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Article

SAK/PLK4 Is Required for Centriole Duplication and Flagella Development

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Summary

Background: SAK/PLK4 is a distinct member of the polo-like kinase family. *SAK*$^{-/-}$ mice die during embryogenesis, whereas *SAK*$^{+/-}$ mice develop liver and lung tumors and *SAK*$^{+/-}$ MEFs show mitotic abnormalities. However, the mechanism underlying these phenotypes is still not known.
Results: Here, we show that downregulation of SAK in *Drosophila* cells, by mutation or RNAi, leads to loss of centrioles, the core structures of centrosomes. Such cells are able to undergo repeated rounds of cell division, but display broad disorganized mitotic spindle poles. We also show that *SAK* mutants lose their centrioles during the mitotic divisions preceding male meiosis but still produce cysts of 16 primary spermatocytes as in the wild-type. Mathematical modeling of the stereotyped cell divisions of spermatogenesis can account for such loss by defective centriole duplication. The majority of spermatids in *SAK* mutants lack centrioles and so are unable to make sperm axonemes. Finally, we show that depletion of SAK in human cells also prevents centriole duplication and gives rise to mitotic abnormalities.
Conclusions: SAK/PLK4 is necessary for centriole duplication both in *Drosophila* and human cells. *Drosophila* cells tolerate the lack of centrioles and undertake mitosis but cannot form basal bodies and hence flagella. Human cells depleted of SAK show error-prone mitosis, likely to underlie its tumor-suppressor role.

Introduction

Polo-like kinases (Plks) belong to a conserved family of mitotic serine-threonine protein kinases that play key roles in centrosome function and are misregulated in many human tumors [1]. Two branches of the family have emerged in metazoans, and they are represented in *Drosophila* by Polo and SAK (also called Plk4) [2]. Whereas much is now known about the mitotic functions of the founder member of the family, *Drosophila* Polo, and its related mammalian counterparts (Plk1–3) [1, 3, 4], the precise mitotic roles of SAK remain obscure. Conservation of the structures of the two types of Plks throughout evolution [2] suggests that different roles may have been preserved. Both branches of the family have an amino-terminus kinase domain and a regulatory C-terminal domain that contains conserved polo boxes (PBs; Figure 1A). However, whereas the two PB domains of mammalian Plk1 interact with each other to create a positively charged cleft able to bind phosphopeptides, the SAK PB forms an intermolecular homodimer in which different sequences are exposed [2, 5]. Moreover, SAK has a second very divergent PB (cryptic PB) [6, 7] that does not bind to its conserved PB [6, 7]. The Plk1–3 group are more akin to the single Plks found in the yeasts, such as Cdc5 in *S. cerevisiae*, and expression of either Plk1 or Plk3, but not SAK, rescues the mitotic defects of temperature-sensitive *cdc5-1* mutant cells [7, 8].

Drosophila Polo and its closest mammalian counterpart, Plk1, are associated with centrosomes, kinetochores, and the late-mitotic central spindle, reflecting their functions in centrosome maturation, in the metaphase-anaphase transition, and in cytokinesis [1, 9]. SAK also localizes to the centrosome, and *SAK*$^{-/-}$ mice die shortly after gastrulation, showing a 20-fold increase in cell death [6, 10]. It was recently reported that elderly *SAK*$^{+/-}$ mice display a 15-times-higher incidence of spontaneous liver and lung cancers than their wild-type littermates [11]. Similarly, the human *SAK* gene maps to a chromosome region, 4q28, that is frequently rearranged in hepatocellular carcinomas (reviewed in [7]). Multipolar mitotic spindles were reported in livers and MEFS of *SAK*$^{+/-}$ mice, suggesting that haploinsufficiency for tumor suppression may result from chromosome instability in the oncogenic pathway. Here, we address the underlying cause of these mitotic defects, showing that in both *Drosophila* and human cells, SAK is required for centriole duplication. It is therefore essential for centrosome integrity and thereby fidelity of the mitotic apparatus. Moreover, SAK is also required for development of axonemal structures, reflecting the dual nature of centrioles and basal bodies.

Results

SAK Is Required for Centrosome Integrity

To examine SAK function, we first depleted more than 70% of SAK mRNA in cultured *Drosophila* cells. We observed a 12-fold increase in the percentage of mitotic cells that had no γ-tubulin at the poles (1.6%, controls versus 24.7%, *SAK* RNAi; Figures 1B and 1C, and Figure S1D in the Supplemental Data available with

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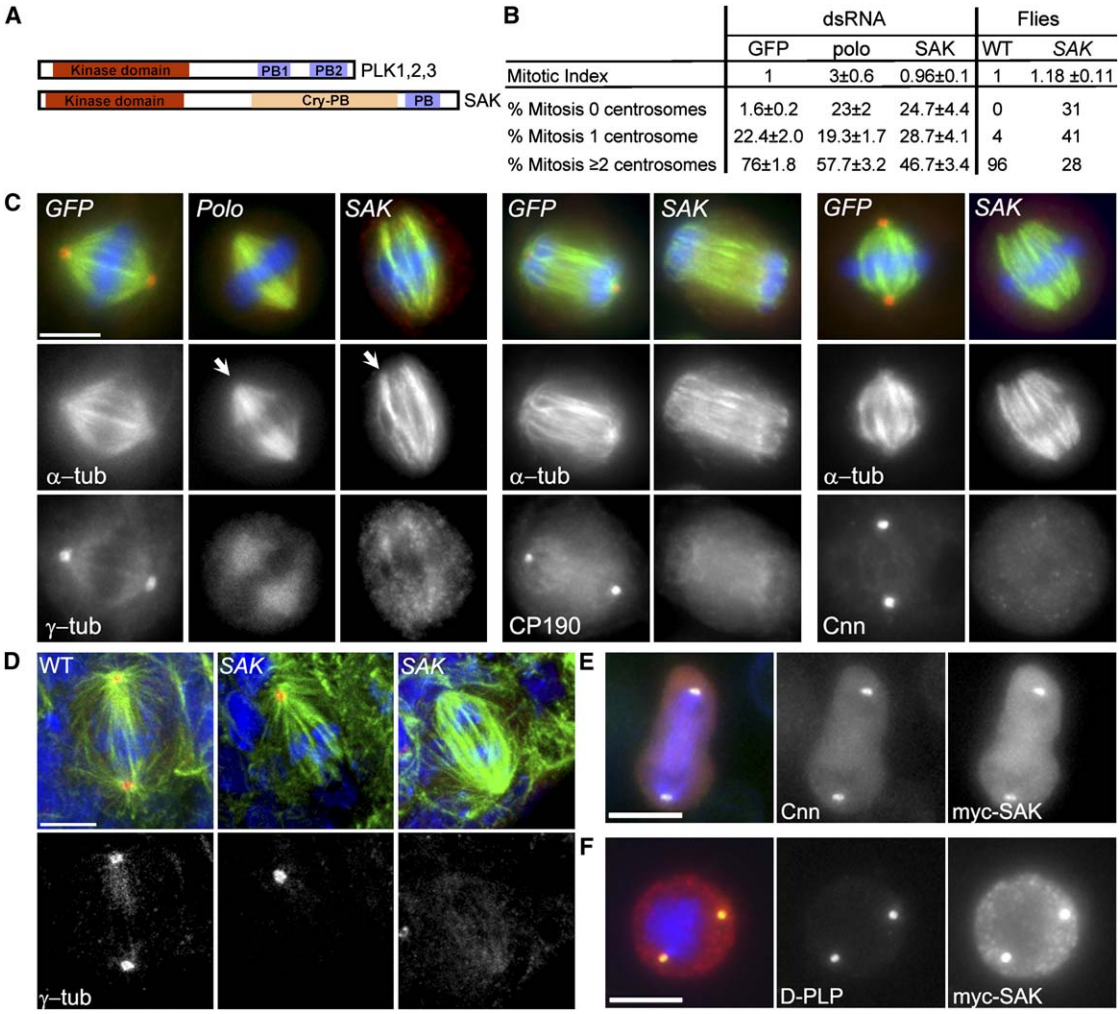


Figure 1. *Drosophila* SAK Is Necessary for Centrosome Integrity

(A) Domains of polo-like kinases. PB denotes polo box; Cry-PB denotes cryptic PB.

(B) Quantification of mitotic defects (mitotic index as a percentage) 4 days after RNAi in S2 cells or in wild-type (WT) and *SAK* mutant larval brains. GFP dsRNA is the control. Data are the average of 3–4 RNAi experiments ± standard error of the mean (SEM) or are from four independent preparations of wild-type and *SAK* brains (n = 100 mitotic cells). S2 cells show high background of centrosome defects [14, 41]. γ-tubulin was used as a centrosome marker.

(C) *SAK* RNAi leads to loss of centrosome markers. The targeted gene is indicated on the top-left corner of each image. Note broader poles (arrows) and disorganized spindle after *SAK* compared to *polo* RNAi: α-tubulin (green), DNA (blue), and γ-tubulin/CP190/Cnn (red). The γ-tubulin antibody used only recognizes *Drosophila* centrosomes in late G2 or mitosis; it does not recognize centrioles [18].

(D) Mitotic cells in larval brains of *SAK* flies can lack centrosomes and show both focused (right panel) and splayed poles (lower pole, central panel): α-tubulin (green), DNA (blue), and γ-tubulin (red).

(E and F) Myc-SAK fusion protein (red) stably expressed in S2 cells localizes to centrosome. (E) shows late anaphase; Cnn is in green, and α-tubulin is in blue. (F) shows an interphase cell. D-PLP is in green, and DNA is in blue. The same localization was found for a C-terminal fusion. Scale bars represent 5 μm.

this article online). We also observed the absence of other proteins typically recruited to *Drosophila* centrosomes in mitosis: CP190 [12] and Cnn [13] (Figure 1C). In a recent survey of the cell-cycle function of all the protein kinases in *Drosophila* [14], loss of function of only one other kinase, Polo, led to loss of γ-tubulin from the centrosome, consistent with the known function of Polo/Plk1 in centrosome maturation [15, 16] (Figures 1B and 1C). However, in contrast to the striking metaphase arrest following *polo* RNAi (Figure 1B), there was no change in mitotic index (Figure 1B) or in the flow-cytometry profile of DNA content (Figure S1E) after *SAK* RNAi. Thus, in S2 cells, SAK is required for centrosome integrity but not for progression through the cell cycle or cell survival.

We then examined dividing cells in the central nervous system of *SAK* mutant larvae (See Figure S1 for details on the hypomorphic allele used). Such cells showed an extremely similar phenotype to *SAK* RNAi cultured cells: a notable absence of γ-tubulin from the poles of spindles that were often disorganized and splayed (Figures 1B and 1D). Meiotic spindles in testes of such mutants also often lacked γ-tubulin and Cnn at the poles (Figure S2). We found that *Drosophila* SAK localized to centrosomes in both interphase and mitosis of wild-type cells (colocalization in 98% of the cells, n = 141

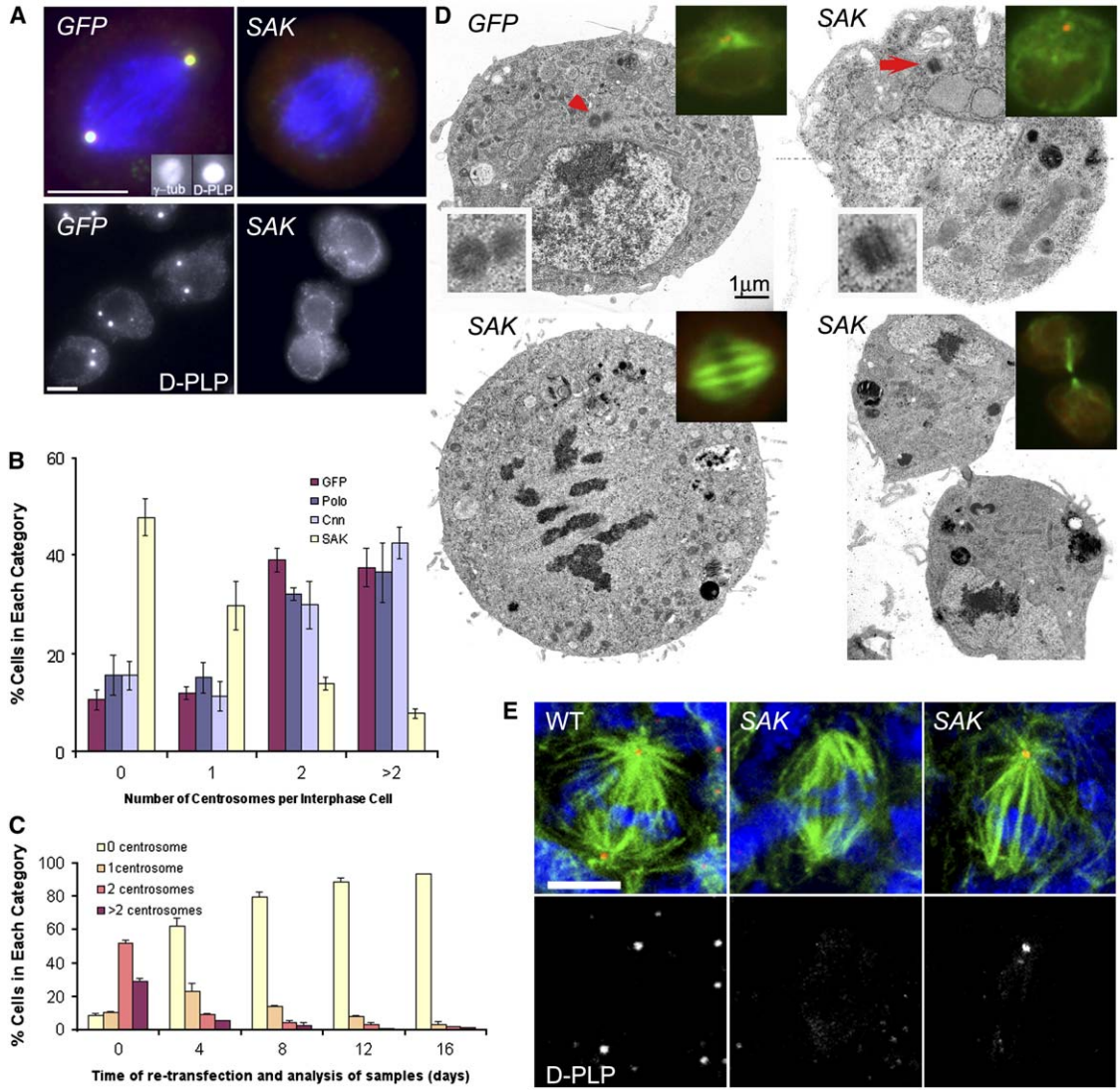


Figure 2. RNAi and Mutation of SAK Lead to Loss of Centrosomes and Centrioles

(A) *SAK* RNAi leads to the absence of D-PLP foci. The top panel shows mitotic cells: D-PLP (green), γ-tubulin (red), and α-tubulin (blue). Insets are 2× top pole. The lower panel shows interphase cells. Note that D-PLP labels wild-type (WT) centrosomes in S2 cells as a single dot.

(B) Quantification of D-PLP-stained centrosomes in interphase cells 4 days after RNAi. Note that a significant increase in cells with zero and one centrosome is only seen after *SAK* RNAi.

(C) Cells were transfected at indicated intervals, and centrosome number was quantitated as in (B). Control samples (GFP dsRNA) were similar at 0, 4, 8, 12, and 16 days, but we only show for time = 0 days. Data in (B) and (C) represent the average of three independent experiments ± SEM.

(D) Transmission electron micrographs (TEM) reveal missing centrioles in *SAK* RNAi cells after serial sectioning. Upper-right insets represent same cell previously stained to reveal α-tubulin (green) and centrin (red). The arrowhead indicates a normal centrosome with two centrioles. The arrow indicates a centrosome with a single centriole after *SAK* RNAi. Lower-left insets are 3× centrioles. Lower panels show *SAK* RNAi anaphase and cytokinetic cells devoid of centrioles.

(E) Reduced number of D-PLP foci in *SAK* mutant larval brains. D-PLP (red; single dot per spindle pole), α-tubulin (green), and DNA (blue). Figures are maximum-intensity projections of optical sections acquired at 0.3 μm intervals. Scale bars represent 5 μm unless indicated.

cells), consistent with its having a function in ensuring centrosome integrity (Figures 1E and 1F).

In contrast to Polo-depleted cells, the poles of *SAK*-depleted cells were broad (Figure 1C, white arrows), similar to the mitotic figures of a *Drosophila* acentriolar cell line [17]. This led us to ask whether the innermost centrosomal structures, the centrioles, might be disrupted by depletion of SAK. We found that the majority of mitotic cells that showed no γ-tubulin at the poles following *SAK* RNAi had no detectable pericentrin-like protein (D-PLP) [18], normally present in both centrioles and the pericentriolar material (PCM) (88%; Figure 2A, top panel). This was in contrast to *polo* or *cnn* RNAi cells, where D-PLP was still present in more than 75% of cells lacking γ-tubulin at both poles, reflecting the roles of Polo and Cnn in centrosome maturation [16, 19, 15] but not centriolar integrity. In interphase, the majority of *SAK* RNAi cells had either zero or a single centrosome, whereas interphase *polo* or *cnn* RNAi cells did not show a significant change in centrosome number (Figure 2A, lower panel, and Figure 2B). The absence of D-PLP in a large proportion of interphase *SAK* RNAi cells

suggested that they might be missing both centrioles and PCM. To confirm loss of centrioles after *SAK* RNAi, we first stained such cells for the centriole marker centrin and then embedded and serially sectioned them for transmission electron microscopy (TEM). In SAK-depleted cells in which centrin staining was absent at the spindle poles, we were unable to detect centrioles by TEM (Figure 2D). Thus, the downregulation of *SAK* leads to loss of centrioles from affected cells, with no effect on the ability of those cells to proliferate.

To determine whether loss of centrosomes also occurred in *SAK* mutants and still permit proliferation of diploid cells, we first examined the central nervous system of mutant larvae. This revealed cells with two, one, or zero centrosomes (Figure 2E), a phenotype identical to *SAK* RNAi. Moreover, the brains of mutant larvae were of normal size and the proportion of brain cells in mitosis was comparable to the wild-type, indicating no obvious defects in cell-cycle progression (Figure 1B). *SAK* mutants were able to pupate, and adults eclosed from the pupae (Figure S1). However, the majority of adults were uncoordinated and died after getting stuck in the food (Figure S1). This phenotype is similar to *D-PLP* mutations that cause defects in basal bodies [18], the centriolar-derived structures required for formation of cilia in neurons of type-I sensory organs that function in transduction of sensory stimuli [20]. The lack of centriole markers in cells of the larval central nervous system of *SAK* mutants suggests that the uncoordinated adult phenotype is likely to reflect absence of basal bodies in sensory neurons [18].

SAK Is Needed for Centriole Duplication in *Drosophila*

We wondered whether the above defects could reflect a failure to assemble new centrosomes. This could account for the increased frequency of cells with only one centrosome in *SAK* mutants (Figure 1B, Figures S2D and S2I), if new centrosomes are not formed but cells continue to cycle. Our prediction was that if cultured cells were allowed to continue dividing while being exposed to SAK dsRNA, the average number of centrosomes per cell would decrease with time. We tested this by repeatedly transfecting cells with SAK dsRNA at 4-day intervals. Successive transfections led to a progressive increase in the proportion of cells with no centrosomes, rising to greater than 93% of cells after 16 days (Figure 2C). This dilution is consistent with SAK's having a role in centrosome assembly. Such a role was further substantiated by the finding that transfection of S2 cells with the active, but not inactive, kinase led to an increase in cells with more than two D-PLP foci (Figure S1F; 67% ± 6.2 versus 29% ± 4.5). These foci are unlikely to result from aborted cell division because the majority of those cells had a single nuclei (91%, n = 119). These foci behaved as microtubule organizing centers clustering at the poles of mitotic spindles (Figure S1G). Although additional experiments will be needed to verify the origin of the multiple foci, these results suggest that overexpression of SAK may result in multiple centrosomes.

To determine whether the phenotypes observed in *SAK* mutant flies were associated with defective centrosome separation, abnormal centrosome inheritance, or problems in centriole duplication [21], we examined spermatogenesis in *SAK* mutant males. The germline has a stereotyped pattern of mitotic and meiotic divisions, a pattern that allows the cellular history of centrosomes and centrioles to be deduced (Figure 3A) [22]. Additionally, their centrioles are approximately 10-fold longer than those found in other *Drosophila* cells [23] and can be easily visualized by fluorescence of a GFP-PACT fusion protein harboring the centriole-targeting domain of D-PLP protein [18].

A very high proportion of primary spermatocytes from *SAK* mutants had no centrioles at one or both spindle poles in meiosis I (Figures 3B and 3C). When centrioles were absent, the spindle poles were broad and there were no astral microtubules (Figure 3B, arrowhead), or very disorganized spindles were formed (Figure 3B, arrow). Transverse TEM sections of wild-type sperm tails revealed the classic "9 + 2" axonemal microtubules (Figure 3D, yellow arrow) that were missing from the majority of elongating *SAK* spermatids (Figure 3D, red arrowheads). Accordingly, the majority of sperm from *SAK* mutant testes were nonmotile, and males were sterile (Figure S1). Frequently, cells that had no axonemes had irregular size and numbers of mitochondrial derivatives per cell (Figures 3D and 3F). This is usually associated with defective chromosome segregation and cytokinesis during meiosis [22]. The lack of a strong spindle-assembly checkpoint in meiosis [24] makes cells more likely to progress all the way through both meiotic divisions even in the presence of abnormalities. When axonemes were present, they appeared to be normal in structure (Figures 3D and 3E).

Wild-type primary spermatocytes enter meiosis I (MI) with a pair of centrioles at each spindle pole (Figure 3A). Daughter cells inherit a pair of centrioles that are not duplicated in *Drosophila melanogaster* [23]. These centrioles separate, each generating a centrosome at the pole of the second meiotic spindle (Figure 3A). Thus, each daughter spermatid inherits a single centriole. Our failure to observe more than a single centriole in the products of meiosis II in the *SAK* mutant (Figures 4A and 4B) allowed us to discard the possibility of defects in centriole separation.

The cysts of 16 primary spermatocytes encapsulate the history of centriole duplication in the four preceding cell divisions. We therefore asked whether the number of centrioles per cell in *SAK* mutant cysts could reflect abnormal centriole duplication. Mature cysts of primary spermatocytes in both wild-type and *SAK* mutant testes always contained 16 spermatocytes of comparable size (Figure 4C), indicating success in the four rounds of premeiotic mitosis. However, whereas wild-type primary spermatocytes contained four centrioles (64 per cyst), the majority of spermatocytes from *SAK* mutants (68%, n = 239) had no centrioles, and a smaller proportion of cells had intermediate numbers between one and four (Figures 4C and 4D). The absence of cells having more than two centrosomes/four centrioles (0%, n = 352) provided a second indication of the lack of defects in centrosome segregation to daughter cells. To test whether the distribution of centrioles observed resulted from a defect in centriole duplication, we generated a mathematical model to describe the centriole-duplication cycle and adjusted it for the four mitotic divisions of

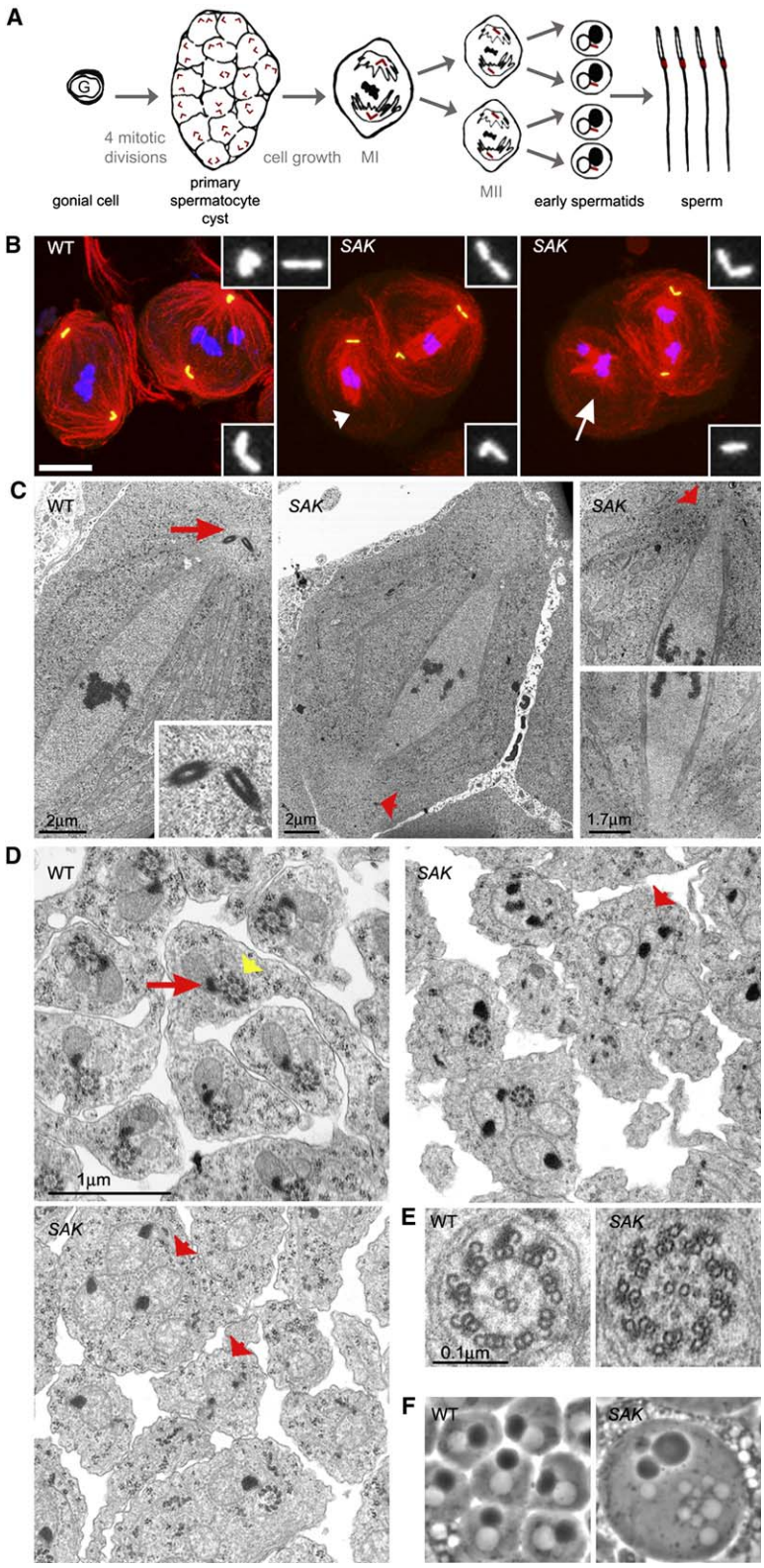


Figure 3. *SAK* Mutants Lack Centrioles and Axonemes in *Drosophila* Spermatogenesis

(A) Schematic of spermatogenesis in flies. Each stem cell division produces a gonial cell that undertakes four rounds of mitosis to produce a cyst of 16 primary spermatocytes connected through 15 ring canals, the result of incomplete cytokinesis. Each of these cells has four centrioles (red bars) and undertakes a prolonged G2 phase. Meiotic divisions produce a cyst of 64 interconnected spermatids, each with one centriole. Early spermatids have a single nucleus (white sphere) and mitochondrial derivative (Nebenkern, black sphere) of similar sizes. The spermatid centriole differentiates into a basal body to organize the flagellar axoneme of sperm.

(B) Primary spermatocytes in meiosis I. Red indicates α-tubulin, green indicates GFP-PACT, and blue indicates DNA. There is an absence of centrioles at unfocused poles (white arrowhead) and abnormal spindles (white arrow). The insets show 3× GFP-PACT staining. The scale bar represents 10 μm.

(C) TEM of serial sections of primary spermatocytes in metaphase/early anaphase of meiosis I. The righthand image is a composite from two sections at poles. Note the absence of centrioles at poles of mutant cells (red arrowheads). The red arrow shows centrioles in a wild-type (WT) cell, (3× in inset).

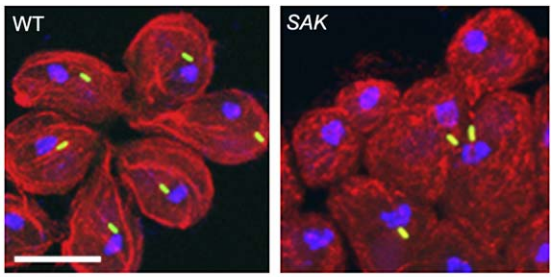
(D) TEM of cysts of elongating spermatids. Sections were taken at the level of the Nebenkern (dark body; red arrow). The yellow arrowhead shows axoneme. Note the absence of axonemes in many *SAK* cells (red arrowhead).

(E) Axonemes. Note WT axoneme is an at earlier stage than mutant, and accessory microtubule hooks have not yet closed fully.

(F) Phase-contrast image of spermatids showing irregular nuclei and Nebenkern in *SAK* mutants.

germ cells in a cyst. We used multitype branching-process theory to evaluate the distribution of cells with a given number of centrioles (see Appendix S1 for details). The model has some analogies with previous analytical work on plasmid copy number in bacteria [25]. It assumes that centrosome separation and segregation is perfect, and that the variable number of centrioles in G2 after the four germline divisions is due to partially defective centriole duplication having a probability θ. We then built generating functions to follow the dynamics of the mean number of cells with a given number of centrioles. The proportion of cells with a given number of

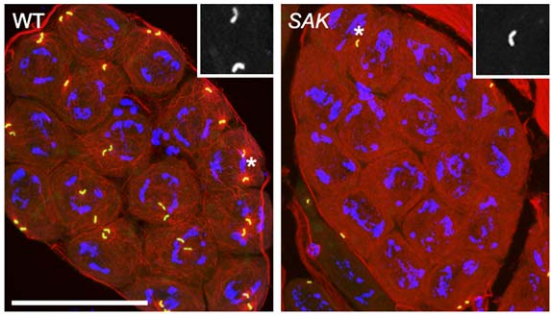
A



B

	Percentage of cells (post MII)		
	0 centrioles	1 centriole	2 centrioles
WT(n=154)	0	100	0
SAK(n=207)	65	35	0

C



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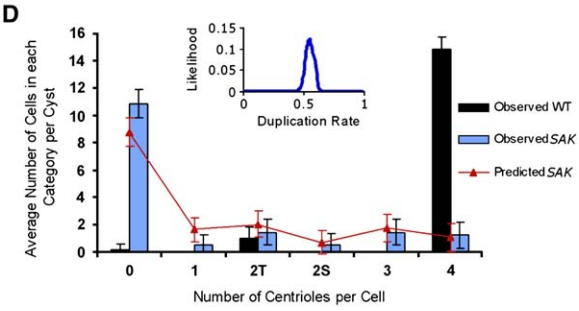


Figure 4. Defects in Centriole Replication and Not Separation in *SAK* Mutant Testes

(A and B) Centrosomes can separate in *SAK* mutants. Post-MII centrioles were counted. Note that quantitation of centrioles in *SAK* mutant spermatocytes may show an underestimate of cells lacking centrioles, as a result of their irregularity of nuclear number, size, and cell shape. Green shows GFP-PACT; red shows α-tubulin; and blue shows DNA. The scale bar represents 10 μm.

(C and D) The four mitotic divisions of the gonial cell in the spermatocyte cyst were used to model the centrosome cycle (see Appendix S1 for details). (C) shows wild-type (WT) and *SAK* mutant cysts in G2. Green shows GFP-PACT, red shows α-tubulin, and blue shows DNA. Both cysts have 16 cells but *SAK* cysts show a small number of centrioles. The inset shows GFP-PACT (2×, asterisk at low power). The scale bar represents 50 μm. (D) Observed (wild-type [n = 15 cysts in G2] and mutant [n = 22 cysts in G2]) and predicted number of cells with a given number of centrioles following the model presented in Appendix S1. Cells in cysts with two centrioles are subdivided into classes: 2T (always seen in WT), with two centrioles close together, or 2S (seen in mutant), with two isolated centrioles. All three centriole cells were 2T + 1; all four centrioles were 2 × 2T. Note that some WT cells had zero or two centrioles (2T), and so we incorporated a WT error rate into the model (see Appendix S1). Bars represent observed values (mean ± standard deviation [SD]); red triangles represent expected values under the best statistical model. The inset reports the maximum-likelihood function for centriole duplication rate between 0 and 1. The function is strongly peaked around a maximum of 0.55.

centrioles in G2 was evaluated after four cell divisions. Finally, we found a value of θ that best fit the empirical data (see Appendix S1 for details and statistical analyses) (Figure 4D, inset). The function is very peaked around 0.55 (Figure 4D, inset), fitting the empirical data very well (Figure 4D) and giving high confidence in the estimated duplication rate. Thus, we were able to model the reduction in centriole number during the premeiotic divisions of *SAK* hypomorphic mutants solely by assuming reduced success of centriole duplication.

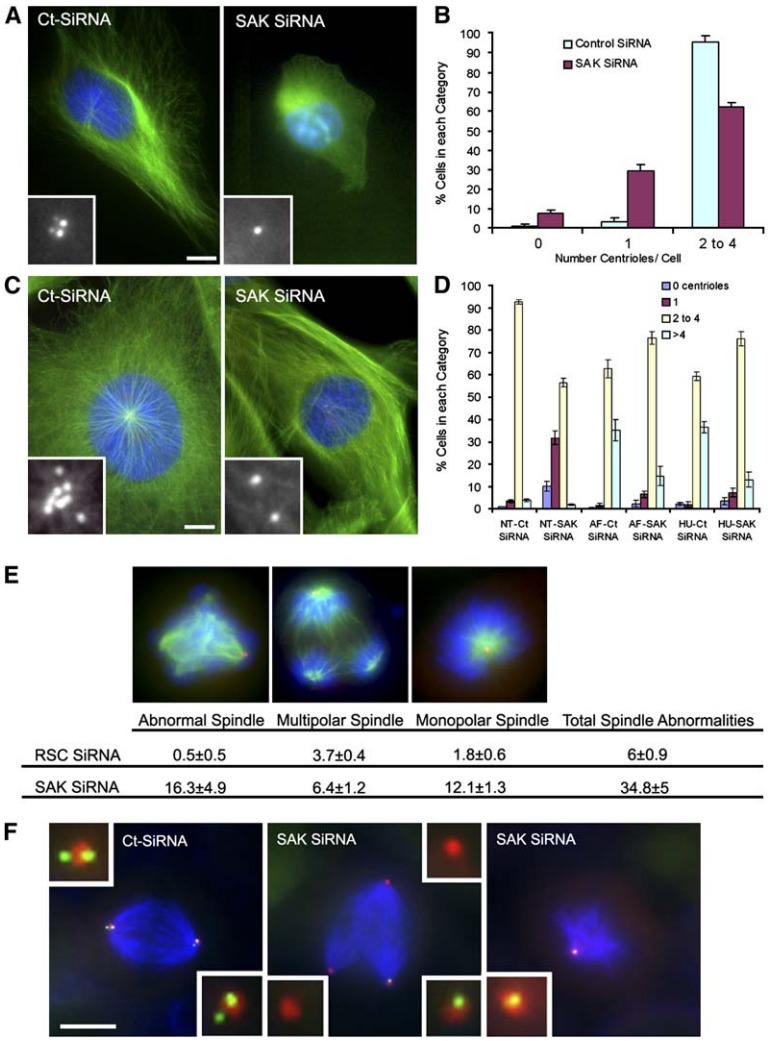
SAK Is Necessary for Centriole Duplication and Mitotic Fidelity in Human Cells

Finally, we asked whether the role of SAK in centriole duplication was conserved in human cells and developed SAK siRNA conditions that reduced levels of SAK transcripts by more than 70% (Figure S3A). Such depletion resulted in a 10-fold increase of HeLa cells with just one centriole (Figures 5A and 5B). This was associated with an increased mitotic index (58% increase as compared to wild-type [WT] cells; Figure S3D) and a higher-than-2-fold increase in apoptosis (Figures S3B and S3C), leading to a decrease in cell number. We observed a similar reduction in centriole number after SAK RNAi in U2OS cells (Figure 5D). It is known that centrioles continue to replicate when U2OS cells are blocked in S phase by treatment with the DNA polymerase α-inhibitor aphidicolin (AF) or the ribonucleotide reductase inhibitor hydoxyurea (HU) [26–28]. Following SAK RNAi, we found the number of cells accumulating supernumerary centrosomes in an S phase block to be less than half that of control cells (Figures 5C and 5D), as shown before for other molecules required for centriole duplication [26–28]. Thus, downregulation of SAK reduces centriole duplication in cells blocked in S phase. There was no increase in the proportion of cells with a single centriole after SAK RNAi when cells were inhibited from dividing by treatment with AF or HU (Figure 5D), a result consistent with inhibition of centriole duplication. We therefore conclude that the human SAK kinase is required for centriole duplication in both HeLa and U2OS cells and for centriole reduplication in AF- or HU-treated U2OS cells.

Reduced centriolar number was associated with a 6-fold increase in abnormal mitotic spindles 72 hr after SAK SiRNA in HeLa cells (Figures 5E and 5F). These included monopolar and multipolar spindles (Figures 5E and 5F). We have observed similar defects in U2OS cells (Figure S3E). Curiously, in a few cases, we saw recruitment of γ-tubulin to the acentriolar poles of multipolar spindles (Figure 5F, middle panel), suggesting that acentriolar poles may organize some PCM in mammalian cells. We have not observed such cases in *Drosophila*. In summary, spindle organization is affected in the absence of two canonical centrosomes having two centrioles, and this is likely to contribute to the reduced chromosome-transmission fidelity that has been suggested from observations of *SAK*-deficient mouse cells [11].

Discussion

Human SAK/PLK4 has been described as a tumor suppressor in humans (reviewed in [7]) and mice [11], and



	Abnormal Spindle	Multipolar Spindle	Monopolar Spindle	Total Spindle Abnormalities
RSC SiRNA	0.5±0.5	3.7±0.4	1.8±0.6	6±0.9
SAK SiRNA	16.3±4.9	6.4±1.2	12.1±1.3	34.8±5

Figure 5. SAK Is Essential for Centriole Duplication in Human Cells

(A and B) SiRNA of control (Ct) and SAK in HeLa cells. Cells were fixed and stained after 48 hr. (A) Green shows α-tubulin, blue shows DNA, and red shows centrin-2. The insets show centrin staining at 6×.

(B) Quantification of centrioles. Note the increase in percentage of cells with zero and one centrioles after depletion of SAK.

(C and D) HsSAK SiRNA reduces centriole number observed in reduplication assays. Twenty-four hours after transfection of U2OS cells with SiRNAs, aphidicolin (AF) or hydroxyurea (HU) was added. Cells were fixed and immunostained at 72 hr for centriole number counting. (C) Green shows α-tubulin, blue shows DNA, and red shows GT335 (polyglutamilated tubulin). The insets show GT335 staining at 4×. (D) Quantification of centrioles. NT denotes not treated with drugs. Note the increase in the percentage of cells with zero and one centrioles in nontreated cells transfected with SiRNA for HsSAK; SiRNA for HsSAK reduces the percentage of cells with more than four centrioles after treatment with AF or HU (light blue bars) by 50%.

(E and F) Examples of mitotic defects in HeLa cells 72 hr after HsSAK RNAi. Green shows α-tubulin, blue shows DNA, and red shows centrin-2. Mitotic defects were scored as in [14]. Results show average of at least three experiments ± SEM. (F) Blue shows α-tubulin, red shows γ-tubulin, and green shows GFP-centrin. The insets show γ-tubulin and GFP-centrin at 4×. Scale bars represent 10 μm.

it has been suggested that correct levels of SAK are essential for mitotic fidelity [11]. Here, we show for the first time that SAK is essential for centriole duplication in both *Drosophila melanogaster* and human cells. We show that the reduced centriole number arising in the absence of SAK in human cells leads to the formation of abnormal mitotic spindles, providing the first mechanistic insight for the tumorigenic role of this molecule.

Drosophila has just two PLK family members: Polo, involved in centrosome maturation and mitotic progression, and SAK, not previously studied in this organism. Depletion of SAK, but not Polo, led to cells with a reduced number of centrioles. Centriole loss in a cycling population of cells can arise through defective centrosome duplication, abnormal separation of centrosomes at entry to mitosis, or abnormal centrosome segregation to the daughter cells in cytokinesis. We show in both human and *Drosophila* cells that there is no abnormal centrosome segregation in the absence of SAK because there is always loss but never gain of centrosomes/centrioles after cell division (Figures 1–5). Moreover, centriole reduplication in S phase is reduced after SAK knockdown in the absence of cell division in human cells (Figure 5D). Our analysis of centriole distribution in *Drosophila SAK* spermatids revealed no defects in centrosome separation. Moreover, the distribution of centrioles in cysts of 16 primary spermatocytes was consistent with a mathematical model assuming defects in centriole duplication. Together, these observations point to a conserved role of this member of the PLK family in centriole duplication. However, we cannot entirely exclude an additional function for SAK in mitosis until we isolate a complete loss-of-function mutant.

Experiments in *C. elegans* have suggested five proteins to be important for centrosome duplication in embryogenesis [29, 30], and these include one protein kinase, ZYG1 [31]. Although ZYG-1 has only low sequence similarity to *Drosophila* SAK, it is the closest homolog in a BLAST search [32], and SAK may thus represent the ortholog of ZYG-1 in flies and vertebrates.

What could be the role of SAK in centriole duplication? We have never observed structurally compromised centrioles either in *SAK* mutants or after SAK depletion in either *Drosophila* or human tissue-culture cells. Moreover, overexpression of SAK, but not of the inactive kinase, leads to the formation of multiple D-PLP foci, suggestive of overduplication of centrioles. Together, these data suggest that SAK has a regulatory role in centriole duplication. ZYG-1 has been found to be high in the hierarchy of molecules necessary for centriole assembly and

essential for the recruitment of SAS-6/SAS-5 to the centriole [27], but it remains to be discovered whether this role has been conserved in *Drosophila* and vertebrates.

The perdurance of centrosomal structures in *SAK* mutants studied here may result from some residual SAK function due to the hypomorphic nature of this allele and/or from remaining wild-type maternal SAK protein provided by the heterozygous mother. Such perdurance of maternal protein to the third larval-instar stage is a common feature of mitotic mutants in *Drosophila* [33, 34]. Nonetheless, *SAK* mutants provide the first opportunity to assess the consequences of the absence of centrioles and centrosomes upon the development of an organism. Our examination of the larval central nervous system suggests that as many as 72% of dividing cells have fewer centrosomes than expected, with 28% possessing no centrosomes at all. In cysts of primary spermatocytes, only 8% of the cells have the correct number of centrioles, whereas 68% have none. It has been proposed that centrosomes are not required for the formation of mitotic spindles, but do provide speed and fidelity to this process (reviewed in [35]) and so may be necessary for proper cell-cycle progression (reviewed in [36]). We conclude that in cultured *Drosophila* cells and in the whole organism, centrosomes are not essential for mitotic progression or cell survival. However, the absence of centrioles, and hence basal bodies, compromised both meiotic divisions and the formation of sperm axonemes. The only cilia and flagella known in the fly are found in the peripheral nervous system and in the male germline [37]. Accordingly, we found *SAK* mutants to be both uncoordinated and sterile.

What could be the consequences of the lack of SAK in vertebrates? Mammalian cells appear to be more sensitive to depletion of SAK and the lack of centrosomes than *Drosophila* cells. There was a significant increase in abnormal mitoses, in mitotic index, and in apoptosis after depletion of SAK in human cells. Mitotic abnormalities and an increase in cell death were also observed after depletion of SAS-6, a protein involved in centriole replication, in U2OS cells [27] and may thus be a general consequence of centriole loss in vertebrates. Mice homozygotes for *SAK* die very early during embryogenesis [10], hindering study of the effects on cilia formation and development. However, these embryos showed increased cell death and a higher mitotic index [10]. $SAK^{+/-}$ MEFs also show abnormal spindles and chromosome segregation, and, significantly, $SAK^{+/-}$ mice are more prone to develop cancer [11]. This link between centrosome duplication, embryonic survival, and haploinsufficiency for tumor suppression is also seen with nucleophosmin, a previously suggested regulator of centrosome duplication [38, 39]. Together, these studies reinforce the link established by Boveri in 1914 between centrosome defects and oncogenesis [40]. The sensitivity with which two tumor cell lines undergo aberrant mitosis and cell death after downregulation of the SAK kinase suggests that it may be a valuable target for cancer therapy.

Supplemental Data

Supplemental Data include Supplemental Experimental Procedures, three figures, one table, and one appendix and are available with this article online at: http://www.current-biology.com/cgi/content/full/15/24/2199/DC1/.

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Note Added in Proof

While this manuscript was under review, another study reported a role for SAK/PLK4 in centrosome duplication: Habedanck, R., Stierhof, Y.-D., Wilkinson, C.J., and Nigg, E.A. (2005). The Polo kinase Plk4 functions in centriole duplication. Nat. Cell Biol. 7, 1140–1146. Published online October 23, 2005. 10.1038/ncb1320.

This version differs slightly from the one originally published online in that italics and uppercase letters have been added in a few places to suit the correct genetic nomenclature.