Sgt1 acts via an LKB1/AMPK pathway to establish cortical polarity in larval neuroblasts

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A B S T R A C T

Drosophila neuroblasts are a model system for studying stem cell self-renewal and the establishment of cortical polarity. Larval neuroblasts generate a large apical self-renewing neuroblast, and a small basal cell that differentiates. We performed a genetic screen to identify regulators of neuroblast self-renewal, and identified a mutation in sgt1 (suppressor-of-G2-allele-of-skp1) that had fewer neuroblasts. We found that sgt1 neuroblasts have two polarity phenotypes: failure to establish apical cortical polarity at prophase, and lack of cortical Scribble localization throughout the cell cycle. Apical cortical polarity was partially restored at metaphase by a microtubule-induced cortical polarity pathway. Double mutants lacking Sgt1 and Pins (a microtubule-induced polarity pathway component) resulted in neuroblasts without detectable cortical polarity and formation of “neuroblast tumors.” Mutants in hsp83 (encoding the predicted Sgt1-binding protein Hsp90), LKB1, or AMPKα all show similar prophase apical cortical polarity defects (but no Scribble phenotype), and activated AMPKα rescued the sgt1 mutant phenotype. We propose that an Sgt1/Hsp90–LKB1–AMPK pathway acts redundantly with a microtubule-induced polarity pathway to generate neuroblast cortical polarity, and the absence of neuroblast cortical polarity can produce neuroblast tumors.

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Introduction

The precise regulation of stem cell self-renewal versus differentiation is essential for normal development, required for tissue homeostasis, and may suppress tumorigenesis. Despite its importance, the molecular mechanisms regulating stem cell self-renewal are only beginning to be uncovered. Recently, Drosophila larval neuroblasts have proven to be an effective model for characterizing the mechanisms regulating stem cell self-renewal (Doe, 2008; Januschke and Gonzalez, 2008).

Drosophila larval neuroblasts undergo repeated asymmetric cell divisions that involve formation of molecularly distinct apical and basal cortical domains, and alignment of the mitotic spindle along the apical/basal polarity axis. The apical cortex contains two protein complexes: the Par complex (Bazooka, Baz; atypical protein kinase C, aPKC; and Partitioning defective-6, Par-6) and the Pins complex (Partner of Inscurable, Pins; Goi/o, and Discs large, Dlg). These two complexes are thought to be linked by the protein Inscurable (Insc) (Doe, 2008; Knoblich, 2008). The differentiation factors Numb, Brain tumor (Brat), and Prospero (Pros) accumulate on the basal surface of neuroblasts; the adaptor protein Miranda (Mira) is required for both Brat and Pros localization (Doe, 2008; Knoblich, 2008). In larval neuroblasts, apical polarity is first established at late G2/early prophase, whereas basal cortical polarity is first detectable at the prophase/metaphase transition (Siller et al., 2006). The apical domain is partitioned into the larger daughter cell which self-renews as a neuroblast, while the basal domain segregates into the smaller daughter cell which has a more restricted developmental potential (Doe, 2008; Knoblich, 2008).

Despite the importance of the apical polarity proteins in regulating neuroblast self-renewal, it remains unknown exactly how the Par and Pins complexes are initially localized to the apical cortex. The conserved polarity protein Cdc42 binds Par-6 and is required for apical localization of Par-6 and aPKC, but cdc42 mutants still localize Baz to the apical cortex, albeit weakly (Atwood et al., 2007). The only mutant reported to abolish Baz apical localization is lkb1 (Bonaccorsi et al., 2007), which encodes a serine/threonine kinase in the Par-4 kinase family; however, the authors only observed weak Baz localization in wild type neuroblasts, so the significance of the mutant phenotype is difficult to interpret.

Here we describe a forward genetic screen that identified a mutant with a reduced number of brain neuroblasts and defects in the earliest steps of generating neuroblast cortical polarity. We mapped the mutant to a region of the third chromosome and used a novel method to identify the mutant lesion, revealing a small deletion in the sgt1 gene. Sgt1 is an evolutionarily conserved protein that directly interacts with Hsp90 using its “CHORD/Sgt1” domain (Lee et al., 2004); it regulates kinetochore assembly in yeast and mammalian...
cells; cell cycle progression in yeast, mammals, and Drosophila; and pathogen sensing in plant and animal cells (da Silva Correia et al., 2007; Kitagawa et al., 1999; Martins et al., 2009; Mayor et al., 2007; Shirasu, 2009; Steensgaard et al., 2004).

Results

A genetic screen for neuroblast self-renewal mutants identifies sgt1

To find potential regulators of neuroblast self-renewal, we screened homozygous P element induced mutations for altered larval neuroblast numbers, using Miranda (Mira) and Deadpan (Dpn) to identify neuroblasts and Scribble (Scrib) to outline the neuroblast cell cortex and highlight general neuroanatomical landmarks (Lee et al., 2006c) (Fig. 1A). One mutant that showed a modest decrease in neuroblast number, and larval/pupal lethality, was l(3)s2383 which contains a P element at 66E1–66E2 on the left arm of the third chromosome (Figs. 1A,B). To confirm that the P element insertion caused the phenotype, we assayed the l(3)s2383 chromosome in trans to a deficiency that removed chromosomal region 66E1–66E2. Surprisingly, these larvae were completely normal for neuroblast number and viability, showing that the mutation mapped to elsewhere on the third chromosome. We used deficiency mapping to localize the mutation to the 84F6–84F13 region of the right arm of the third chromosome (Fig. 1B). We found that l(3)s2383/Df(3R)6147 larvae showed the same neuroblast number and larval/pupal lethality phenotypes as l(3)s2383 homoygotes. Phenotypic analysis of overlapping deficiencies in the region allowed us to map the mutation to a 60 kb interval, and we confirmed the mutation was in this interval by genomic DNA rescue of the phenotype (data not shown; see Methods).

To identify the DNA lesion responsible for the phenotype, we used a novel “sequence capture” strategy combined with Illumina deep sequencing (Fig. 1B) to identify an in frame 15 nucleotide deletion in the first exon of the sgt1 gene, also known as CG9617 (Fig. 1C; and see Methods). We henceforth call this allele sgt1c01428. To confirm that the sgt1c01428 lesion is responsible for the observed neuroblast and viability phenotype, we crossed sgt1c01428 with the known sgt1s2383 mutation (Martins et al., 2009). We found that sgt1c01428 fails to complement sgt1s2383, and that sgt1c01428/sgt1s2383 larvae have the same phenotype as sgt1s2383 homozygotes. Furthermore, the fact that the phenotype of sgt1c01428/sgt1s2383 is identical to that of sgt1s2383/Df(3R)6147 indicated that sgt1s2383 is a strong or null allele for CNS function. The lesion in sgt1c01428 resulted in a five amino acid deletion in the Chord and Sgt1 (CS) domain that mediates Sgt1–Hsp90 protein interactions in other organisms (Zhang et al., 2010). We conclude that Drosophila Sgt1 is required for maintaining normal numbers of larval neuroblasts and for viability.

sgt1 is required for apical protein localization in prophase neuroblasts

To investigate the cellular origin of the neuroblast depletion phenotype in sgt1 mutants, we assayed neuroblast cell cycle progression and neuroblast cell polarity. Prophase neuroblasts were identified as PH3 + without a bipolar spindle; metaphase neuroblasts were identified as PH3 + with a bipolar spindle. We confirmed a previous report (Martins et al., 2009) that sgt1 mutants have slower cell cycle progression, cytokinesis failure, polyploidy, multiple centrosomes, and malformed mitotic spindles (Supplemental Fig. 1 and data not shown). In this paper, we focus on the previously unreported cell polarity aspects of the sgt1 mutant phenotype. First, we assayed neuroblast apical cortical polarity (“apical cortex” is defined as the cortical domain that is partitioned into the sibling neuroblast, whereas “basal cortex” is defined as the domain segregated into the GMC). The first sign of neuroblast asymmetry occurs during late G2 and early prophase when Par complex proteins (e.g. Baz, aPKC), Pins complex proteins (e.g. Pins), and the Par/Pins linker protein Insc are localized to an apical cortical crescent (Fig. 2A; quantified in A’). In contrast, sgt1 mutant prophase neuroblasts typically showed cytoplasmic or undetectable localization of these proteins (Fig. 2B; quantified in B’). Interestingly, during metaphase we found a substantial rescue of apical cortical polarity (Figs. 2C,D; quantified in C ’D’); the basal proteins Miranda and Numb were also fairly normal at metaphase (Supplemental Fig. 2). We conclude that Sgt1 is required for establishing apical cortical polarity during prophase, and during metaphase an Sgt1-independent pathway can generate apical/basal cortical protein localization.

We next analyzed Dlg and Scrib cortical localization in wild type and sgt1 mutant neuroblasts, for two reasons. First, we had observed a complete lack of Scrib cortical localization in our original screen (Fig. 1A), and second, Dlg is part of the Pins complex (Siegrist and Doe, 2005) so we wanted to know if it was delocalized in prophase neuroblasts similar to Pins. In wild type interphase and mitotic neuroblasts, Scrib was always uniform cortical (Fig. 3A; 100% uniform cortical, n=50), while Dlg was invariably uniform cortical throughout the cell cycle plus enriched with Pins at the apical cortex during mitosis (Fig. 3B; 100% uniform cortical n=53, 94% apical enriched, n=34). In contrast, sgt1 mutant neuroblasts showed a strong loss of cortical Scrib localization during metaphase (Fig. 3C; 3% cortical, n=75) and interphase (Fig. 1A, and data not shown); they also showed a loss of the uniform cortical pool of Dlg protein at interphase and mitosis, with retention of the pool of Dlg asymmetrically co-localized with Pins at the apical cortex at metaphase (Fig. 3D; 2% uniform cortical n=45, 83% apical enriched, n=29). We conclude that Sgt1 is essential for targeting Dlg/Scrib to the neuroblast cortex, except where Dlg is co-localized with Pins.

Martins et al. (2009) showed that overexpression of wild type Polo kinase can rescue aspects of the sgt1 neuroblast cell cycle defects; we found that Polo overexpression increases the number of mitotic neuroblasts in both wt and sgt1 mutant brains (Supplemental Fig. 1B), but does not rescue neuroblast prophase cortical polarity (7% normal Pins, n=45; 4% normal aPKC, n=27) or Scrib cortical localization (0% normal Scrib, n=75).

Microtubules induce apical cortical polarity in sgt1 metaphase neuroblasts

What is the Sgt1-independent pathway that generates Pins and Par complex apical cortical localization in metaphase neuroblasts? We previously defined a microtubule-induced cortical polarity pathway that could induce Pins/Goi/Dlg cortical polarity (Siegrist and Doe, 2005), so here we test whether this pathway induces Pins and Par complex apical cortical polarity at metaphase in sgt1 mutant neuroblasts. We used the microtubule inhibitor colcemid to abolish spindle microtubules in sgt1 mutant and control neuroblasts. Wild type neuroblasts treated with the colcemid retain apical cortical crescents of Dlg, Pins and Insc (Fig. 4B, top row; quantified in figure legend). Strikingly, sgt1 mutant neuroblasts treated with colcemid lacked all detectable apical cortical polarity (Fig. 4B, bottom row; quantified in figure legend). We conclude that microtubules are required to establish apical cortical polarity in the absence of Sgt1.

pins sgt1 double mutants neuroblasts lack all detectable cortical polarity and form neuroblast brain "tumors"

The developmental consequence of a complete loss of neuroblast polarity has never been assayed, and the loss of both Sgt1-dependent and microtubule-dependent cortical polarity pathways is predicted to generate a completely apolar neuroblast (see earlier discussion). We cannot assay sibling cell fate in sgt1–colcemid-treated neuroblasts because they are arrested in mitosis, so we examined a
double mutant lacking Sgt1 and the microtubule-induced polarity component Pins. We tested (a) whether sgt1 pins double mutants abolish both Par and Pins complex polarity, and if so (b) what is the fate of such apolar neuroblasts. We found that sgt1 pins double mutant neuroblasts establish little or no apical or basal cortical polarity (Fig. 5B and data not shown). The developmental consequences of this loss of polarity are the formation of ectopic neuroblasts throughout the brain (Fig. 5F). This is in striking contrast to the sgt1 and pins single mutant brains, which are each smaller with reduced neuroblast numbers (Figs. 5D,E). We propose that loss of all cortical polarity in

Fig. 1. Identification of a mutant in sgt1 that is required for larval brain development. (A) Wild type and l(3)s2383/Df(3R)Exel6147 (l(3)s2383/Df) third instar larval brains stained for the neuroblast markers Miranda (Mira) and Deadpan (Dpn) plus Scribble (Scrib) which decorates the cortex of all cells in the brain. Right: histogram of the average neuroblast number per brain lobe (bar, standard deviation) in each genotype. Note that the mutant brains have fewer neuroblasts and Scrib protein is cytoplasmic. (B) Schematic of the “sequence capture” and deep sequencing strategy used to identify the lesion in the l(3)s2383 mutant chromosome. Although the chromosome was generated in a P element mutagenesis, we found a 15 nucleotide deletion unrelated to the P element insertion that showed the mutant phenotype when transheterozygous to Df(3R)Exel6147. (C) The l(3)s2383 mutation resulted in an in-frame five amino acid deletion in the Drosophila Sgt1 protein, within the N-terminal CS domain (green); the C-terminal Sgt1-specific SGS domain is shown (white).
these double mutants results in symmetric neuroblast division to form two neuroblasts and expand the pool of brain neuroblasts (see Discussion).

Reducing Hsp90 levels mimics the sgt1 mutant phenotype

To determine the mechanism by which Sgt1 promotes apical cortical polarity and Dlg/Scrib cortical localization, we started by determining if Sgt1 worked together with its evolutionarily conserved binding partner, Hsp90. Sgt1 directly interacts with Hsp90 via its CS domain in all organisms tested (Catlett and Kaplan, 2006; Lee et al., 2004; Lingelbach and Kaplan, 2004; Nony et al., 2003), and our sgt1^{s2383} allele generates an Sgt1 protein lacking 5 amino acids within CS domain (Fig. 1C). We obtained mutants in hsp83 (which encodes the Hsp90 protein) and examined the larval brain neuroblast phenotype in hsp83^{13F3}/hsp83^{582} transheterozygotes (Lange et al., 2000). We find that hsp83 mutants have neuroblast phenotypes that are similar to that of sgt1 mutants: loss of apical cortical polarity at prophase (Figs. 6B,C; quantified in Fig. 6G). However, there is no loss of cortical Scrib in hsp83 mutants (Fig. 6C), suggesting that Sgt1 promotes Dlg/Scrib localization by an Hsp90-independent mechanism (see Discussion). The similarity of the sgt1 and hsp83 apical cortical polarity phenotypes, and the fact that the Sgt1^{s2383} protein has a deletion within the Hsp90-binding CS domain, leads us to hypothesize that Sgt1/Hsp90 act together to promote apical cortical polarity in prophase neuroblasts.

Sgt1 acts via an LKB1/AMPK pathway to generate apical cortical polarity, but not for Scrib cortical localization

Sgt1 has been shown to act through Polo kinase to regulate neuroblast cell cycle progression and cytokinesis (Martins et al., 2009). We confirmed that misexpression of Polo rescues the neuroblast cell cycle phenotypes, but found no rescue of the neuroblast cortical polarity phenotypes (data not shown). Thus, we investigated other downstream effectors of Sgt1 for a role in establishing apical protein localization.

lkb1 mutants are reported to have defects in neuroblast apical polarity that are similar to the sgt1 mutant phenotype (Bonacorsì et al., 2007). We confirm that lkb1 mutants had a nearly identical neuroblast polarity phenotype as sgt1 mutants, with the striking exception of showing normal Scrib cortical localization (Fig. 6D; quantified in Fig. 6G). In addition, lkb1 mutants – similar to sgt1 mutants – have
slower cell cycle progression, cytokinesis failure, polyploidy, multiple centrosomes, and malformed mitotic spindles (Bonaccorsi et al., 2007) (data not shown). We conclude that Sgt1 and LKB1 are both required to establish apical cortical polarity in neuroblasts.

LKB1 kinase can activate multiple members of the AMP-activated kinase (AMPK) family, such as Par-1, AMPK, Sik, NUAK, and others (Lizcano et al., 2004). Both Par-1 and AMPK activity are required for generating cell polarity in multiple cell types and organisms (Goldstein and Macara, 2007; Williams and Brennan, 2008), so we tested whether Par-1 or AMPK functions downstream of Sgt1/LKB1 in generating apical localization of Par complex proteins in neuroblasts. First we examined the localization and function of Par-1; we observed high levels of the protein in cortex glia that ensheath the neuroblast, but no sign of polarized localization within the neuroblast and no change in neuroblast polarity in par-1 mutant MARCM clones (data not shown). We conclude that loss of Par-1 from larval neuroblasts has no detectable effect on cell polarity.

We next assayed AMPKα mutants, and found that they – like sgt1 and lkb1 mutants – showed a loss of apical protein localization at prophase (Fig. 6E; quantified in Fig. 6G), slower cell cycle progression, cytokinesis failure, polyploidy, multiple centrosomes, and malformed mitotic spindles (Lee et al., 2007) (data not shown). As with lkb1 mutants, we saw normal cortical localization of Scrib, confirming that Sgt1 acts in an LKB1/AMPK-independent pathway to promote Scrib cortical localization. Importantly, we found that expression of constitutively activated AMPKα (Lee et al., 2007) (tub-gal4 UAS-ampkαTD) substantially rescued the sgt1 mutant phenotype (Fig. 6F; quantified in Fig. 6G). We conclude that Sgt1 acts in an LKB1/AMPK-independent pathway to promote Scrib cortical localization.

The AMPK pathway promotes myosin contractility in epithelia, and an activated myosin regulatory light chain, called Sqh in...
Drosophila (Sqh20E21E), can rescue the ampka embryonic epithelial polarity phenotype (Lee et al., 2007; Mirouse et al., 2007). Thus, we tested if active Sqh21E (Jordan and Karess, 1997) could rescue the sgt1 neuroblast cortical polarity phenotype, or whether loss of sgt1, lkb1, or ampka altered cortical myosin activity in larval neuroblasts. First, we found that expression of activated Sqh21E or the non-activatable Sqh20A21A (Jordan and Karess, 1997; Lee et al., 2007) both failed to rescue the sgt1 neuroblast cortical polarity phenotype (Supplementary Fig. 3A). Second, we found that all three pathway mutants (sgt1, lkb1, and ampka) showed an increase in uniform or patchy cortical activated Sqh20E21E (Supplementary Figs. 3B–E). In addition, time-lapse imaging of sgt1 mutant larval neuroblasts showed highly abnormal cortical protrusions, misshapen cells, and failure of cytokinesis (Supplemental Fig. 3F, Movie 1); these phenotypes are never seen in wild type larval neuroblasts (CABERNARD and DORE, 2009; Cabernard et al., 2010). The failure to execute cytokinesis is consistent with a loss of myosin activity, whereas the elevated cortical phospho-Sqh is consistent with an increase in myosin activity; thus all three pathway mutants (sgt1, lkb1, and ampka) have defects in the regulation of cortical myosin activity. However, the lack of cortical polarity phenotype in neuroblasts homozygous for zipper, which encodes myosin heavy chain (Barros et al., 2003; Peng et al., 2000), and the inability of Sqh21E to rescue the cortical polarity phenotype (this work) suggests that simple loss of myosin activity can not explain the sgt1, lkb1, or ampka mutant neuroblast polarity defects (Fig. 6H).

Discussion

Here we present evidence that the evolutionary-conserved protein Sgt1 acts with Hsp90, LKB1 and AMPK to promote apical localization of the Par and Pins complexes in prophase neuroblasts. We propose that Sgt1/Hsp90 proteins function together based on multiple lines of evidence: (1) they show conserved binding from plants to humans (Catlett and Kaplan, 2006; Lee et al., 2004; Lingelbach and Kaplan, 2004; Nony et al., 2003); (2) our sgt121383 mutant results in a five amino acid deletion within the CS domain, which is the Hsp90 binding domain (Catlett and Kaplan, 2006; Zhang et al., 2008); (3) Sgt1 and hsp83 have similar cell cycle phenotypes (Supplemental Fig. 1)(Lange et al., 2000); and (4) sgt1 and hsp83 have similar neuroblast polarity phenotypes (Figs. 6B,C). The Sgt1/Hsp90 complex either stabilizes or activates client proteins (Zuehlke and Johnson, 2010); we suggest that Sgt1 activates LKB1, rather than stabilizing it, because we were unable to rescue the sgt1 mutant phenotype by simply overexpressing wild type LKB1 protein (data not shown). We have not tested for direct interactions between Sgt1 and LKB1 proteins, and thus the mechanism by which Sgt1 activates LKB1 remains unknown.

LKB1 is a “master kinase” that activates at least 13 kinases in the AMPK family (Lizzano et al., 2004). We suggest that LKB1 activates AMPK to promote neuroblast polarity because overexpression of phosphomimetic, activated AMPKα can rescue the lkb1 and sgt1 mutant phenotype (Fig. 6F and data not shown). It remains unclear how AMPK activity promotes apical protein localization. An antibody to activated AMPKα (anti-phosphoT385-AMPKα, Mirouse et al., 2007) shows spindle and cytoplasmic staining that is absent in ampka

Fig. 6. sgt1, hsp83, LKB1, and AMPKα have similar neuroblast polarity phenotypes. Prophase larval brain neuroblasts stained for the Par complex protein Bazooka (Baz), the Pins complex protein Pins, and the cortical protein Scribble (Scrib). Quantiﬁcation in (G), Scale bar, 5 μm. (A) Wild type. Baz and Pins form apical cortical crescents; Scrib is uniform cortical. (B) sgt121383/3R;3R/6147 (sgt1). Baz and Pins are delocalized; Scrib is cytoplasmic. (C) hsp831277/T.TB;hsp832542 (hsp83). Baz and Pins are delocalized; Scrib is uniform cortical. (D) lkb14A4-2/lkb14A4-2 (lkb1). Baz and Pins are delocalized; Scrib is uniform cortical. (E) ampka/ampka (ampka). Baz and Pins are delocalized; Scrib is uniform cortical. (F) sgt1s2383/hsp831277/3R;6147; tub-gal4 UAS-ampka98 (sgt1 + AMPK rescue). Baz and Pins form apical cortical crescents; Scrib is cytoplasmic. (G) Quantification of the Baz and Pins phenotypes for the genotypes listed in A–F. Dark green, normal asymmetric light green, expanded asymmetric; red, cytoplasmic. Number of neuroblasts scored shown in each bar. (H) Model for Sgt1 regulation of neuroblast cortical polarity. Sgt1/Hsp90 activates LKB1 which activates AMPK to promote apical Par/Pins complex localization by an unknown mechanism; spindle microtubules provide a redundant pathway generating Par/Pins apical localization at metaphase. Sgt1 acts by an Hsp90/LKB1/AMPK-independent mechanism to promote Scribble (Scrib) uniform cortical localization.
mutants, and centrosomal staining that persists in AMPKα null mutants, but no sign of asymmetric localization in neuroblasts (data not shown). AMPK activity is thought to directly (Lee et al., 2007) or indirectly (Bultot et al., 2009) activate myosin regulatory light chain to promote epithelial polarity (Lee et al., 2007; Miranda et al., 2010). AMPK is activated by a rise in AMP/ATP levels that occur under energy stress or high metabolism; AMP binds to the γ regulatory subunit of the heterotrimeric complex and results in allosteric activation of the α subunit (Hardie, 2011). ampkα mutants grown under energy stress have defects in apical/basal epithelial cell polarity in follicle cells within the ovary (Mirouse et al., 2007). In contrast, AMPKα mutants grown on nutrient rich food still show defects in embryonic epithelial polarity (Lee et al., 2007), neuroblast apical polarity (this work), and visceral muscle contraction (Bland et al., 2010). Larval neuroblasts, embryonic ectoderm, and visceral muscle may have a high metabolic rate, require low basal AMPK activity, or use a different mechanism to activate AMPK than epithelial cells. What are the targets of AMPK signaling for establishing apical cortical polarity in larval neuroblasts? AMPK could directly phosphorylate Baz to destabilize the entire pool of apical proteins, but currently we have no evidence supporting such a direct model. AMPK may act via regulating cortical myosin activity; we have seen clear defects in cortical motility, ectopic patchy activated myosin at the cortex, and failure of cytokinesis in sgt1, lkb1, and ampkα mutants (Movie 1; Supplementary Fig. 3). This strongly suggests defects in the regulation of myosin activity, but how or if gain/loss/mispositioning of myosin activity leads to failure to establish apical cortical polarity remains unknown. Lastly, the defects in apical cell polarity seen at prophase could be due to the prometaphase cell cycle delays.

What activates the Sgt1-LKB1-AMPK pathway to promote cell polarity during prophase? In budding yeast, Sgt1 requires phosphorylation on Serine 361 (which is conserved in Drosophila Sgt1) for dimerization and function (Bansal et al., 2009); this residue is conserved in Drosophila Sgt1 but its functional significance is unknown. Sgt1/Hps90/LKB1/AMPK are all required for apical Par/Pins complex localization, but Sgt1 must act via a different pathway to promote Dlg/Scrib cortical localization, because only the sgt1 mutant affects Dlg/Scrib localization, and overexpression of activated AMPKα is unable to restore cortical Scrib in sgt1 mutants (Fig. 6). The mechanism by which Sgt1 promotes Dlg/Scrib cortical localization is unknown.

We have shown that sgt1 mutants lack Par/Pins apical polarity in prophase neuroblasts, but these proteins are fairly well polarized in metaphase neuroblasts. The rescue of cortical polarity is microtubule dependent, probably occurring via the previously described microtubule-dependent cortical polarity pathway containing Pins, Dlg and Khc-73 (Siegist and Doe, 2005). The weak polarity defects still observed in sgt1 metaphase neuroblasts may be due to the poor spindle morphology (Supp. Fig. 1). The lack of microtubule-induced polarity at prophase, despite a robust microtubule array in prophase neuroblasts, suggests that the microtubule-induced cortical polarity pathway is activated at metaphase. Activation of the pathway could be via expression of the microtubule-binding protein Khc-73; via phosphorylation of Pins, Dlg or Khc-73 by a mitotic kinase like Aurora A (Johnston et al., 2009); or via a yet unknown pathway.

It was somewhat surprising that the sgt1 pins double mutants had increased numbers of brain neuroblasts, because each single mutant had reduced neuroblast numbers (this work)(Lee et al., 2006b). The double mutant phenotype may be due to loss of both Pins and cortical Dlg/Scrib, as the sgt1 pins double mutant phenotype is similar to the dgl pins double mutant phenotype (data not shown). It could also be due to a change in an unknown downstream effector of both Sgt1 and Pins. A not mutually exclusive possibility is that the sgt1 pins double mutant phenotype is due to loss of all Par/Pins cortical polarity. This model is consistent with our observation that sgt1 or pins single mutants retain some neuroblast cortical polarity, whereas the sgt1 pins double mutants lack all known neuroblast cortical polarity. We propose that the apolar double mutant neuroblasts partition cell fate determinants equally to both siblings, and that both siblings frequently assume a neuroblast identity. This is supported by our recent finding that when the neuroblast spindle is aligned orthogonal to a normal apical/basal polarity axis, such that both siblings inherit equal amounts of apical cortical proteins, the siblings always acquire a neuroblast identity (Carnabard and Doe, 2009). Thus, equal partitioning of apical/basal cell fate determinants (in spindle orientation mutants) or failure to establish any cortical polarity (sgt1 pins mutants) may result in neuroblast/neuroblast siblings and an expansion of the neuroblast population.

Methods

Identification and sequencing of the sgt122383 mutant

The sgt122383 mutant allele was originally found in a P element screen of the 3rd chromosome. The P element mapped to 66E1–2, but deficiency mapping revealed that the lethality and CNS phenotype mapped to Df(3R)6147 at 84F6–F13. To further define the region, rescue constructs were made using gap-repair and U3C3 mediated transgenesis (Venken et al., 2009). Using the tilling BAC 33N15, we made two overlapping 60 kb and 28 kb rescue constructs; only the 60 kb construct rescued the l(3)sc2383 phenotype, narrowing the relevant region to approximately 13 genes. To identify the lesion in this interval, we used a biotin mediated “sequence capture” technique followed by deep sequencing. Genomic DNA from both wild type and mutant larva were prepared for deep sequencing. Using BAC33N15 as a template, biotin incorporated “probes” were created. The biotin labeled probes were individually hybridized with wild type or mutant DNA and purified over streptavidin beads. The genomic DNA was then eluted from the beads, and sequenced using Illumina deep sequencing, revealing a 15 nucleotide in frame deletion within the first exon of sgt122383, this allele will be called sgt1 for simplicity. In addition, a P element allele of sgt1 (C01428; from the Harvard Exelixis collection) failed to complement and showed the same phenotype as sgt122383.

Fly stocks

Df(3R)6147 was obtained from the Bloomington stock center. The lkb1144–2 and lkb1144–11 mutant stocks (Mirouse et al., 2007) were kindly provided by Daniel St. Johnston (Cambridge, UK); the strong or null ampkα mutants ampkα1, ampkα2 and the UAS-GFP-lkb1 and UAS-ampkα(T–D) stocks were gifts from Jay Brennan (Chapel Hill, NC); UAS-ampkRNAi was obtained from the Vienna Stock Center. pins1626 was obtained from Bill Chia (Singapore); hsp8313F3 and hsp8313F3 were obtained from Howard Lipshitz (Toronto); sgt114F1, sgt114F1 were obtained from the Bloomington stock center; UAS-polo was obtained from Claudio Sunkel (Porto, Portugal), and wormni-gal4-UAS-miranda:GFP (Carnabard and Doe, 2009) was used for live imaging. The sgt122383 pins1626 double mutants were generated by recombination, insc-gal4 (aka 1407-gal4), wormni-gal4, and tubulin-gal4 were all used for rescue experiments. Mutant larvae were identified by lack of balancer chromosome markers Tubby or GFP.

Antibody staining, drug treatment, and imaging

Third instar larval brains were dissected and fixed as previously described (Lee et al., 2006a). Neuroblast counting and BrdU labeling were done as previously described (Lee et al., 2006a). Dissected brains were washed several times in PBS-T (PBS with 0.1% TritonX-100) and blocked for 1 h in PBS-BT (PBS with 0.1% Triton and 1% BSA). Brain preparations were incubated overnight at 4 C with primary antibodies diluted in PBS-BT. All analyses were performed with the
mitotically marked rat anti-α-tubulin (1:3000; Serotec, Kidlington, Oxford, UK) or rabbit anti-phospho-histone H3 (1:1000, Upstate, West Grove, PA) and one or more of the following antibodies: guinea pig anti-Bazooka (1:1000, Doe lab), rabbit anti-aPKC (1:1000; Sigma, St. Louis, MO), rabbit anti-Par-6 (1:100; Doe lab), rat anti- Pins (1:500; Doe lab), rabbit anti-Insucutable (1:500; Bill Chia), rabbit anti-Goi (1:500; Doe lab), mouse anti-Discs Large (1:500; Developmental Hybridoma Studies Bank [DHSB], Iowa), anti-rabbit Scribble (1:5000; Doe lab), guinea pig anti-Miranda (1:400; Doe lab), rabbit anti-Miranda (1:1000; Doe lab), rabbit anti-phosphoThr385AmpKα (1:100; Cell Signaling, Beverly, MA), anti-phospho-Ser19 human MLCK (Cell Signaling, no. 3671) 1:100 to detect phospho-Ser22 Sqh (Myosin regulatory light chain), and guinea pig anti-Numb (1:100; Jim Skeath). After 3 washes with PBS-BT the samples were incubated for 2 h at room temperature with the appropriate secondary antibody (FITC-conjugated IgG, Rhodamine Red-X-conjugated IgG, and Cy5-conjugated IgG; Invitrogen, Eugene, OR). All secondary antibodies were diluted from a 0.5 mg/ml stock solution and used at 1:400. Brains were washed in PBS, mounted in VectaShield medium, and examined with a Bio-Rad Radiance 2000 confocal microscope using a 63 × 1.3NA oil immersion objective. Image processing was performed using ImageJ (NIH) and Photoshop (Adobe), and figures were assembled in Illustrator (Adobe).

To inhibit microtubule formation, dissected wild type and sgt1 mutant brains were placed in 10 μg/ml Colcemid (Sigma, St. Louis, MO) diluted in Schneider’s medium (Sigma, St. Louis, MO) for 1 h. Brains were fixed and antibody stained as described earlier.

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References


