Mutations in *Drosophila Greatwall/Scant* Reveal Its Roles in Mitosis and Meiosis and Interdependence with Polo Kinase

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Polo is a conserved kinase that coordinates many events of mitosis and meiosis, but how it is regulated remains unclear. *Drosophila* females having only one wild-type allele of the polo kinase gene and the dominant Scant mutation produce embryos in which one of the centrosomes detaches from the nuclear envelope in late prophase. We show that Scant creates a hyperactive form of Greatwall (Gwl) with altered specificity in vitro, another protein kinase recently implicated in mitotic entry in *Drosophila* and *Xenopus*. Excess Gwl activity in embryos causes developmental failure that can be rescued by increasing maternal Polo dosage, indicating that coordination between the two mitotic kinases is crucial for mitotic progression. Revertant alleles of Scant that restore fertility to polo–Scant heterozygous females are recessive alleles or deficiencies of gwl; they show chromatin condensation defects and anaphase bridges in larval neuroblasts. One recessive mutant allele specifically disrupts a Gwl isoform strongly expressed during vitellogenesis. Females hemizygous for this allele are sterile, and their oocytes fail to arrest in metaphase I of meiosis; both homologues and sister chromatids separate on elongated meiotic spindles with little or no segregation. This allelic series of gwl mutants highlights the multiple roles of Gwl in both mitotic and meiotic progression. Our results indicate that Gwl activity antagonizes Polo and thus identify an important regulatory interaction of the cell cycle.

Introduction

Reversible protein phosphorylation and periodic protein destruction play major roles in regulating the eukaryotic cell division cycle. The major protein kinase that directs cell division is cyclin-dependent kinase 1 (Cdk1), the active component of Maturation Promoting Factor, first found to promote meiotic entry in amphibian oocytes. The cyclical inactivation of Cdk1 prior to mitotic exit is brought about in part through destruction of its cyclin partner (reviewed by [1]). Two other protein kinase families, the Polo and Aurora families, are known to have critical functions in progression into and through M phase (mitosis and cytokinesis) and functionally interact with each other and also with Cdk1 to mediate their functions.

Polo, originally discovered in *Drosophila* [2,3], exemplifies an evolutionarily conserved mitotic protein kinase. Polo, as well as its close orthologs, has been shown to function in multiple events essential for cell division. Polo was initially found to be essential for centrosome maturation and separation [2]. It promotes recruitment of the γ-tubulin ring complex and phosphorylates Asp to facilitate nucleation of an increased number of dynamic microtubules on mitotic entry (reviewed by [4]). At the G2/M transition, Polo (Polo-like kinase 1 in vertebrates) phosphorylates and activates the Cdc25 phosphatase responsible for removing inhibitory phosphates on Cdk1; this promotes mitotic entry [5]. It also functions at the kinetochore-microtubule interface to monitor tension; the 3F3/2 phospho-epitope seen on kinetochores in the absence of tension is a consequence of Plk1/Plk1 kinase activity in vertebrates [6,7]. Removal of cohesins from chromosomal arms in mitosis and meiosis also requires phosphorylation of cohesin subunits by Polo kinases (reviewed by [8]). In *Drosophila* mitosis II, Polo phosphorylates and inactivates the centromeric cohesion protector protein Mei-S332 [9]. In addition, Polo is required for cytokinesis [10]. The growing list of Polo kinase substrates is evidence of its role in multiple mitotic events.

It is clear that protein kinases such as Cdk1 and Polo are only part of a large network of protein kinases that regulate cell cycle progression, many of which are as yet poorly characterized. A genome-wide survey found that up to one-third of the protein kinome of *Drosophila* has some cell cycle role [11]. Depletion of the Gwl kinase from S2 cells by RNA interference (RNAi) led to a mitotic delay characterized by formation of long spindles and scattered chromosomes [11].

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**Abbreviations**: Cdk1, cyclin-dependent kinase 1; FISH, fluorescence in situ hybridization; GFP, green fluorescent protein; Gwl, Greatwall; mtrm, matrimony; RNAi, RNA interference; Scant, Scott of the Antarctic; Sr, Scant revertant; wt, wild type

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Yu and colleagues (2004) also found a mitotic role for Gwl kinase by characterizing missense hypomorphic mutations. Reduced gwl function results in mitotic defects in larval neuroblasts and tissue culture cells, including delay between late G2 and anaphase onset and chromosome condensation defects. Gwl has close homologs across eukaryotes and more distant homologs in budding and fission yeasts. Indeed, Yu and colleagues recently reported a function for Gwl and neighboring genes in early syncytial embryos, which are initiated by the detachment of a single centrosome from the nuclear envelope in prophase. Loss-of-function mutants of greatwall reveal that the kinase is required for proper chromosome structure and segregation in mitosis and meiosis. One of these mutations results in the loss of Greatwall specifically during vitellogenesis (building up the egg’s contents) and leads to a failure of meiosis I characterized by the premature loss of sister chromatid cohesion. This study shows that the Greatwall kinase fulfills multiple crucial functions in the different cell cycles of a developing animal and will be the foundation for further investigations.

**Author Summary**

Coordination of cell division in development requires a complex interplay between protein kinases, which catalyze the transfer of phosphates to specific substrate proteins to modify their activities. One of these kinases is the conserved Polo, which is the target of anticancer drugs. Using genetics in Drosophila (the fruit fly), we have identified Greatwall, another conserved protein kinase, as an antagonist of Polo. Studies of Scant, a dominant mutation of the greatwall gene, lead us to examine the effects of overexpressing wild-type Greatwall. Too much Greatwall activity relative to Polo leads to developmental defects in early syncytial embryos, which are initiated by the detachment of a single centrosome from the nuclear envelope in prophase. Loss-of-function mutants of greatwall reveal that the kinase is required for proper chromosome structure and segregation in mitosis and meiosis. One of these mutations results in the loss of Greatwall specifically during vitellogenesis (building up the egg’s contents) and leads to a failure of meiosis I characterized by the premature loss of sister chromatid cohesion. This study shows that the Greatwall kinase fulfills multiple crucial functions in the different cell cycles of a developing animal and will be the foundation for further investigations.

**Results**

**Scant, a Dominant Enhancer of polo Female Effect**

**Lethality, Is an Allele of greatwall**

**greatwall** is an ethyl methanesulfonate-induced mutation on the right arm of Chromosome III that causes greatly reduced female fertility when heterozygous with polo homozygotes. These females produce embryos that develop characteristic mitotic abnormalities [14]. Scant shows no interaction with other mutants known to affect progression through embryonic cycles, namely gnu, mgr, asp, and stq [14]. The original Scant-bearing chromosome is also recessive female sterile [14]; however, although they are close, we were able to separate the dominant Scant from the recessive(s) by crossing- over (see Text S1). One recombinant that retained the dominant Scant interaction but was recessive female fertile was recovered; this and its derivatives were used in all the experiments reported here.

**greatwall** is a dominant enhancer of polo female sterility for all polo alleles, though the strength of the interaction varies with the defectiveness of the polo allele. Scant was originally described in combination with polo, a hypomorphic allele; polo homozygotes are viable though females are completely sterile. Typically, polo+/+ Scant females produce 4% as many progeny as controls, but polo+/+ Scant and Df(3L)rdgC-co2, polo−/− Scant females are completely sterile (Figure 1A). polo−/− is a breakpoint allele, In(3L)polo−/−, 77E1-3;77E1-2 (see Text S1), and therefore probably amorphic; homozygotes are prepupal lethal. This suggests that the interaction depends on how much functional maternal Polo protein is available in the egg. However, the reciprocal question—whether the interaction depends on how much functional Scant protein is in the egg—cannot be asked; Scant is not only homozygous viable and fertile, it is viable and fertile over all deficiencies of its region, and none of these deficiencies affect fertility of heterozygous polo mutations. This suggests that the Scant mutation is hypermorphic or neomorphic. If it is, then the dominant interaction with polo mutations should be alleviated by mutations that inactivate its gene, and those mutations may have recessive phenotypes that can be deficiency mapped. We therefore recovered Scant revertants by mutagenizing homozygous males with X rays, mating them to polo/Balancer females, and testing the + Scant+/polo + daughters for fertility. We recovered three classes of revertants (Figure 1B):

- The first class is duplications of polo; we recovered two, a tandem duplication and a 3:3 duplication transposition. This confirms the deduction from the genetic analysis that, in the presence of the hyper- or neomorphic Scant mutation, two maternal doses of polo are needed for full female fertility.

- The second class is a second-site suppressor because it is a 3L deficiency and independent overlapping deficiencies of its
region also suppress the \textit{polo–Scant} interaction; we have yet to identify the relevant gene.

The third class is represented by two recessive mutants in \textit{gwl}, \textit{polo}, or both genes (\textit{polo}, \textit{Scant}). The extent of imprecise excisions was approximately mapped by the ability to PCR-amplify the 1 kb-long regions of genomic DNA defined by the tick marks. doi:10.1371/journal.pgen.0030200.g001

Two female progeny were tested for fertility. \textit{Scant revertant} \textit{gwl2}, \textit{gwl3a} homozygous for \textit{B}) Genetic screen to generate imprecise excisions. Figure 1C; Text S1). The chromosome carrying the second-site suppressor complements the \textit{polo} mutation, and both \textit{polo} and \textit{gwl} are semilethal and produce a “\textit{Scant}-revertant (Sr) phenotype” when placed over \textit{Sr} alleles (generated in B). The extent of imprecise excisions was approximately mapped by the ability to PCR-amplify the 1 kb-long regions of genomic DNA defined by the tick marks.

gwl 

Figure 1. Generation of \textit{gwl} Alleles

(A) Females heterozygous for both the \textit{Scant} mutation and a loss-of-function \textit{polo} mutation (such as \textit{polo}1) lay embryos that die during development.

(B) Genetic screen to generate \textit{Scant revertant} (\textit{Sr}) alleles. Males homozygous for \textit{Scant} were x-rayed and crossed to \textit{polo}1 heterozygous females. Female progeny were tested for fertility. \textit{Scant revertant} mutations restore female fertility and can be duplications of the \textit{polo} gene, third-site suppressor mutations (\textit{su}), or recessive mutations allelic to each other. See Text S1 for details.

(C) Hopping a \textit{P} element inserted directly upstream of the \textit{gwl} gene generated imprecise excisions disrupting either \textit{gwl} (\textit{gwl}P, \textit{gwl}PA, and \textit{gwl}PB), \textit{CG7718} (\textit{CG7718P}, \textit{CG7718PA}, and \textit{CG7718PB}), or both genes (\textit{gwl}PA). \textit{gwl}P, \textit{gwl}PA, and \textit{gwl}PB are semilethal and produce a “\textit{Scant}-revertant (Sr) phenotype” when placed over \textit{Sr} alleles (generated in B). The extent of imprecise excisions was approximately mapped by the ability to PCR-amplify the 1 kb-long regions of genomic DNA defined by the tick marks.

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The chromosome carrying the second-site suppressor complements the \textit{polo} gene; this was confirmed by sequencing \textit{gwl} in them (see “\textit{Scant} Encodes a Hyperactive Gwl Kinase with Altered Specificity” and “The Allelic Series of Recessive \textit{gwl} Alleles Reveals Multiple Mitotic Functions” below). \textit{gwl}P and \textit{gwl}PA are probably deletions into \textit{gwl}, and \textit{gwl}PB clearly is; both \textit{5'} upstream sequence and \textit{N}-terminal coding sequence have been deleted. All three of these small deletions give escapers homozygous and over deficiencies on nutritious food, with the \textit{Sr} constellation of phenotypes. Thus, the \textit{gwl} gene is not absolutely vital.

Finally, we carried out a second X-ray mutagenesis, now of \textit{polo}1. \textit{Scant} recombinant chromosome made from the fertile \textit{polo}1/Dp(\textit{polo}) \textit{Scant} females and tested over a third-chromosome balancer (Figure S1). This gave ten positives, all of class three; four are cytologically visible deficiencies each of which removes all or part of 91C5–6 (plus more), one is a translocation with one breakpoint in 91C, and the remaining five have no obvious relevant cytological defect. For two of these, all or part of the \textit{gwl} gene fail to PCR up, so they are probably additional deletions. All seven of these fail to complement \textit{Sr} for both phenotype and viability. The final three, \textit{Sr17}, \textit{Sr19}, and \textit{Sr18}, complement \textit{Sr} and deficiencies for phenotype but females have reduced fertility or are sterile. \textit{Sr17} and \textit{Sr19} also have reduced viability over \textit{Sr3} in the presence of \textit{polo}1 and some DNA defect \textit{5'} of the \textit{gwl} coding region (probably an insertion based on PCR, see Text S1). Both alleles reduce the amount of Gwl protein produced but have not been studied further. \textit{Sr18} is perfectly viable but is absolutely maternal-effect female sterile; this allows us to separate somatic \textit{gwl} function from its role in producing a functional egg. All further studies of \textit{Sr18} used a chromosome from which \textit{polo}1 had been removed by recombination.

Thus we identified three categories of \textit{gwl} alleles: the
Figure 2. Scant Interacts Genetically with polo, Leading to Mitotic Defects in Embryos

(A) Aberrant mitotic figures in embryos derived from females heterozygous for polo and Scant. Embryos were collected for 2–3 h, dechorionated, and fixed for immunofluorescence. Stainings are α-tubulin (green), γ-tubulin (red), and DNA (blue). Representative examples of mitotic phenotypes are shown for the indicated genotypes. Arrows in the center panel indicate the displacement of centrosomes from one pole. Scale bars are in μm.

(B) Typical displacement of one centrosome observed in embryos derived from polo¹⁺/+ Scant heterozygous females. The scale bar represents 10 μm.

(C) Quantitation of aberrant mitotic figures observed. Embryos were treated as in (A) and syncytial single embryos with mitotic nuclei were scored for the percentage of defective nuclei (having lost at least one centrosome). Numbers are average percentages (± standard error of the mean).

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dominant Scant allele, recessive zygotic plus germline alleles, and a recessive maternal-effect allele. Our characterization of these three groups gives new insights into the mitotic and meiotic functions of the protein kinase that gwl encodes.

Scant Enhances polo Defects at Spindle Poles
The severity of the dominant Scant mutant phenotype increases in relation to the decrease in polo function. Not only is the dominant effect overcome by two doses of polo¹⁺ in the presence of a polo mutation (see above), but also the severity of the phenotype in the presence of one copy of polo¹⁺ is proportional to the strength of the polo allele. Weak hypomorphic alleles such as polo¹⁻, a viable allele that shows maternal effect lethality, give some progeny when heterozygous with Scant. Amorphic alleles such as polo¹⁻ (see above) and polo¹⁻ deficiencies are completely sterile; these females lay normal numbers of eggs that do not hatch but do begin to develop and turn brown.

The archetypal Scant phenotype is shown by embryos derived from polo²⁰⁺⁺ Scant females. The mitotic figures of such embryos frequently display centrosome disassociation from one pole (Figure 2A and 2B). To compare severity of phenotypes we counted the number of defective mitotic nuclei (showing detachment of at least one centrosome) in syncytial embryos derived from mothers of different genotypes (Figure 2B and 2C). There is a slight but significant increase in defective spindles in embryos derived from polo¹⁺⁺, polo¹⁺⁻, Scant¹⁺⁻, and Scant/Scant females relative to wild-type females, indicating that a single mutant copy of these genes in mothers leads to mitotic defects at a low frequency (Figure S2). However, these embryos always hatched and developed fully, indicating that such low frequencies of defects can be tolerated. The mitotic spindles in embryos derived from polo¹⁺⁻ Scant females are more frequently aberrant than those derived from polo¹⁺⁺ Scant females. Thus the severity of the maternal effect phenotypes observed at the cellular level is consistent with the relative strengths of the polo alleles as homozygotes, indicating that, in the context of the early embryo, Scant enhances mitotic defects resulting from a decrease in polo function.

To examine the formation of the aberrant mitotic figures in polo¹⁻ Scant-derived embryos, we used time-lapse microscopy to follow mitosis in embryos that also expressed green fluorescent protein (GFP)-β-tubulin constitutively from the ubiquitin promoter (Figure 3; Videos S1 and S2). No defects were observed in embryos derived from the wild-type GFP-β-tubulin stock (Video S1). In contrast, polo¹⁻ Scant/⁺-derived embryos show an initial detachment of one centrosome early in mitosis, before nuclear envelope breakdown. All 33 cases where the filming was continuous from before the centrosome detached show that the detachment occurs prior to nuclear envelope breakdown and involves only one centrosome. The free centrosome drifts away from the nucleus, and astral microtubule formation usually appears normal, though there is no asymmetric microtubule enhancement. A half-spindle is established by microtubules forming connections between the chromosomes and the centrosome still associated with the nuclear envelope. However, spindle bipolarity is often attained by microtubules growing from the chromosomes outwards. If a free centrosome is sufficiently close to this second half-spindle, it can reattach to it to form a normal bipolar spindle containing two centrosomes and nuclear...
division completes normally (Figure 3A; Video S2). However, if the free centrosome drifts too far away from its spindle, it cannot be recaptured, and the monoastral spindle that forms initially is unfocussed at the pole lacking a centrosome. In some cases, monoastral bipolar spindles fuse with neighboring spindles (Figure 3B; Video S2) and degenerate to give interconnecting arrays of microtubules (Figure 2A). In other cases, the acentrosomal pole eventually focuses and anaphase occurs (Video S2) (unpublished data) as previously observed in sak mutants, which lack centrosomes [15]. The primary defect in these mitoses is therefore premature centrosome detachment; subsequent spindle abnormalities reflect secondary, mechanical problems. Somehow the Polo-Gwl kinase balance is important for maintaining centrosome-nuclear envelope propinquity until nuclear envelope breakdown.

Free centrosomes always show the presence of γ-tubulin, pericentrin-like protein (PLP) and centrosomin (CNN) when the attached one does, while the acentrosomal spindle poles always lack all three antigens (unpublished data)—free centrosomes seem to mature normally. Indeed, free centrosomes are mature by the functional test as well, since they can always lack all three antigens (unpublished data)—free centrosomes seem to mature normally. Indeed, free centrosomes are mature by the functional test as well, since they can be recaptured if they become reassociated with a spindle (Figure 3A).

Loss of centrosomes in Drosophila embryos has been shown to occur in response to DNA damage; nuclei then drop from the cortex into the interior of the syncytial embryo [16]. Both centrosome loss and nuclear fallout are suppressed in embryos lacking the Chk2 protein kinase [17]. Centrosomes still detach in embryos derived from chkh2/scant females (Figure S3), suggesting that the loss of centrosomes in embryos laid by poloa Scant females is not the consequence of DNA damage inducing its response pathway. This independence from Chk2 as well as the enhancement of poloa phenotypes by the Scant mutation suggests that coordinated activity of the protein kinases encoded by these genes is required directly to coordinate centrosome attachment to the nuclear envelope before spindle assembly.

Scant Encodes a Hyperactive Gwl Kinase with Altered Specificity

To identify the nature of the Scant mutation, we sequenced gwl on both the Scant chromosome and on several non-Scant mutant chromosomes that resulted from the same mutagenesis [14]. The sole difference is an A to T base change in Scant, which changes amino acid residue 97 from lysine to methionine. The gwl sequence in the Sr mutations retains the K97M substitution codon as well as additional changes, consistent with their recessive reduction in function (see “The Allelic Series of Recessive gwl Alleles Reveals Multiple Mitotic Functions” below).

To confirm that the K97M substitution in Gwl is indeed responsible for the Scant phenotype, we reconstituted the genetic interaction with polo using a synthetic gwl-K97M generated in a wild-type gwl sequence and carried as a transgene. Because the Scant mutation appears to be hyper- or neomorphic, we also asked whether expressing wild-type (wt) Gwl kinase at higher levels than normal generates the characteristic embryonic mitotic defects from polo mothers. There are two isoforms of Gwl; the female vitellogenic ovary expresses only the long form (see below). We therefore made transgenic flies expressing either the longer isoform of Gwl-Wt or Gwl-K97M (otherwise identical to wild type) under the control of the UASp promoter, driven by Gal4 expressed from the maternal α-tubulin promoter (Mat α-Tub Gal4). Expression of the transgenes (checked by western blot; unpublished data) was driven at comparable levels in the germline in poloa/polo+ and poloa+/polo+ females, and in both cases the transgenic expression exceeds the endogenous Gwl by approximately 3-fold (unpublished data). Female germline expression of Gwl-K97M in poloa+/polo+ heterozygotes causes complete sterility (Figure 4A). These embryos show loss of centrosomes from very early mitotic spindles (Figure 4BIV), a much stronger
and earlier phenotype than Scant itself. Overexpression of Gwl-wt in heterozygous polo+/polo- females allows partial egg hatch (around 4%), and these embryos also show loss of centrosomes from spindles in early syncytial divisions (Figure 4BIII). Fertility is also reduced (to 23%) in polo+/polo+ females expressing Gwl-K97M where centrosome loss usually occurs in the later divisions of the syncytial blastoderm stage (Figure 4BII). However, polo+/polo- females overexpressing Gwl-wt are fully fertile and their embryos do not show significant centrosome detachment (Figure 4BI). Thus embryonic mitotic defects and maternal-effect lethality arise from overexpression of either the wild-type or K97M mutant forms of Gwl kinase in the female germline when Polo kinase function is reduced, but only the K97M form induces a phenotype when Polo function is normal.

The above results show that the Scant phenotype in embryos with compromised Polo function can arise just from increased maternal expression of Gwl kinase but also indicate that the Gwl-K97M protein has additional properties, perhaps increased stability or altered activity. Western blots of gwl+ and Scant-derived embryos show no increase in levels of Gwl resulting from the Scant mutation; if anything there is less Gwl in homozygous Scant, so an increase in stability is unlikely (Figure 4C). To ask whether Scang's K97M mutation affects the kinase activity of Gwl, we expressed Myc-tagged forms of Gwl (WT or K97M or kinase-dead [K87R]) in

Figure 4. Gwl-K97M Is Hyperactive

(A) Effects of overexpressing gwl-long-wt or gwl-long-K97M (Scant) on female fertility. Mat α-Tub-Gal4 and the UASP-gwl transgenes were present in only one copy on the same chromosome in all flies tested. Single females of the indicated genotypes with 3 WT (Oregon R) males laid eggs for 3 consecutive d. Numbers are averages of hatched adult progeny (± standard error of the mean) per day for 12 females of each genotype (three females of each of four independent transgenic lines per genotype = 36 samples). The 100% reference comes from the observation of 100% egg hatch and no larval or pupal lethality.

(B) Effect of overexpressing gwl-long-wt or -K97M (Scant) on embryonic mitosis. Embryos were laid by mothers of the indicated genotypes and treated as in Figure 2A. Scale bars are in μm.

(C) Endogenous Scant protein is not more abundant than Gwl-wt in embryos. Western blots for Gwl and Polo from embryos from mothers of the indicated genotypes. *, cross-reactive band that serves as a loading control.

(D) Gwl-K97M is hyperactive in vitro with altered specificity. Myc-tagged forms of Gwl-wt, K87R (kinase dead), and K97M (Scant) were expressed in Dmel stable cell lines. Myc immunoprecipitations were carried out and the kinase activity on Histones H1, H3, Casein, and Myelin Basic Protein was assayed on beads. Note that the Gwl-K97M was always expressed at lower levels in stable cell lines, suggesting toxicity for this protein.

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**Drosophila** cells in culture and purified the fusion proteins for in vitro kinase assays (Figure 4D). Myc-Gwl phosphorylates myelin basic protein and casein more efficiently than histones H1 or H3, although the signals are only marginally increased above the K87R, presumably kinase-dead, control (the K87R mutation may not effectively abolish the kinase activity or phosphorylation could result from traces of copurifying kinases). Myc-Gwl-K97M specifically phosphorylates myelin basic protein with dramatically increased efficiency, while showing only slight increases for casein and H1 and no increase for H3. This high activity on myelin basic protein was repeatedly observed despite the lower amount of kinase present in the reaction for Myc-Gwl-K97M compared with Myc-Gwl or Myc-Gwl-KD. Indeed we found that Gwl-K97M was reproducibly expressed at a lower level than its WT equivalent in several independent stable cell lines. This suggests that the hyperactive K97M mutation is toxic to the cell line used, so the only stable transformants that survive are those that can keep it downregulated. Both our genetic and biochemical results show that the Scant mutation (Gwl-K97M) results in a hyperactive enzyme with altered specificity.

Taken together, several pieces of evidence indicate that Gwl and Polo have antagonistic activities in the early embryo. The Scant phenotype is not seen in the presence of wild-type levels of polo, and its strength is dependent upon the strength of the mutant polo allele. The failure of embryos to develop upon overexpression of overactive Gwl-K97M and, to a lesser extent, of Gwl-wt, is strongly dependent on reduced Polo dosage. Two of the mutations that restore fertility to polo-Scant heterozygous females are duplications of polo (Figure 1B; Text S1). These results suggest negative regulatory interactions between Gwl and Polo and indicate that the balance between these two protein kinase activities is paramount for success of the rapid mitotic cycles of early embryonic development.

**The Allelic Series of Recessive gwl Alleles Reveals Multiple Mitotic Functions**

The hypomorphic recessive alleles of gwl allowed us to study the roles of the Gwl protein kinase in several of the different cell cycle types in Drosophila development. We compared their mutant phenotypes to those of the null allele gwl<sup>rm</sup>, a P-hop deletion that removes the N-terminal end of the Gwl protein and its 5' upstream sequence (described above). Two close bands are detectable around 100 kDa in Gwl western blots from larval brains, and both bands are absent in hemizygous gwl<sup>rm</sup> larval brains (Figure 5B).

DNA sequencing revealed that all of the nondeficiency Scant revertant alleles (Sr) retained the K97M Scant mutation (Figure 5A). gwl<sup>Sr3</sup> has, in addition, a 27-bp deletion corresponding to amino acids 156-164 (Figure 5A). This short deletion maps to the predicted kinase fold in the N-terminal portion (not shown). The gwl<sup>Sr3</sup> allele is a hypomorph since gwl<sup>Sr3</sup>/gwl<sup>rm</sup> is more viable than gwl<sup>Sr3</sup>/Df; and gwl<sup>Sr3</sup>/Df is in turn more viable than gwl<sup>rm</sup>/Df. This predicts that the kinase encoded by the gwl<sup>Sr3</sup> allele retains some activity. However, western blots of extracts of gwl<sup>Sr3</sup>/Df larval brains reveal little or no protein, suggesting that the protein encoded by gwl<sup>Sr3</sup> is unstable (Figure 5B). gwl<sup>Sr3</sup>/Df has an A to T substitution that changes lysine 689 into the ochre termination codon (STOP; Figure 5A). gwl<sup>Sr3</sup> also behaves as a hypomorphic allele suggesting either that this STOP codon can be suppressed to some extent or that the truncated protein retains partial function. We see no Gwl protein in western blots of gwl<sup>Sr3</sup>/Df larval brain extracts (Figure 5B) but our anti-Gwl antibodies were raised against the C-terminal part of the protein that is predicted to be truncated in this mutant, so this test is unreliable. The weak hypomorphs gwl<sup>Sr17</sup> and gwl<sup>Sr19</sup> show a slight reduction in levels of both forms of Gwl in brains (unpublished data). Finally, the female-sterile-only allele, gwl<sup>Sr16</sup>, has one base deleted in the splice acceptor sequence of exon 4. This change in sequence from AAAGGCT to AAAGGCT (Figure 5A) leaves the same splice-acceptor sequence but changes the reading frame after it to encode a string of 61 different amino acids followed by a series of STOP codons. This mutation has lost the slower migrating isoform of Gwl (Gwl-long) in neuroblasts of gwl<sup>Sr18/Df</sup> larvae without affecting the faster form (Gwl-short) (Figure 5B). Both isoforms of Gwl are also expressed in S2 cells (Figure S4) [13]. We cloned both Gwl cDNAs from total S2 cell mRNA; sequencing revealed that the shorter cDNA lacks exon 4 precisely. RNAi treatment of S2 cells using double-stranded (ds)RNA targeting exon 4 depleted the upper but not the lower Gwl band seen on western blots (Figure S4), whereas RNAi to common exons depleted both isoforms. Thus gwl<sup>Sr18</sup> has a mutation in a splice site used in the synthesis of only one of the two isoforms.

The mutations fall into an allelic series. The amorphic genotype gwl<sup>Sr17/Df</sup> dies predominantly in the pupal stage, and the rare escaper adults have rough eyes, ragged wings, cuticle defects, missing bristles, and are sterile, phenotypes typical of cell division cycle mutants. We found similar, but less severe, phenotypes in gwl<sup>Sr3/Df</sup>, gwl<sup>Sr6/Df</sup>, and gwl<sup>Sr6/Df</sup> animals, a greater proportion of which survive to adulthood (Figure S5). In contrast, gwl<sup>Sr18/Df</sup> flies are fully viable and show no such morphological defects but females are sterile (males are fertile).

The severity of developmental defects is paralleled by the cellular phenotype of third instar larval neuroblasts in the gwl mutants (Figures 6 and S6). Strikingly, the viable but female-sterile splice acceptor site mutant that expresses no long isoform, gwl<sup>Sr18</sup>, shows no significant mitotic defects. The other Scant revertant alleles (illustrated here by gwl<sup>Sr3</sup>/gwl<sup>Sr6</sup>) all show mitotic defects that are exaggerated in hemizygotes, and gwl<sup>Sr6</sup> is usually more affected than gwl<sup>Sr3</sup> (Figure S6). In Figure 6, the strongest phenotypes are seen in gwl<sup>Sr6/Df</sup>. There is an increase in the mitotic index; more cells are pre-anaphase (Figure 6B). RNAi targeted to the first exon of gwl in cultured S2 cells depletes both isoforms and also results in an increase in mitotic cells, confirming earlier findings [11]. These cells show BubR1 staining on kinetochores and high cyclin B levels (Figure S4), indicating that they are delayed in prometaphase. gwl mutant neuroblasts also show a high frequency of defective chromatin condensation. However, in contrast to the undercondensation reported in other gwl alleles by Yu and colleagues [13], we consistently observe that some regions of chromosomes appear undercondensed and other regions are overcondensed (Figure 6A). In some cells the chromosomes are uniformly overcondensed, suggesting lengthy pre-anaphase delay (Figure 6A). In S2 cells depleted of Gwl by RNAi, we do not observe strong undercondensation, but rather chromosomes are scattered along extensively elongated spindles (Figure S4), as also found by Bettencourt-Dias et al. [11]. The small number of cells that manage to
Greatwall/Scant in Mitosis and Meiosis

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Figure 5. gwl Alleles Generated and Used in This Study

(A) Molecular map of gwl alleles. Gwl's highly conserved kinase domain (white) is predicted to be split by a less-conserved intervening sequence of yet unknown function (light gray). The N-terminal and C-terminal ends are also less conserved (dark gray). This diagram is inspired from the one proposed in Yu et al. [13]. The Scant mutation changes lysine residue 97 to methionine, making Gwl hyperactive and altering its specificity in vitro. Mutation Scant revertant 3 (gwlSr3) removes exactly nine codons coding for residues 156–164 predicted to be part of the kinase fold. Mutation Scant revertant 6 (gwlSr6) introduces a premature ochre termination codon instead of lysine 689. Genetically, both mutations gwlSr3 and gwlSr6 seem to have residual Gwl function. Mutation gwlSr18 changes the reading frame following the splice acceptor site at the beginning of exon 4, which encodes residues 382–424 in Gwl-long. Exon 4 is spliced out from Gwl-short and, therefore, Gwl-short is unaffected by the gwlSr18 mutation. Mutations gwlSr7, gwlSr6a, and gwlSr3a are null alleles generated by imprecise excisions of a P-element (see Figure 1C).

(B) Anti-Gwl western blot on larval brain extracts of the indicated genotypes. Gwl-long and Gwl-short are both visible in the WT lane (+/Df). Two gwlSr18/Df samples were loaded to confirm that the absence of the top Gwl band is not artifactual.

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enter anaphase in mutant neuroblasts frequently show anaphase bridges (Figure 6A). The gwl null gwl6a/Df, unlike the hypomorphs, has a significant proportion of polyploid mitotic figures (Figure 6A and 6B). Conditions that block anaphase, such as colchicine treatment, also induce polyploidy; eventually the hypercondensed metaphase chromosomes decondense, and the cell reenters interphase, bypassing the mitotic checkpoints. Since gwl6a/Df cells show significant pre-anaphase delay with hypercondensed chromosomes, polyploidy is in fact expected from this normal interphase reentry. The absence of polyploid cells in the hypomorph gwl6a/Df suggests that enough active Gwl is available to complete mitosis normally—eventually.

Expression of UASp-gwl-long or UASp-gwl-short as transgenes driven ubiquitously by Actin 5C-Gal4 rescues the viability and somatic integrity of gwl6a, gwl6a/Df, and gwl6a/Df flies (Figures 6C and S5) (unpublished data) to similar degrees. Therefore, both forms of Gwl (long and short) are active kinases that have redundant functions in somatic tissues where they are both present (Figure 5B).

The Long Isoform of Gwl Is Required for Female Meiosis and Is Provided to the Egg
gwl6a, gwl6a, and gwl6a, in which levels of Gwl are reduced (at least in neuroblasts; Figure 5B), are all sterile in both males and females. This sterility reflects cell-proliferation failure of the germlines and probably of their supporting somatic tissues as well. In contrast, gwl6a/Df shows normal viability and has no somatic defects including normal ovaries and testes, despite expressing only the short isoform of Gwl (Figure 5B). Therefore, the short form is sufficient for mitosis in general, and it is both necessary and sufficient for the mitotic divisions of germline cells in ovaries and testes. gwl6a/Df females lay lots of eggs but they remain white; males are fertile. Since gwl6a produces eggs, the Gwl-long isoform has a specific role late, rather than early, in germline function. Western blotting of mature wild-type ovaries or unfertilized eggs does show high levels of Gwl (Figure 7A and 7B); a single band in unfertilized eggs (Figure 7B) and a thick band in ovaries (Figure 7A). In contrast, gwl6a/Df females show a great reduction in Gwl signal in these tissues. Since our molecular analysis (cloning and sequencing) and biochemical analysis (in neuroblasts) reveal that only the long form is affected by the gwl6a mutation and since Gwl signal disappears from gwl6a/Df eggs and ovaries, the western signal in wild-type eggs and ovaries arises from the long form only. Gwl-short was not detected in eggs or mature ovaries, although Gwl-short must be present in gwl6a/Df ovarian follicle cells and premeiotic germline mitotic cells, since these do divide normally. Gwl-long appears as a thick band in western blots of wild-type ovary extracts (Figure 7A); this presumably corresponds to activated phosphoforms of Gwl-long analogous to those seen in [12], while it appears as a thin band in blots of unfertilized eggs (Figure 7B), where Gwl-long may not be activated.

When we examined the ovaries of gwl6a/Df females we found them fully developed (Figure 7C). However, preliminary observations reveal that late (stages 13–14) eggs often
have irregularly distributed yolk, in no fixed pattern. Earlier stages have uniform yolk distribution but appear to have less of it than same-stage controls (our unpublished observations).

The *Drosophila* ovary comprises 15 or so ovarioles each containing multiple egg chambers at successive stages of development. Cystoblasts in the germaria of the ovarioles divide four times to form cysts of 16 cells that remain interconnected by ring canals derived from the cleavage furrows of incomplete cytokinesis. Of the two cells interconnected by four ring canals, one will become the oocyte although formation of synaptonemal complex is complete in both of them. Once the oocyte is determined, the other 15 cells undergo endoreduplication cycles and associated cell growth to become the nurse cells. The morphology of egg chambers of *gwl*Sr18/Df females appears normal; they have 15 nurse cells and one oocyte indicating that the preceding mitoses had progressed normally.

We detect strong staining for Gwl (the antibody recognizes both isoforms) in wild-type egg chambers from stage 8 of oogenesis onwards but not in *gwl*Sr18/Df females (the faint signal in *gwl*Sr18/Df ovaries is probably Gwl-short present in the follicle cells surrounding the egg chamber but some may be background). In wild-type egg chambers, Gwl protein is present in the oocyte and four neighboring nurse cells; actin staining shows that all four nurse cells connect directly to the oocyte by ring canals and that they have the expected numbers of ring canals to be the oocyte’s sister and daughters (four, three, two, and one; unpublished data). In each of these cells Gwl accumulates in the nucleus, although there is some cytoplasmic staining. The long isoform of Gwl is contributed maternally to the embryo where it also concentrates in interphase syncytial nuclei. During mitosis Gwl is depleted in the nucleus and enriched around the outside of the spindle envelope before accumulating in the nucleus once again during the next interphase (Figure S7). Thus Gwl-long accumulates in a subset of polyploid nuclei of nurse cells, the prophase I nucleus of the oocyte and interphase nuclei of the embryo.

Gwl accumulation in the oocyte led us to ask whether *gwl*Sr18/Df oocytes encounter problems during meiosis per se. To examine female meiosis I, we performed immunostaining (for α-tubulin and DNA) in inactivated vitellarial eggs at stages 13 and 14. Wild-type oocytes normally arrest in metaphase I with the larger chromosomes with chiasmata compacted into a single mass at the metaphase plate, while...
the small fourth chromosomes have moved halfway to the poles as the result of distributive segregation (see WT control in Figure 8Aa and [18,19]). We scored the number of DNA masses (ignoring the tiny fourth chromosome) in WT and gwlSr18/Df oocytes. In contrast to the WT oocytes, which mostly have only one DNA mass at the metaphase plate, 78% of gwlSr18/Df oocytes have widely scattered chromosomes, and the number of chromosome masses varies from two to 12 (Figure 8B). Complete homologue separation would give six large chromatin masses; about a quarter of the mutant meioses have more masses than that. Multiple chromatin masses might be caused by reduced meiotic exchange, or precocious loss of sister-chromatid cohesion, or broken chromosomes resulting possibly from faulty meiotic exchange. If meiosis I lacks chiasmata then metaphase I arrest does not occur [18,19], although in wild type anaphase I and II proceed precociously but normally; although some anaphase movements probably occur precociously in gwlSr18/Df, they are certainly not normal, so simple reduced exchange does not explain this phenotype though it could be compounded with other problems. A few of the meiotic spindles are aberrantly shaped (Figure 8C; 8% of the total oocytes), but all of the multipolar spindles contain multiple DNA masses. Since the wild-type spindle is nucleated from the central mass of chromatin in meiosis I, these abnormal spindles probably
reflect nucleation of individual “mini-spindles” from the multiple chromosome masses.

The fertility of gwlsr18/Df females is rescued completely by driving transgenic UASp-gwl-long in the maternal germline with Mat α-Tub-Gal4 (Figure 8D), and nearly complete egg hatch for all fertile genotypes shown, demonstrating that the sterility and meiotic defects of gwlsr18 are due solely to the loss of Gwl-long in ovaries. Similar expression of UASp-gwl-short in a separate experiment partially rescues the fertility of gwlsr18/Df females (Figure 8E). That this rescue is only partial is confirmed by partial egg hatch (around 50%) compared with nearly complete hatch in all fertile control genotypes. Gwl-long is more competent than Gwl-short for providing maternal function; that there is no significant increase in nondisjunction (Figure 8, legend) suggests that rescue of meiosis per se by Gwl-short is complete or nearly so.

**Figure 8. Gwl Is Required for Female Meiosis**

(A) Examples of meiotic defects observed in gwlsr18/Df oocytes. Alpha-tubulin is stained green and DNA is red. Note the small, probably nondisjoined Chromosomes 4 in b-b’. In c’, arrows indicate likely separated sister chromatids of the fourth chromosome. A typical wild-type (Oregon R) meiotic figure arrested in metaphase I is shown for comparison (a). The longer spindles in b and c are a typical feature of the bipolar spindles formed in gwlsr18/Df oocytes. Scale bar is 10 μm.

(B) Quantitation of chromosomal defects observed in metaphase and anaphase oocytes. In wild-type oocytes, only 2% appear to have progressed into a normal anaphase (not included in this quantitation). Chromosome defects were characterized by scattered chromosome masses. The number of DNA masses, excluding the tiny fourth chromosome, was counted in defective figures. The percentage of defective figures presenting two to six DNA masses (as in Ab–c) or seven to 12 DNA masses (as in Ad–e) is shown. Normal figures showed either one mass in metaphase or two masses in anaphase.

(C) Quantitation of spindle defects observed in metaphase and anaphase oocytes. Defective, multipolar, and occasionally monopolar spindles are observed in gwlsr18/Df oocytes (as in Ad–e). 149 Oregon R oocytes and 142 gwlsr18/Df oocytes were scored in these experiments (B and C).

(D and E) Rescue of gwlsr18/Df female fertility with the expression of UASp-gwl-long (D) or UASp-gwl-short (E) driven from the Mat α-Tub-Gal4 driver (in two separate experiments). The average numbers of adult progeny per female from 6 d (D) or 7 d (E) of egg laying by females of the indicated genotypes are shown (± standard error of the mean). See Text S1 for details. Females of the genotypes used in (E) were also tested for meiotic chromosome nondisjunction by crossing to Y;Y, v f X, v f B;0; C(4)RM, ci eyR/0 males. Numbers of (nullo-4, diplo-X+nullo-X)/total progeny are, from top to bottom: (0, 0)/432; (2, 0)/671; no progeny; (0, 0)/646; (0, 0)/397; and (2, 0)/294.

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eggs collapse on handling; this may be related to the yolk-distribution problem. Very gently handled single dechorionated early embryos have all failed to reach cellular blastoderm (our unpublished observations).

Gwl Is Required for Sister Chromatid Cohesion in Oocytes

The presence of more than six DNA masses in \( gwl^{Sr18/Df} \) oocytes could be caused by the absence of sister chromatid cohesion or by DNA damage causing chromosome fragmentation. To distinguish between these two possibilities, we performed fluorescence in situ hybridization (FISH), probing for satellite DNA near the centromere of the X chromosome. In wild type, we observed that 92% of oocytes have two large foci, corresponding to the two homologous pairs of sister centromeres that are under tension in prometaphase and metaphase I while held together as chiasmate chromosomes (Figure 9Aa–c and 9B). In \( gwl^{Sr18/Df} \) oocytes, we often observed three or four centromeric foci, indicating the absence of sister chromatid cohesion. Panels d and e show examples of figures with six or fewer DNA masses; panels f–h show examples of figures with more than six DNA masses. Scale bar is 10 µm.

(B) Quantitation of the number of centromeric FISH foci relative to the number of DNA masses in wild-type and \( gwl^{Sr18/Df} \) oocytes (as in [A]). In \( gwl^{Sr18/Df} \) oocytes, the presence of more than two centromeric FISH signals strongly correlates with the presence of more than six large DNA masses, consistent with the loss of sister chromatid cohesion. Numbers of oocytes scored are 75 for wild type, 98 for \( gwl^{Sr18/Df} \) with six or fewer DNA masses, and 32 for \( gwl^{Sr18/Df} \) with seven or more DNA masses.

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Discussion

The Gwl kinase seems to have multiple roles in progression through mitosis and meiosis. The phenotypes shown by \( gwl \) mutants differ at different stages of development reflecting both the nature of different alleles and the variety of ways in which the cell cycle is regulated in \( Drosophila \). Indeed it is these different modes of cell cycle regulation as development proceeds that allow us to tackle Gwl’s multiple functions in cell division.

Gwl Antagonizes Polo

Our starting point was the strong genetic interaction between the \( Scant \) mutation and \( polo \) mutations; heterozygous females lay embryos that die, presumably as a consequence of mitotic failure whose first observed defect is that a single centrosome moves away from the nucleus before nuclear envelope breakdown in all cases examined. This centrosome misbehavior is probably the primary defect; developmental failure probably results from secondary defects of abnormal spindles. It will be interesting to reexamine the phenotype of other maternal-effect mutants showing free centrosomes to see if they disassociate from the nuclear envelope in the same manner. It will also be interesting to find out whether the detaching centrosome always contains either the older or the younger centriole, since they may harbor different amounts of biochemical factors in their pericentriolar material. If so, the history of the centrosome determines its vulnerability to detachment when the Gwl/Polo balance is compromised. The \( Scant-polo \) genetic interaction is moderately specific since a
screen for mutants reverting the maternal-effect embryonic lethality generated only one third-site interactor among two independent polo" duplication events plus two revertants of the Scant allele itself. Scant encodes a Gwl kinase with a K97M substitution that results in hyperactivity in vitro (albeit with altered specificity on the artificial substrates tested); a wild-type transgene with just this amino acid mutated interacts dominantly with polo mutants, so this amino acid substitution is Scant. Moreover, mothers overexpressing wild-type Gwl kinase in the presence of reduced Polo kinase function produce embryos with the same kinds of defects as Scant. Therefore, the increased activity of Gwl-K97M and not its altered substrate specificity is responsible for the functional interaction between Scant and polo. It follows that a balance between Gwl and Polo activities in embryos is crucial, but because there does not seem to be any such interaction in cell cycles at later stages (in proliferating larval, pupal, or adult tissues), since polo"/+ Scant itself has normal viability, the balance appears particularly important for these early embryonic cell cycles. The syncytial nuclear division cycles are unusual in that they comprise rapidly alternating cycles of S phase and M phase without intervening gap (either G1 or G2) phases. A G2 phase is only introduced following cellularization when String (the Cdc25 dual-specificity phosphatase that activates Cdk1) is degraded; its expression then comes under transcriptional regulation in a spatio-temporally defined pattern. The critical balance of Polo and Gwl kinase activities in the syncytiotium may reflect the absence of a G2 state; mitotic proteins are held on continual standby, awaiting their use in the next cycle, rather than being degraded and resynthesized each cell cycle as is the case for cycles with a G2. Alternatively, centrosome detachment may be frequent in other tissues of polo-Scant flies where it may be better tolerated. However, we consider this unlikely because we observe only normal centrosome positioning in polo-Scant testes (unpublished data).

This antagonism between Polo and Gwl was not predicted from studies of these enzymes in Xenopus cell-free systems, which have been used to model the entry into mitosis from G2 through the activation of the Cdc25 phosphatase. There the evidence indicates that both Gwl and Plk1 kinases participate in the autoregulatory loop that activates the Cdk1/cyclin B MPF kinase complex [5,12]. This apparent cooperation of the two kinases in this process suggests that the Xenopus cell-free system may be assessing a different aspect of cell cycle progression than our in vivo studies on the syncytial cycles of Drosophila embryos. The apparent differences in results may also reflect the different aspects of mitosis under study; activation of Cdk1 in one system and the integrity of the mitotic apparatus in the other. We have few clues about the directionality of the antagonism we observe for Polo and Gwl function in fly embryos or whether it involves direct interactions between the two protein kinases. Yu et al. [12] observed that xPlk1 is capable of phosphorylating xGwl, but they did not observe changes in xGwl activity or a synergistic effect in combination with cyclin B-Cdc2-mediated phosphorylation. However, any inhibitory effect of Polo kinase on Gwl need not be mediated through regulation of kinase activity but could also occur by regulating Gwl's localization or stability. In this case, reduced dosage of Polo in the fly embryo might provide only a subthreshold activity, insufficient for the efficient down-regulation of the hyperactive Gwl kinase encoded by gwlScant. That Gwl needs to be downregulated is suggested by its subcellular localization in mitosis. Gwl is enriched in the nucleolus in interphase, but it is excluded from the nucleus during prophase, before nuclear envelope breakdown. This could occur through active nuclear export or through degradation. In favor of the latter, we observe that Gwl is ubiquitinated (our unpublished observations).

Gwl could also inhibit the function of Polo. Our in vitro experiments suggest that Gwl does not readily phosphorylate Polo (unpublished observations), but it is also possible that phosphorylation of an intermediate substrate by Gwl mediates the hypothetical inhibitory effect. Since Scant causes a decrease in female fertility that is stronger in stronger polo mutant alleles, and the meiotic divisions occur in the embryonic cytoplasm, it is possible that Scant lowers Polo's activity during meiosis. Scott Hawley and colleagues have recently observed a functional interaction between polo and matrimony (mtrm); heterozygous mtrm/+ females have an elevated frequency of chromosome nondisjunction in meiosis, and this is suppressed by lowering the polo dosage [20]. Therefore, if Scant acts to lower Polo's activity in female meiosis, then Scant might suppress the increased level of nondisjunction in mtrm heterozygotes. Indeed it does (Tables S1-4), though Scant needs to be homozygous for the suppression to be detectable, so the possibility that this suppression reflects homozygosity of some closely linked third player rather than Scant itself cannot be eliminated (see Text S2). Furthermore, even in Scant/Scant, the suppression of mtrm/+ is much weaker than halving the dosage of polo directly. This is consistent with the effects of Scant on fertility; homozygous Scant in a homozygous polo" background is much more fertile than polo11 Scant/+ , so regardless of how Scant acts to reduce the functional level of polo, one copy of Scant does not reduce the activity of one copy of polo completely. Nevertheless, the Scant-mtrm interaction result strongly suggests that the polo-Scant (and probably polo-gwl) interaction occurs during female meiosis as well as embryonic mitosis, and the unexpected Scant-mtrm interaction lowering female fertility implies a role for mtrm in embryogenesis.

That Gwl downregulates Polo's function in the embryo is also suggested by the cellular phenotype, which is in line with the known functions of Polo at the centrosome. Moreover, the Scant phenotype is increased by the severity of the polo mutant. Occasional displacement of centrosomes early in mitosis is seen in syncytial embryos derived from heterozygous polo mutant females themselves (Figure S2), and Polo promotes centrosome separation, maturation, and integrity. In Drosophila, Polo phosphorylates Asp and together they promote the recruitment of γ-tubulin to the centrosome [21]. In mammalian cells, Plk1 phosphorylates Nlp, triggering its dissociation from the centrosome and recruitment of several factors [22–24]. Plk1 also phosphorylates Kizuna, which is required to preserve centrosome cohesion [25]. The detached centrosomes observed in the polo-Scant-derived embryos do not show a reduction in γ-tubulin staining, and astral microtubules nucleate normally. Similar centrosome detachment was observed in Scant/+ and Scant/Scant-derived embryos, albeit with much lower frequencies. Therefore, it seems likely that the partial loss of Polo activity and the gain of Gwl activity both weaken centrosome function in a similar fashion; this is consistent with the mutually antagonistic
interaction between polo and Scant mutations. Furthermore, we note that centrosome detachment occurs before nuclear envelope breakdown (Figure 3), a time when both Polo and Gwl are enriched around the nuclear envelope in syncytial embryos ([26] and Figure S7). This suggests that coordination between the centrosome, microtubules, and the nuclear envelope before nuclear envelope breakdown is sensitive to the balance between Polo and Gwl. Gwl (or cyclin B-Cdk1, which it activates in frog extracts) may share substrates with Polo and regulate them antagonistically in early mitosis.

Centrosome loss can also occur in response to DNA damage, allowing damaged nuclei to fall into the interior of the syncytial embryo and be discarded from the developing fly [16]. This response depends on Mnk/Chk2 [17]. The centrosome detachment observed in polo-Scant-derived embryos does not depend on Chk2 (Figure S3) and thus seems to arise from a more direct effect on the centrosome–nuclear envelope association.

Gwl Controls Chromosome Dynamics in Mitosis

Our hypomorphic gwl mutants do not appear to impact directly upon centrosome behavior in the more conventional cell cycles of the larval central nervous system. Previously, Yu and colleagues reported a long delay in late G2 to anaphase in gwl mutant neuroblasts in addition to chromatid condensation defects [13] and have suggested that these defects, particularly undercondensation of chromatin, could all be attributed to the function of Gwl in activating cyclin B-Cdk1, although no direct substrate of Gwl is known [12,13]. However, the prevalence of condensation defects and anaphase bridges that we observed in gwl mutant neuroblasts, together with the nuclear localization of Gwl in interphase, suggests to us that Gwl may act directly at the chromosome level. The anaphase bridges observed could be a consequence of tangled chromatids or dicentric chromosomes resulting from telomere fusion or other aberrant DNA damage repair events. When Gwl is depleted from cultured cells, they delay at the spindle assembly checkpoint with high levels of cyclin B and checkpoint proteins at kinetochores. The chromosomes of these cells are scattered upon an elongated spindle as though they have defects in kinetochore function. Since metaphase cells with highly condensed chromosomes accumulate in the larval CNS of gwl mutants, prolonged checkpoint arrest probably also occurs here. However, the polyploidy cells seen in the null mutant indicate that cells can slip past the checkpoint without segregating their chromosomes; since this also happens in wild-type neuroblasts in the presence of colchicine, polyploidy is probably not a direct consequence of Gwl failure.

Gwl Is Needed for Sister Chromatid Cohesion in Meiosis

A major role for Gwl kinase in regulating aspects of chromosome behavior is also suggested by the meiotic phenotype seen in gwl018/Df females. gwl018 disrupts the only form of Gwl expressed during vitellogenesis without disrupting the second mitotic isofrom of Gwl. Therefore gwl018 provides a unique opportunity to study how loss of Gwl kinase affects vitellogenesis and meiosis. Although gwl018/Df oocytes develop normally, yolk distribution is abnormal in stages 13–14, females are sterile, and the severe meiotic defects include scattered chromosomes with separated chromatin and elongated spindles.

Scattered chromosomes could result from a number of problems. One possibility is that Gwl is required for proper meiotic recombination; if so, the multiple DNA masses observed could correspond to chromosome fragments resulting from failure to complete chromatid exchange and to repair double-strand breaks. This would also lead to failure to arrest at metaphase I because bivalents would not be held together by chiasmata [18,19]. This is unlikely for several reasons. First, if the masses were fragmented chromosomes, they should vary widely in size; they do not. Second, Gwl accumulates in the oocyte nucleus and the nuclei of the nurse cells directly connected to the oocyte at (but not before) stage 8, which is much later than the time of meiotic recombination. However, we note that if Gwl is involved in meiotic recombination, the tiny amounts of it present in pachytene (germinal) nuclei could be below the detection limit of our antibody. Third, two of the five nuclei that accumulate Gwl never entered pachytene. Finally, our FISH data (Figure 9) prove that chromatid cohesion fails in gwl018 oocytes, and this is sufficient to account for the scattering of DNA masses observed (Figure 8). The number of DNA masses was often higher than six, the maximum expected number for disassociated bivalent chromosomes, disregarding the tiny fourth chromosomes. Therefore, Gwl is required for sister chromatid cohesion in meiosis I. In the absence of Gwl-long, the premature loss of (or failure to establish) arm cohesion would lead to the release of chiasmata if indeed any are formed.

However, neither of these defects alone is expected to lead to complete female sterility. For example, mutants in c(3)G [27] prevent all meiotic recombination but are still partially fertile. Mutants in ord do not keep sister chromatid cohesion yet show only a partial loss of female fertility [28–30]. While the dissolution of sister chromatid cohesion in ord leads to progression through metaphase I into meiosis II, we have seen no normal meiosis II figures in gwl018 oocytes, though it is possible that the elongated bipolar spindles represent attempts to do meiosis II after a failed anaphase I. Thus, the absence of Gwl-long in meiosis does not lead to a simple lack of meiotic recombination nor does it lead only to a premature dissolution of cohesion. The lack of Gwl could lead to a combination of both defects or to yet some other kind of defect that leads to full female sterility. Even if the occasional meiosis succeeds, it is very likely that these embryos would fail to develop because maternal Gwl-long is expected to be required for early embryonic mitoses; indeed, these embryos fail to reach cellular blastoderm.

Most female meioses in gwl018 have scattered chromosomes on a single elongated spindle; we think that the minority (8%) that have multiple bundles of spindle are just the extreme of this scattering, since microtubules are nucleated by the chromatin in the acentriolar oocyte [31]. Failure of karyosome formation might cause this scattering; however, oocytes of earlier stages do at least often form a single karyosome (unpublished data). Mutants that affect the spindle directly such as those affecting the microtubule-associated protein Msps show more spindle defects than chromosome scattering [32]. A mutant disrupting the female germline-specific Cdk1-adaptor Cks30A disrupts the integrity of meiotic spindles in addition to showing chromosome alignment defects, but in this case the chromosome scattering observed is much more modest than that in the gwl018 mutant [33].

How does Gwl regulate sister chromatid cohesion? Our
results suggest that Gwl antagonizes Polo, which is known to negatively regulate sister chromatid cohesion. It is therefore possible that the absence of Gwl during meiosis results in excessive and/or premature Polo activity, leading to premature loss of sister chromatid cohesion. In budding yeast, Polo (Cdc5) promotes the cleavage of the cohesin Scc1 by direct phosphorylation [34]. In meiosis, sister chromatid cohesion is protected at centromeres until anaphase II by Mei-S332 in Drosophila (Shugoshin in other organisms; reviewed by [35]). Indeed, mei-S332 mutants show premature sister separation in meiosis I [36]. In budding yeast, Shugoshin prevents cleavage of the cohesin Rec8, which replaces Scc1 in meiosis (reviewed by [35,37]), and Cdc5 is required in meiosis for cleavage of Rec8 [38,39]. In Drosophila, Polo also negatively regulates Mei-S332 activity and localization [9]. Thus, the lack of Gwl in meiosis could lead to premature activity of Polo, which could negatively regulate Mei-S332 and lead to precocious sister separation in meiosis I. We have examined Mei-S332’s localization in gwls15 hemizygous oocytes and found that Mei-S332 was largely properly localized to centromeres (unpublished data). However, Mei-S332 can be inactivated even when it remains localized at centromeres [9]. Therefore, we cannot rule out the possibility that Mei-S332 is being negatively regulated in the absence of Gwl. Alternatively, Gwl could promote sister chromatid cohesion by directly phosphorylating effectors of cohesion. Gwl-long is better than Gwl-short at performing a maternal function and we suspect that Gwl-long will be a better kinase for a yet unknown maternal substrate.

Gwl Has Multiple Roles in Cell Division

In conclusion, it appears that Gwl, in common with the other major mitotic kinase proteins, has multiple roles in mitotic and meiotic progression. These have been revealed through a series of gwl alleles that exhibit different characteristics and reveal aspects of Gwl kinase function in the different types of cell cycle during Drosophila development. A gain-of-function allele of gwl reveals a requirement for coordinated activity of the Gwl and Polo kinases in the rapidly oscillating M and S phase cycles of early embryos. Partial and total loss of Gwl function leads to frequent chromosome condensation defects and anaphase bridge formation in the conventional division cycles of cells in the larval CNS. Finally, loss of Gwl function in the female germline leads to severe meiotic abnormalities including loss of sister chromatid cohesion. It will be of interest to identify potential binding partners of the Gwl protein kinase both in interphase, when it is present predominantly in the nucleus, and in mitosis, when it moves out to the cytoplasm. This may in turn facilitate the identification of its substrates; this is crucial for understanding exactly how it regulates these various aspects of cell division.

Materials and Methods

Genetics. The isolation of the Scant mutation, the generation of Scant revertant alleles by X-ray mutagenesis, and the mapping of Scant revertants and of Scant using complementation tests, imprécise excision of F-elements, and sequencing are all described in detail in Text S1. Details of the rescue experiments are also provided in that section.

Plasmids. Plasmids were constructed following the Gateway technology. The gwl-long cDNA was PCR-amplified from cDNA LD35132 (Flybase sequence) with primers including attB BP recombination sites at both ends, permitting cloning into the pDONR221 vector, entry vector to generate pDONR-gwl-long (with STOP codon or without it for C-terminal tagging). The gwl-short cDNA was cloned using the same primers from total cDNA from S2 cells to generate pDONR-gwl-short. pDONR-gwl-long-K97M and -K87R (kinase dead) were made by PCR-based point mutagenesis. Entry clones were sequenced; gwl-long was found to match the Flybase entry, the K97M and K87R mutants differed from gwl-long by those single mutations only, and gwl-short was found to lack exon 4 exactly. For transcription, lines, pUASP-gwl-long, pUASP-gwl-long-K97M, pUASP-gwl-long-EGFP, pUASP-gwl-long-K97M-EGFP, and pUASP-gwl-short were made by LR recombination of the entry clones into a pUASP or pUASP-C-termEGFP Gateway destination vector. For cell lines, pAC5-myc-gwl, pAC5-myc-gwl-K97M, and pAC5-myc-gwl-K87R were made by LR recombination into pAC5-N-termMy.

Generation of Gwl antibodies. The sequence encoding Gwl residues 352–846 was PCR’d from cDNA LD35132 and was inserted into pET23b vector to produce the protein in fusion with a C-terminal His(6) tag in Escherichia coli after IPTG induction. The protein was purified under denaturing conditions (BugBuster, Novagen). Antibodies were raised after injection of the purified protein in rabbit (Harlan Sera-Lab).

Immunofluorescence. Embryos were collected every 2–3 h, dechorionated by incubation in 50% bleach for 2 min, rinsed in water, and incubated with agitation for 5 min in permeabilizing-fixative solution (55% heptane, 15% formaldehyde, PBS). The vitelline membranes were then removed by repeated washes in methanol, and embryos were rehydrated initially in methanol/PBS (1:1) and then in PBS containing 0.1% Tween 20 (PBT). For immunofluorescence, blocking was performed with PBT containing 1% BSA (PBTB) for at least 30 min. Antibodies (diluted in PBTB): monoclonal rat anti-γ-tubulin YL1/2 (diluted 1:20); mouse monoclonal anti-γ-tubulin GTU88 (Sigma T6557, diluted 1:50); anti-rat-FITC (Sigma F-6285, diluted 1:100); and anti-mouse-Rhodamine Red (Jackson Immunochemicals 715-295-151, diluted 1:100). Embryos were incubated with primary antibodies overnight at 4°C, rinsed in PBT, and washed repeatedly in PBT at RT, incubated with secondary antibodies for 2 h at RT, rinsed in PBT, and washed repeatedly in PBT. To stain the DNA, embryos were washed with PBS, incubated with a 1:1,000 solution of Toto-3 dye in PBS, and washed in PBS. Immunofluorescence in S2 cells was performed essentially as described [11,40]. To visualize female meiosis, embryos were fixed and stained for immunofluorescence essentially as described [41] except that BSA (1%) was used as the blocking agent and DNA was stained with propidium iodide.

Cell culture. Dmel cells were maintained in SFM medium supplemented with 1 mM Glutamine, penicillin, and streptomycin. S2 cells were maintained in SFM supplemented with 10% calf serum, penicillin, and streptomycin.

Immunoprecipitations and kinase assays. Cells (around 1 x 10⁸) stably expressing Myc-tagged proteins were lysed in 1 ml of extraction buffer (50 mM Tris-Cl [pH 7.5], 110 mM NaCl, 50 mM β-glycerophosphate, 5 mM MgCl₂, 1 mM Na₂VO₃, 0.1% NP-40, Complete Protease Inhibitors [Roche] at its recommended dilution, 0.1 mg/ml DN-EP DNase [Sigma]) with four cycles of freeze-thaw on dry ice and cold water. Lyastes were centrifuged at 14,000 rpm on a Sorvall centrifuge. A total of 50 µl of 9101 (Invitrogen) and 25 µl of Protein G-conjugated Dynabeads (Invitrogen) were added to the clarified supernatants, which were then incubated with agitation at 4°C for 2 h. Beads were washed four times 5 min with 1 ml of extraction buffer without DNase and once with kinase buffer (20 mM K-HEPES [pH 7.4], 2 mM MgCl₂, 1 mM DTT) and resuspended in 30 µl of kinase buffer. Kinase assays were performed on beads in kinase buffer using 1 µg of either Histone H1 (Roche), Histone H3 (Roche), Casein (Sigma), or Myelin Basic Protein (Sigma) in the presence of 1 mM ATP and 10 µCi of 32-P-gamma-ATP in a total volume of 20 µl. Reaction mixtures were incubated for 20 min at 30°C, with occasional agitation, and reactions were terminated by the addition of 2X Laemmli SDS-PAGE sample buffer. Samples were then resolved by SDS-PAGE, transferred to nitrocellulose, and analyzed by autoradiography.

Microscopy and flow cytometry. Fixed embryos and ovaries were examined with a Nikon Eclipse microscope equipped with BioRad MRC1024 confocal scanner and software. Fixed S2 cells were visualized on a Zeiss Axiovert 200 fluorescence microscope with Metamorph software. Videos of embryos were made on a Zeiss Axiovert 200 fluorescence microscope equipped with the PerkinElmer UltraVIEW confocal scanner and software. Flow cytometry was performed as described [10].

Brain squash and oocyte staining. Brains were dissected from third-instar larvae in 0.7% NaCl, fixed in a solution of methanol, acetate, and water (1:1:1:2) for 1 min, transferred to 8% of orcein dye on a
coverslip, stained for 1–2 min, covered with a glass slide, flipped over, and squashed manually. Only fields with abundant cells were counted.

**FISH.** FISH for satellite DNA near the centromere of the X chromosome (1.688 g/cm² satellite on the left arm) was performed essentially as described [29,42]. DNA was stained with propidium iodide.

**Supporting Information**

**Figure S1.** Second Round Generation of Scant Revertant Alleles
(A) Females heterozygous for both the Scant mutation and a loss-of-
function polo mutation (such as polo11), here in cis lay embryos that die
during development.
(B) Second genetic screen to generate Scant revertant (Sr) alleles. Unlike in the first round of mutagenesis in which trans-heterozygous males were mutagenized (Figure 1B), here a polo11 Scant recombination
chromosome made from the fertile polo11 Diplo(polo11) Scant females was
mutagenized and tested for fertility over a third-chromosome
balancer. In this screen, all ten recessive mutations recovered were
allelic to each other and to glo. See Results and Text S1 sections for
details.

Found at doi:10.1371/journal.pgen.0030200.sg001 (40 KB PPT).

**Figure S2.** Centrosome Disassociation Is Occasionally Observed in Embryos Derived from Females Heterozygous for polo or Scant Singly
Embryos were collected for 2–3 h, dechorionated, and fixed for immunofluorescence. Stainings are α-tubulin (green), γ-tubulin (red), and DNA (blue). The scale bar represents 10 μm.

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**Figure S3.** The Centrosome Disassociation of polo-Scant-Derived
Embryos Is Still Observed in the Absence of Mnk/Chk2
Embryos were collected for 2–3 h, dechorionated, and fixed for
immunofluorescence. Stainings are α-tubulin (green), γ-tubulin (red),
and DNA (blue). Two images are shown to illustrate the range of
phenotypes observed for this genotype. Centrosome disassociation
was observed in all embryos of this genotype. The aberrant DNA
masses and microtubule bundling sometimes seen in mnk/mnk; polo5
Scant/Scant-derivé embryos (A) A, B) B(III)] are the mnk mutation alone
(provided by W. Theurkauf), which we verified by PCR.

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**Figure S4.** Gwl Depletion by RNAi in S2 Cells and Associated
Phenotypes
(A) Western blot for Gwl after RNAi in S2 cells using double-stranded
RNA targeting total Gwl (double-stranded RNA targeting exon 1) or
Gwl-long only (double-stranded RNA targeting exon 4); GFP double-
stranded RNA is the control. Y indicates a cross-reacting band serving
as a loading control.
(B) Chromosomes are scattered along an elongated spindle, and
BubR1 is present at kinetochores in total Gwl-depleted S2 cells
undergoing abnormal mitosis. The control cell is in early anaphase.
(C) Cyclin B levels remain high in Gwl-depleted S2 cells undergoing
abnormal mitosis. Depletion of Gwl-long only had no detectable
effect. Scale bars are 10 μm.

Found at doi:10.1371/journal.pgen.0030200.sg004 (2.8 MB PPT).

**Figure S5.** Examples of Somatic Defects Observed in glo Mutant Flies
Rough eyes and ragged wings observed in glo(glo) glo(glo) mutant flies are
rescued by UASp-glo-long driven by Actin 3C-Gal4.

Found at doi:10.1371/journal.pgen.0030200.sg005 (1.2 MB PPT).

**Figure S6.** Quantification of Defects Observed in glo Mutant Neuro-
blasts of Additional Genotypes (to Be Compared with the Data in
Figure 6)
Quantification of defects observed in larval neuroblasts following
orc1n DNA staining (as in Figure 6) for glo(glo) glo(glo) glo(glo) glo(glo) (both partial loss of function genotypes), Mitotic cells were
recognized by their condensed chromatin. The MA ratio corresponds
to the number of prometaphase and metaphase cells over the number
of anaphase and telophase cells (F, number of fields scored), %
condensation defects, number of mitotic cells with under-
overcondensation/total number of mitotic cells (v); % chromatin
bridging, number of bridged anaphases/total number of anaphases
(n); % polyploidy, number of polyplid mitotic cells/total number of
mitotic cells (n).

Found at doi:10.1371/journal.pgen.0030200.sg006 (149 KB PPT).

**Figure S7.** The Localization of Gwl Is Cell Cycle-Regulated in Embryos
The strong genetic interaction between Scant and polo mutations
suggests that Glo and Polo activities have to be coordinated in the
cell division cycle. We used time-lapse to examine the cell cycle
localization of Glo-EGFP in embryos, using Matα-Tub Gal4 to drive
expression in the germline. In syncytial embryos (left), Glo-EGFP is
concentrated in the nucleus in interphase and concentrated around
the nucleus in early mitosis, presumably before nuclear envelope
breakdown (the arrow indicates the direction of the mitotic wave). In
cellularized blastoderm, Glo-EGFP is also concentrated in the
nucleus in interphase (Tb), but then becomes concentrated around
the nuclear envelope and largely disappears from the nucleus (below
cytoplasmic levels) shortly before nuclear division (9–12 min). It then
becomes more evenly distributed throughout the cell, presumably
after the nuclear envelope has become broken or fenestrated (12–18
min). Glo-EGFP becomes clearly nuclear again at the end of nuclear
division (21–23 min). Glo-K97M-EGFP behaves identically to Glo-
EGFP (unpublished data), ruling out the possibility that the genetic
effect of Scant (on polo mutants) could be due to abnormal subcellular
localization of Glo caused by the K97M substitution. This localization
pattern suggests that Glo is being actively imported into the nucleus
in interphase and either degraded in the nucleus or exported to the
cytoplasm in early mitosis.

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**Table S1.** Progeny from Females with Normal X Chromosomes and the
Indicated Third-Chromosome Genotype Crossed to Y;Y, fem B/C;
C(4)RM, ci ey7/0
Males
Parental crosses were FM7/+; sr Scant females x w; mtrm126/TM3126 or
y w/Y; mtrm126/TM3126 males and FM7/B(II;1)16D, w females by both
genotypes of mtrm males. y w/DJ females from the latter cross
produce only half the expected number of regular males, since the
deficiency is recessive lethal; for these progeny, the number of regular
males has been doubled (parentheses) before calculating gametic
frequencies. Although diplo-X ova produce phenotypically distinct
progeny here, they have been included in the calculations as though
they could not be distinguished from mono-X ova, so the frequencies
here will be comparable to those in Table S4. Tests set up on different
days are presented separately.

Found at doi:10.1371/journal.pgen.0030200.s001 (46 KB DOC).

**Table S2.** Progeny from FM7/+ Females with the Indicated Third-
Chromosome Genotype Crossed to Y;Y, fem B/C;
C(4)RM, ci ey7/0
Males
Females tested are sibs of those in Table S1. FM7/0 males are nearly
always lethal.

Found at doi:10.1371/journal.pgen.0030200.s002 (31 KB DOC).

**Table S3.** Progeny from Females with Normal X Chromosomes and the
Indicated Third-Chromosome Genotype Crossed to Y;Y, fem B/C;
C(4)RM, ci ey7/0
Males
Females with the Indicated Third-Chromosome Genotype Crossed to Y;Y, fem B/C;
C(4)RM, ci ey7/0
Males
Parental crosses were FM7/+; sr Scant females x + Scant/TM6B, mtrm13
Scant/TM6B, or mtrm126 Scant/TM6B126 males.

Found at doi:10.1371/journal.pgen.0030200.s003 (30 KB DOC).

**Table S4.** Progeny from FM7/+ Females with the Indicated Third-
Chromosome Genotype Crossed to Y;Y, fem B/C;
C(4)RM, ci ey7/0
Males
Females tested are sibs of those in Table S3.

Found at doi:10.1371/journal.pgen.0030200.s004 (29 KB DOC).

**Text S1.** Supporting Materials and Methods

Found at doi:10.1371/journal.pgen.0030200.s001 (49 KB DOC).

**Text S2.** Supporting Results

Found at doi:10.1371/journal.pgen.0030200.s002 (27 KB DOC).

**Text S3.** Supporting References and Accession Numbers

Found at doi:10.1371/journal.pgen.0030200.s003 (51 KB DOC).

**Video S1.** Wild-Type (Oregon R) Syncytial Embryos (GFP-β-Tubulin):
Control for Video S2
Frames were taken every 20 s. Exposure time was 300 ms. Spacing
between images was 0.5 μm.
Some nuclei undergo an apparently normal mitosis, while for others one centrosome initially disassociates but is eventually recaptured, allowing mitosis to be completed normally. In other nuclei, the free centrosome drifts far away from its spindle. These centrosomes are not recaptured and the mono-centrosomal spindles can eventually fuse with each other or proceed to produce a bipolar spindle and divide. Frames were taken every 20 s. Exposure time was 300 ms. Spacing between images was 0.5 μm.

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References

1. Morgan DO (1997) Cyclin-dependent kinases: engines, clocks, and microprocessors. Annu Rev Cell Dev Biol 13: 261–291.
2. Sunkel CE, Glover DM (1988) polo, a mitotic mutant of Drosophila displaying abnormal spindle poles. J Cell Biol 99: 25–38.
3. Llamazares S, Moreira A, Tavares A, Girdham C, Spruce BA, et al. (1991) polo encodes a protein kinase homolog required for mitosis in Drosophila. Genes Dev 5: 2153–2165.
4. Glover DM (2005) Polo kinase and progression through M phase in Drosophila: a perspective from the spindle poles. Oncogene 24: 239–237.
5. Kumagai A, Dumpy WG (1996) Purification and molecular cloning of Polo, a Cdc25-regulatory kinase from Xenopus egg extracts. Science 273: 1577–1580.
6. Ahonen LJ, Kallajo MJ, Daum JR, Bolton M, Manke JA, et al. (2005) Polo-like kinase 1 creates the tension-sensing 3F32 phosphoepitope and modulates the association of spindle-checkpoint proteins at kinetochores. Curr Biol 15: 1078–1089.
7. Wong OK, Fang G (2005) Plx1 is the 3F3/2 kinase responsible for targeting chromosomes to microtubule plus-ends. Nature 432: 980–987.
8. van de Weerdt BC, Medema RH (2006) Polo-like kinases: a team in control of cell cycle. PLoS Biol. In press.
9. White-Cooper H, Carmena M, Gonzalez C, Glover DM (1996) Mutations in polo11 Scant promote chiasma formation and sister chromatid cohesion in Drosophila melanogaster. Nature 381: 629–637.
10. Kerrebrock AW, Miyazaki WY, Birnby D, Orr-Weaver TL (1992) The sister-chromatid cohesion protein ORD is required for chiasma maintenance in Drosophila oocytes. Curr Biol 12: 925–929.
11. Mason JM (1976) Orientation disruptor (ord): a recombination-defective mutation in Drosophila melanogaster. Genetics 84: 545–572.
12. Clyne RK, Katis VL, Jessop L, Benjamin KR, Herskowitz I, et al. (2003) Polo-like kinase Cdc5 promotes chiasmata formation and cosegregation of sister centromeres at meiosis I. Nat Cell Biol 5: 480–485.
13. Page SL, Hawley RS (1995) Induction of the meiotic mutant c(3)G6 in Drosophila melanogaster. Genetics 137: 367–409.
14. Page SL, Hawley RS (2004) The genetics and molecular biology of the synaptonemal complex. Annu Rev Genet 38: 293–303.
15.emailer WD, Wayson SM, Hawley RS (2005) The meiotic defects of mutants in the Drosophila mps1 gene reveal a critical role of Mps1 in the segregation of achiasmate homologs. Curr Biol 15: 672–677.
16. Leibfritz D, Spurr R, Sunkel CE (1995) Polo-like kinase 1 regulates Nlp, a centrosome protein involved in microtubule nucleation. Dev Cell 5: 115–125.
17. Page SL, Hawley RS (2004) Polo-like kinase Cdc5 promotes sister-chromatid cohesion in meiosis I. Nat Cell Biol 5: 480–485.
18. Pirrotta V, Lin D (1998) The mei-s332 gene promotes sister-chromatid cohesion in meiosis I. Nat Cell Biol 5: 629–637.
19. Mason JM (1976) Orientation disruptor (ord): a recombination-defective mutation in Drosophila melanogaster. Genetics 84: 545–572.
20. Clyne RK, Katis VL, Jessop L, Benjamin KR, Herskowitz I, et al. (2003) Polo-like kinase Cdc5 promotes chiasmata formation and cosegregation of sister centromeres at meiosis I. Nat Cell Biol 5: 480–485.
21. Lee BH, Amon A (2003) Polo kinase–meiotic cell cycle coordinator. Cell 113: 87–99.
22. Casenghi M, Barr FA, Nigg EA (2005) Phosphorylation of Nlp by Plk1 negatively regulates its dynein-dynactin-dependent targeting to the centrosome. J Cell Biol 170: 3191–3198.
23. Page SL, Hawley RS (2004) The genetics and molecular biology of the synaptonemal complex. Annu Rev Genet 38: 293–303.
24. Lee BH, Amon A (2003) Polo kinase–meiotic cell cycle coordinator. Cell 113: 87–99.
25. Page SL, Hawley RS (2004) The genetics and molecular biology of the synaptonemal complex. Annu Rev Genet 38: 293–303.