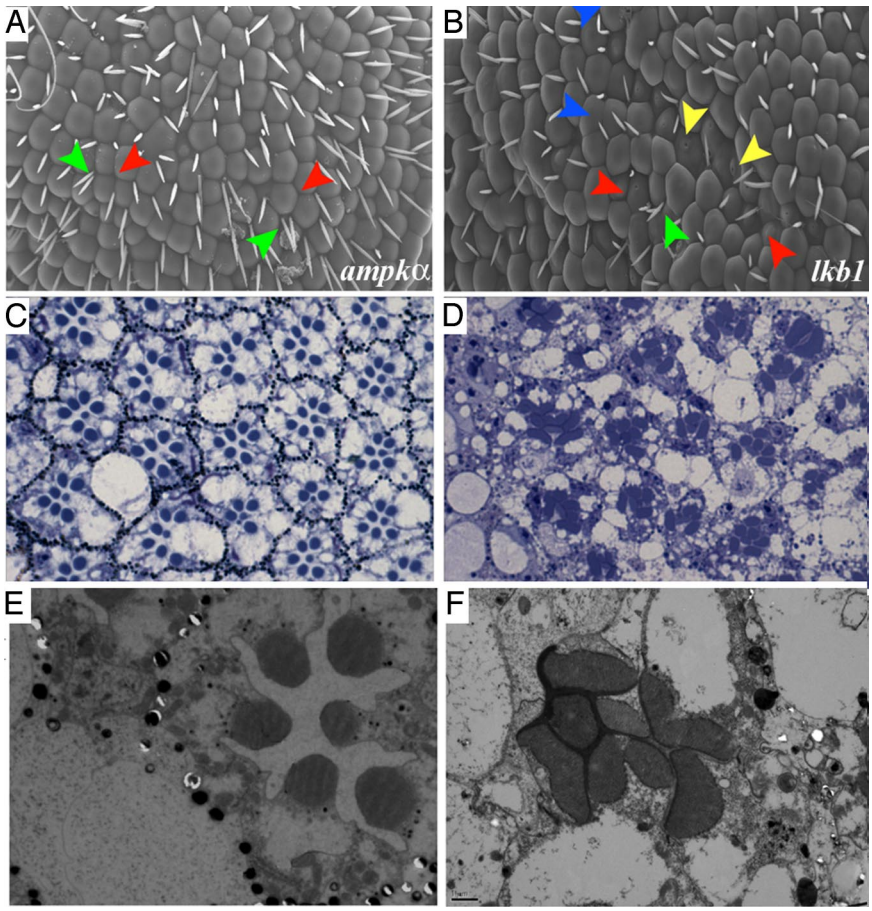

Fig. 5. *lkb1* and *ampkα* mutant adult eyes. (*A–F*) SEMs of adult eyes. *ampkα* and *lkb1* mutant eyes are "rough" (*A* and *B*). *lkb1* mutant ommatidia show pitting of the surface (yellow arrowhead in *B*) and rhabdomere fusion (blue arrowhead in *B*) that is not observed in *ampkα* mutant eyes (*A*). Bristles missing between ommatidia (red arrows in *A* and *B*) or duplicated (green arrows in *A* and *B*) are frequently observed. (*C* and *D*) Light micrographs of *ampkα* (*C*) and *lkb1* (*D*) mutant eyes. Both *ampkα* and *lkb1* mutant eyes show enlarged cell bodies (*C* and *D*), whereas only *lkb1* shows elongation of rhabdomeres (*D* compared with *C*). (*E–F*) TEM of *ampkα* (*E*) and *lkb1* (*F*) mutants. R7 is sometimes absent in sections of ampkα mutants (*E*), whereas the rhabdomere membrane is often enlarged in *lkb1* mutants (*F*).

domains, 2 apical domains (white arrowheads in Fig. 3*D*). These data suggest that LKB1 has a crucial role in developing or maintaining distinct membrane domains in the pupal retina.

Expression of the pan caspase inhibitor, p35, did not ameliorate the polarity defects (Fig. S4*G*), suggesting that defects are not due to cell death. Taken together, these data provide evidence that LKB1 is involved in the global organization of distinct membrane domains during pupal development.

AJ Defects in LKB1 Mutants. In wild-type retinas, AJs are consistently regular in size and placement. Ultrathin (70 nm) sections of retinas at 50% pd show expanded AJs in *lkb1* PRCs compared with controls (Fig. 4*B*), and some cells appear to have >2 AJs (Fig. 4*C*). Some AJs are clearly separate from each other, e.g., where AJs appear at 4 corners of a single cell; in other cases, many smaller junctions appear next to each other, which may be a product of a single AJ that has fragmented (Fig. 4*C*). AJs normally uniformly occupy an apicolateral position. In *lkb1* retinas, as well as exhibiting lateral expansion, ectopic junctions also occasionally appear on the lateral and basal membranes.

Quantitation revealed that the average length of wild-type AJs was very consistent [0.55 ± 0.10 μm; in contrast *lkb1* AJs were longer and variable in length (1.28 ± 0.83 μm; $P < 0.0001$)] (Fig. 4*D*). The shorter junctions are possibly a result of junction breakdown, because they often display as a string of "mini" junctions along the apicolateral membrane (Fig. 4*C*). These data suggest that LKB1 is required for the proper localization of AJs at the apicolateral membrane, for AJ integrity, and for the restriction of AJ length.

***ampkα* Loss-of-Function Clones Do Not Phenocopy *lkb1* in the Retina.** LKB1 has been proposed to regulate polarity primarily via regulation of AMPK in embryos, wing discs, and follicle cells. Surprisingly, examination of adult *ampkα*$^{-/-}$ eyes revealed significant differences from *lkb1*$^{-/-}$ eyes (Fig. 5). *ampkα* eyes do not exhibit any pitting (Fig. 5*A*), and rhabodmeres are not elongated (Fig. 5*C*) In addition, the normal termination of axons is largely intact in *ampkα*, whereas in *lkb1* mutants, the lamina appears to be fused with the medulla (Fig. S6).

ampkα^{3} mutant clones at 43% pd displayed no expansion of Arm under normal conditions (Fig. 6*A*) or under conditions of energy deprivation (Fig. 6*B*). Quantitative analysis of Arm length in control photoreceptors under energy deprivation (0.5 ± 0.2 μm) was similar to *ampk* mutants (0.5 ± 0.1 μm). In addition, expression of MRLC^{EE} did not rescue the disruption of PRC in *lkb1* clones (Fig. 6 *C* and *D*). Thus, unlike in embryos and follicles cells; in the pupal retina, LKB1 does not appear to function primarily through activation of AMPK, and MRLC but, instead, acts on other targets to regulate epithelial polarity.

LKB1 phosphorylates and activates a number of AMPK-like kinases, including Par1. Indeed, Par-1 and LKB1 were first identified in a *C. elegans* screen for genes required for the formation of the anterior–posterior axis during embryogenesis (3, 32, 33). *lkb1* and *par-1* mutants show similar phenotypes in oocytes: defective

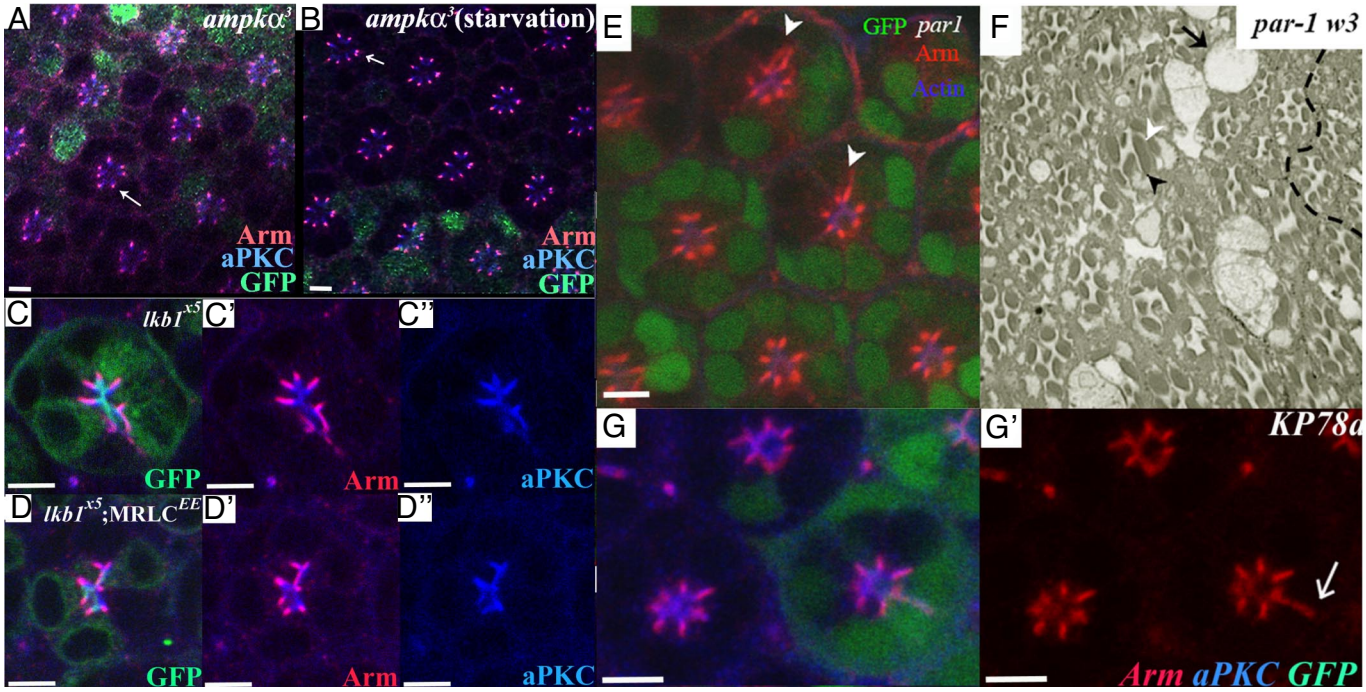

Fig. 6. *ampk*$^{-/-}$ does not alter Arm localization, whereas KP78a loss phenocopies *lkb1*. GFP (green) marks the wild-type tissue in *A*, *B*, and *E*, MARCM clones in *C* and *D*, and flp-out clones in *G* and *H*. *ampkα*^{3} mutant clones do not phenocopy *lkb1* clones raised on normal food (*A*) or starvation food (*B*). *ampkα*^{3} mutant PRCs show discrete Arm (red) localization (arrows in *A* and *B*). Expression of *MRLC*^{EE} in *lkb1* clones does not rescue *lkb1* polarity defects (*D*). (*C*) MARCM *lkb1*^{X5} clones show basolateral spreading of aPKC (blue) and Arm (red). (*D*) MARCM *lkb1*^{X5} clones expressing *MRLC*^{EE} do not show a rescue of the basolateral spreading of apical (aPKC, blue) and junctional markers (Arm, red). (*E*) *par1*^{w3} clones show expansion of Arm (red) toward the basolateral domain of PRCs (arrowheads). (*F*) *par1*^{w3} clones (lack of pigment cells) show elongated rhadomeres (arrowhead) and enlarged cells bodies (arrow); phenotypes also characteristic of *lkb1* loss of function in the eye. (*G*) Expression of *KP78a* RNAi results in basolateral spreading of apical (aPKC, blue) and junctional markers (Arm, red). (Scale bars, 5 μm.)

polarization of the cytoskeleton and mislocalization of polarized mRNAs and proteins (5). We examined *par-1*$^{-/-}$ PRCs and saw basolateral expansion of Arm (ref. 34 and Fig. 6*E*) and enlargement of cell bodies (Fig. 6*F*, arrow) and elongation of rhabodmeres (Fig. 6*F*, arrowhead) as seen in *lkb1* mutant PRCs (Fig. 1*D*).

However, loss of Par1 only weakly phenocopies loss of LKB1, with increases in Arm length similar to the weaker LKB1 allele, *lkb1*$^{4b1\text{-}11}$ (*par1* = 1.4 ± .5 μm; *lkb1*$^{4b1\text{-}11}$ = 1.7 ± 0.5 μm, compared with control Arm length of 0.9 ± 0.2 μm, all under normal energy conditions). The defects in Arm localization in the stronger LKB1 allele are so dramatic that they are difficult to quantitate reliably and are frequently lost from the developing retina. Because loss of Par1 does not fully phenocopy LKB1 loss, we wondered whether other AMPK-like kinases regulate polarity in the eye. There are no available null alleles to these genes, and their functions have not yet been studied in *Drosophila*. We therefore used RNAi to knock down expression of all AMPK-like kinases in the retina. Expression of RNAi to 4 other AMPK-like kinases led to defects in PR development [CG15072 (similar to mammalian SIK and QSK), CG11871 (homologous to mammalian NUAK), CG6715 (KP78a) and CG17216 (KP78b)]. We observed basolateral spreading of Arm (Fig. 6*G* and Fig. S5), similar to that seen in *lkb*$^{4B1-11}$ mutant retinas, although less strongly than in *lkb1*^{4442} mutants.

Quantitation revealed significant extension of Arm staining in these mutants. In controls, Arm length is 0.9 ± 0.2 μm, whereas in the weaker *lkb1*$^{4B1-11}$ mutants, Arm length increases to 1.7 ± 0.5 μm. Similar increases in Arm length are seen in retinas expressing several of the AMPK-like kinases of RNAi (CG16334 = 1.6 ± 0.6 μm; CG39866 = 1.8 ± 0.7 μm, KP78a = 1.7 ± 0.5 μm, and for KP78b = 1.5 ± 0.5 μm). Interestingly the percentage of Arm length relative to the length of the lateral membrane in *lkb1* mutants is significantly shorter, compared with the other lines, consistent with the stronger effect of LKB1 on polarity. In controls, Arm marks 17 ± 3.7% of the lateral membrane, whereas in *lkb1* mutants, Arm occupies 38.5 ± 10.7%. Arm length compared with lateral membrane length is less dramatic in the AMPK-like kinase RNAi lines (CG16334 = 28.6 ± 11.8%; CG39866 = 30.7 ± 12%; KP78a = 33 ± 9.2%; KP78b = 29.4 ± 10%). Together, these data indicate that the regulation of epithelial polarity downstream of LKB1 in the pupal retina is more complex than in embryos or in follicle cells and does not work solely through AMPK or Par1.

Discussion

LKB1 is an important regulator of cell polarity in many systems (3–6, 8, 12, 13), yet how LKB1 regulates polarity is still unclear. The only well-defined targets thus far in *Drosophila* are AMPK and Par-1. We show here that LKB1 is essential during the remodeling of polarity in the fly eye. Apical markers such as aPKC and junctional markers such as Arm lose their normally discrete localization and spread basolaterally. Basolateral markers such as Na^+/K^+ ATPase, extend aberrantly toward the apical membranes. AJs expand, and components of the basolateral membrane mix with apical and junctional markers.

Although recent studies suggested that the major downstream target of LKB1 in the wing and embryo is AMPK, acting through MRLC. (12, 13), examination of *ampkα*^{3} eyes did not reveal defects similar *lkb1* eyes. Furthermore, the expression of activated MRLC did not rescue *lkb1* defects in PRCs, unlike the wing. Thus, polarity establishment and maintenance in the *Drosophila* eye involves a different set of targets than in the embryo or follicle cells.

We found that loss of function of a number of AMPK-related kinases (SIK, NUAK, KP78a, KP78b, and Par-1) partially phenocopy *lkb1* in the pupal retina. In agreement, *par-1* RNAi in the embryo has been shown to lead to the basal expansion of apical and junctional markers and the mislocalization of basolateral markers (35). We were unable to rescue the effects of loss of LKB1 with

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overexpressed Par-1, or a phosphomimetic version of Par-1. Given that we see weak phenocopies of the *lkb1* phenotype with loss of Par-1, SIK, NUAK, KP78a, and KP78b, we speculate that the effects of loss of LKB1 are due to a loss of regulation of a suite of AMPK-like kinases. We note that because RNAi knockdowns are not nulls, our data do not exclude a role for the AMPK-like kinases that had no phenotypic effect on the eye in our assays. Generation of null alleles of all of the AMPK-like kinases will be necessary to fully define the contribution of each kinase to polarity development in the eye.

Taken together, these data argue against the simple model that LKB1 regulates polarity solely through AMPK. Interestingly, in mammals, there are tissues where LKB1 signals through AMPK and other tissues where LKB1 does not affect AMPK activity (36, 37). Thus, tissue-specific modulation of LKB1 function may be a general theme.

Why is the regulation of polarity, downstream of LKB1 more complex in the pupal retina? The embryo and follicle cells are systems in which epithelial polarization is being established (38, 39), whereas pupal PRCs undergo a 90° remodeling of already established polarized membranes (17, 40). We speculate that this remodeling process requires additional mechanisms for its precise regulation and may be why LKB1 acts on additional downstream targets to regulate polarity in the eye.

It is possible that lack-of-polarity defects in the *lkb1* larval disc may be due to perdurance of LKB1 protein. However, we favor a model in which LKB1 acts specifically at the pupal stage, because a number of polarity genes such as *crb*, *par-1*, and *sdt* show polarity defects specifically at pupal stages, when dramatic reorganization of polarity is occurring (20, 27, 34). It is unlikely that these diverse proteins all persist for exactly the same length of time and, instead, suggests a crucial role in the remodeling of the polarity that occurs at this time.

Together, these data suggest that LKB1 can regulate a diverse suite of targets, the regulation of which occurs in a developmental or tissue-specific manner and that more complex tissues, such as the pupal retina, require a more extensive set of targets to develop elaborate cellular polarity.

Materials and Methods

lkb1$^{4A4\text{-}2}$ and *lkb1*$^{4B1\text{-}11}$ *lkb1*^{X5}, *ampk*α^3, *UAS-MRLC*^{EE}, *UAS-RNAi* KP78a (line 47658, VDRC), KP78b (line 51995, VDRC), Sik3 (line 39866, VDRC), and NuAk (line 16334, VDRC) were used. Clonal analysis was performed by using the FRT/Flp technique using *eyFLP* or *hsFLP* with *lkb1 FRT 82b/FRT 82b M*$^+$ *UbiGFP* or *hsFLP* with *ampk*α^3 *FRT 101/FRT 101 UbiGFP*. *hsFLP* clones were induced by heat-shocking the larvae for 1 h at 37 °C. Adult mutant eye clones were generated according to the *EGUF/hid* method. RNAi lines were crossed to *y w hs FLP122;tub>y+>Gal4 UAS-GFP*. Flip-out clones were induced by heat-shocking. The MARCM technique was used to examine *lkb1* clones expressing MRLC^{EE} or Par-1 by using *eyFLP*, *UAS-mCD8::GFP; tubGal80* FRT 82b, *tubGal4*, *UAS-MRLC*^{EE} or *UAS-Par-1*, *lkb1 FRT 82b*. Standard techniques were used for imaging retinas at the light and EM level (details in *SI Materials and Methods*).

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