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Abstract
SUMMARY

Meiosis is a specialized cell cycle limited to the gametes in Metazoa. In Drosophila, oocyte determination and meiosis control are interdependent processes and BicD appears to play a key role in both. However, the exact mechanism of how BicD dependent polarized transport could influence meiosis and vice versa remains an open question. In this article we report that the cell cycle regulatory kinase Polo binds to BicD protein during oogenesis. Polo is expressed in all cells during cyst formation before specifically localizing to the oocyte. This is the earliest known example of asymmetric localization of a cell cycle regulator in this process. This localization is dependent on BicD and the Dynein complex. Loss- and gain-of-function experiments showed that Polo has two independent functions. On one hand, it acts as a trigger for meiosis. On the other hand, it is independently required, in a cell autonomous manner, for the activation of BicD-dependent transport. Moreover we show that Polo overexpression can rescue a hypomorphic mutation of BicD by restoring its localization and its function suggesting that the requirement for Polo in polarized transport acts through regulation of BicD. Taken together, our data indicate the existence of a positive feedback loop between BicD and Polo, and we propose that this loop represents a functional link between oocyte specification and the control of meiosis.

MESH Keywords Animals ; Cell Cycle ; Drosophila ; cytology ; genetics ; growth & development ; Drosophila Proteins ; analysis ; genetics ; metabolism ; Dyneins ; metabolism ; Female ; Meiosis ; genetics ; Mutation ; Oocytes ; chemistry ; growth & development ; metabolism ; Oogenesis ; genetics ; Protein Interaction Mapping ; Protein-Serine-Threonine Kinases ; analysis ; genetics ; metabolism

Author Keywords Polo ; BicD ; oocyte ; meiosis ; polarized transport

INTRODUCTION

A specific feature of germline cells is their ability to undergo meiosis when they differentiate into gametes. However, the mechanisms linking the fate of gamete cells to this specialized cell cycle are still poorly understood. During Drosophila oogenesis, the determination of the oocyte and the first steps of meiosis appear to be closely linked (Huynh and St Johnston, 2004 ; Riechmann and Ephrussi, 2001).

Drosophila oogenesis begins in a structure called the germarium, which is divided into several regions (Fig 2A) (Spradling, 1993). In its anterior part, named region 1, germline stem cell progeny undergoes a precise pattern of divisions to form cysts of 16 cells interconnected by cytoplasmic bridges, the ring canals. Oocyte differentiation is a progressive process that begins in region 2a by the selection of two pro-oocytes corresponding to the first two cells of the cyst. As the cyst enters region 2b and contacts the follicle cells, one cell is selected to become the oocyte, while the other 15 cells will differentiate as nurse cells. This progressive specification can be observed by the accumulation of mRNA and proteins such as Bicaudal-D (BicD), and by the migration of the centrioles (Fig 2A) (Bolivar et al., 2001 ; Cox and Spradling, 2003 ; Ephrussi et al., 1991 ; Keyes and Spradling, 1997 ; Suter et al., 1989 ; van Eeden et al., 2001). When the cyst progresses from region 2b to region 3, it starts to round up, with the oocyte always positioned at the posterior. At this step, centrosomes, mRNAs, proteins and organelles found at the anterior of the oocyte move to the posterior. This early polarization event is important as it prefigures the future antero-posterior axis of the embryo (Huynh et al., 2001).

In each cyst, a germline-specific membranous structure called the fusome extends asymmetrically throughout the ring canals in all 16 cells (Fig 2A) (de Cuevas et al., 1996 ; Lin et al., 1994). This asymmetric distribution is thought to determine which cell becomes the oocyte(de Cuevas and Spradling, 1998 ; Lin and Spradling, 1995 ; Lin et al., 1994 ; Yue and Spradling, 1992). Nevertheless, this initial asymmetry is not sufficient to allow oocyte differentiation, and genetic analyses allow to distinguish different steps in this process.

The polarization of the germline cyst relies on microtubule-dependent transport processes. Microtubules and dynein are required for the accumulation of oocyte determinants such as BicD protein and thus for oocyte differentiation (Theurkauf et al., 1993 ; Bolivar et al., 2001). The transport of mRNA and proteins to the oocyte is also dependent on BicD and Egl proteins (Bolivar et al., 2001 ; Clark and McKearin, 1996 ; Navarro et al., 2004 ; Ran et al., 1994 ; Schupbach and Wieschaus, 1991 ; Suter et al., 1989). These proteins interact together, and both are able to interact with different subunits of the Dynein complex (Hoogenraad et al., 2001 ; Mach and Lehmann, 1997 ; Navarro et al., 2004). BicD may function as an adaptor for cargo molecules such as mRNA, and it has been suggested that Egl is an

important regulator of this function. Finally, the early polarization of the oocyte in region 2b-3 involves many genes including the dynein light chain 8 and, again, egl and BicD (Huynh and St Johnston, 2000 ; Navarro et al., 2004).

All these functional steps are required for establishing or maintaining oocyte fate. Each mutation that disrupts this process leads to the formation of cysts that have neither an oocyte and nor a cell in meiosis, and instead consists of 16 endoreplicative nurse cells. Thus, meiosis control is dependent on oocyte determination.

During Drosophila oogenesis, meiosis starts with homologous recombination that can be recognized through the formation of the synaptonemal complexes (SCs) and the recruitment of proteins such as C(3)G (Huynh and St Johnston, 2000 ; Page and Hawley, 2001 ; Hong et al., 2003 ; Carpenter, 1975). Meiosis begins in region 2a of the germarium, usually in four cells of a cyst (Fig 2A). Meiosis is quickly restricted to the two pro-oocytes, then to the oocyte as the cyst progresses into region 2b. Therefore, meiotic control appears to be spatially and temporally correlated with oocyte determination, and it is difficult to determine whether one process precedes the other.

Functional studies have provided further evidence on the links between oocyte determination and meiosis. Null mutations of egl and BicD have been described to have dramatic and opposite effects on meiosis (Huynh and St Johnston, 2000). In BicD cysts, no cells possess SCs whereas all the cells of egl mutant cysts form SCs in region 2a before all of them exit meiosis simultaneously. Although the initial difference between these two mutants is not yet understood, this observation shows that both are involved in the initial restriction of meiosis to 4 cells. Finally, proteins required for early oocyte polarization are also required for maintaining the oocyte in meiosis after its restriction to one cell (reviewed in Huynh and St Johnston, 2004). Therefore, apart from the essential role of BicD and Egl, the spatiotemporal control of meiosis remains poorly understood.

Obviously, initiation of meiosis is itself under the control of classical cell cycle regulators. Partial loss-of-function mutations in cyclin E, the main cyclin controlling replication and endoreplication, can lead to the formation of 16-cell cysts containing two meiotic cells, both presenting oocyte-like nuclear and cytoplasmic features (Lilly and Spradling, 1996). Conversely, a mutation in p27cip/dacapo , a negative regulator of cyclin E, induces the formation of cysts with 16 endoreplicative nurse cells and no oocyte (Hong et al., 2003). These findings allow the following conclusions. First, meiosis and endoreplication seem to act in competition, since the reduction of a positive or negative determinant of one process promotes or represses the other, respectively. Second, the cell cycle decision of a cell is sufficient to determine its fate, as both oocyte and nurse cells can be led to adopt the other fate by altering the control of the cell cycle. Finally, these results also strongly suggest that the choice between endoreplication and meiosis involves the asymmetric distribution of cell cycle regulators, and this asymmetry may depend on the general process of cyst polarization. One candidate for an asymmetric meiotic determinant is Dacapo, as it is found specifically in the oocyte nucleus in region 3 of the germarium. However, this asymmetric distribution is not observed at earlier stages, and a null mutant for dacapo does not affect meiotic progression in region 2 but only its maintenance in region 3. Many other proteins involved in cell cycle control have been implicated in oocyte specification, thus confirming their influence on cell fate decisions. However, to date, no cell cycle regulator has been found to be asymmetrically localized early enough to explain how the balance between meiosis and endoreplication is initially controlled. Moreover, how cell cycle control influences oocyte cell fate decision remains unknown.

In this article, we show that the Polo kinase, one of the main regulators of the G2/M transition, interacts with BicD protein during oogenesis. Genetic analyses reveal interdependent functions between both proteins during early meiosis control and oocyte specification. Since Polo plays a role in cell cycle control and BicD plays a role in polarized transport to the future oocyte, we propose that their interaction reflects the existing link between meiosis and oocyte determination.

MATERIALS AND METHODS

Molecular Biology

Plasmid constructs were generated by amplification of the desired fragments by PCR, which were sequenced and subcloned into appropriate vectors for yeast two-hybrid analysis (pp7 and pLex12, derived from the original pBTM116 and PGADGH, respectively) and Drosophila transgenes (pUASp) (Rorth, 1998). Details can be provided upon request.

Drosophila Strains and Genetics

All the crosses were produced at 25°C using standard manipulation of fly genetics. Transgenic lines of UASp-polo construct were generated by standard methods and two independent lines were analyzed. Clonal analysis was performed with the FLP/FRT system (Xu and Rubin, 1993) using nuclear GFP as a clone marker.

Yeast Two-Hybrid

The yeast two hybrid screens were performed with Plk1 fragments as baits to screen a human placenta cDNA library using a previously described mating method (Formstecher et al., 2005).

Immunostaining

Tissue stainings were performed according to standard procedures, using the primary antibodies at following dilutions: rabbit anti-C(3)G antibody at 1/1000 (Hong et al., 2003 ; Lilly and Spradling, 1996), rabbit CP309 antibody 1/500 (Kawaguchi and Zheng, 2004), mouse anti-Polo MA294 1/10 (Llamazares et al., 1991), mouse anti-Hts 1B1 1/100 (Developmental Studies Hybridoma Bank), mouse anti-BicD 1B11 plus 4C2 at 1/50 each (Developmental Studies Hybridoma Bank). Cy3, Cy5 (Jackson Immunoresearch) and Alexa 488-conjugated secondary antibodies (Molecular Probes) were used at 1:500.

Ovary immunoprecipitation

Immunoprecipitation was performed as described in Navarro et al. (2004) using polyclonal anti-GFP antibody (Clontech). Details can be provided upon request.

RESULTS

The Polo kinase interacts with BicD

In a yeast two-hybrid screen we found an interaction between the human Polo protein (Plk1) and one of the two human homologs of Drosophila BicD (hBicD2). Several clones corresponding to hBicD2 were obtained in screens with full-length Plk1 (11-596) and with its C-terminal regulatory domain (280-596). Positive clones defined a minimal interacting region corresponding to amino-acids 129-326 of hBicD2. We found a similar interaction between BicD amino-acids 120-350, corresponding to amino-acids 124-358 of hBicD2, and Drosophila Polo, which indicates that this interaction is conserved (Fig. 1A). The N-terminal kinase domain of Polo does not interact with BicD in a two-hybrid assay. The C-terminal regulatory part of Polo, named the Polo-Box domain, is a structural unit composed of two repeats (Polo-Boxes) and an alpha helix in the hinge region between the kinase domain and the Polo-Boxes (Cheng et al., 2003 ; Elia et al., 2003). Deletions at both extremities show that the entire Polo-Box domain is both necessary and sufficient for the two-hybrid interaction with BicD (Fig. 1A).

To date, the only link identified between BicD and cell-cycle concerns entry into meiosis during Drosophila oogenesis (Huynh and St Johnston, 2000). Moreover, the interaction domain of BicD with Polo is particularly well conserved and has been shown to be functionally significant, especially during early oogenesis (Oh et al., 2000). Therefore, we tested the ability of both proteins to interact using co-immunoprecipitation on ovary extracts. We took advantage of flies containing a GFP-Polo transgene that has been shown to reproduce Polo expression and localization in all cell types analyzed, and to rescue polo mutants (Moutinho-Santos et al., 1999). Wild-type flies and flies constitutively expressing an NlsGFP protein were used as negative controls. Anti-GFP antibody efficiently precipitates both NlsGFP and GFP-Polo proteins but BicD was co-precipitated only with the GFP-Polo (Fig. 1B). This experiment shows that Polo and BicD proteins interact in vivo during Drosophila oogenesis.

Polo is gradually restricted to meiotic cells during cyst polarization

We analyzed Polo localization during oogenesis using flies hemizygous for the GFP-Polo construct. In the germarium, Polo was strongly expressed in all the germline cells of region 1, suggesting that the presence of Polo is not cell-cycle-dependent (Fig. 2B). At the subcellular level, GFP-Polo accumulated in several cytoplasmic dots in each cell, generally at the nuclear periphery in region 1 (Fig. 2C). In region 2a, the dots became progressively less bright, except in the more central part of the cysts where they remained particularly intense (Fig. 2D). In regions 2b and 3, Polo was found in one or sometimes a few prominent dots at the posterior of the cyst (Fig. 2B and 2E). This localization was maintained until stages 2–3, and then became undetectable in the germline cells of later stages.

We compared this localization pattern with several markers of germarium structures. Polo accumulated in the pro-oocytes from region 2a and then in the oocyte in region 2b similar to the BicD protein and centrosomes, although it did not colocalize with them (Fig. 2D). In region 3, centrosomes and BicD migrated to the posterior of the oocyte whereas Polo was found generally in its anterior or lateral region (Fig. 2E). We did not observed any significant colocalization between the fusome and Polo, although the Polo dots were often close to it in regions 2a and 2b (Figs. 2F, G and H). Importantly, a comparison of this pattern with SC staining revealed a correlation between the cells in meiosis and the cells that contained Polo speckles from regions 2a to 3 (Figs. 2F and G). The endogenous Polo protein showed a similar expression and localization pattern as the one obtained with GFP-Polo, especially the preferential accumulation in the germ cells that have enter meiosis (Figs. 2I and J). Finally, in situ hybridization of the endogenous polo mRNA reveals that this gene is strongly expressed in the germline in region 1 and that no specific accumulation in the oocyte is detected in the following steps (Fig. 2K). This indicates that the asymmetric distribution of the Polo protein is not due to the localization of its mRNA.

BicD and the Dynein complex are required for meiosis and Polo localization

As Polo interacts with BicD and localizes to the oocyte, we tested whether Polo localization is dependent on BicD and we compared this localization to meiosis progression. First, germline clones of an amorph BicD allele ($BicD^{r5}$) in flies expressing GFP-Polo showed a staining for the SC component C(3)G in all cells of a cyst in region 2a (Fig. 3A). However, this staining was weaker than the one observed in pro-oocytes of wild-type cysts, and did not have the typical morphology of wild-type SCs even if thread-like structures were observed.

C(3)G was no longer detectable in regions 2b and 3. This reveals that, in the complete absence of BicD, all cystocysts enter meiosis but do not progress to the full pachytene and revert back to an endoreplicative nurse cell fate. A similar phenotype was observed in absence of the GFP-Polo transgene, indicating that entry into meiosis in the absence of BicD was not due to overexpression of Polo (data not shown). This result differs from a previous report in which the absence of an other SC epitope in $BicD^{r5}$ clones led the authors to conclude that BicD was required to initiate SC formation (Huynh and St Johnston, 2000). This suggests that this unknown protein is recruited later to the SC than C(3)G during meiosis. In $BicD^{r5}$ clones, Polo has a normal spotted distribution in region 1 of the germarium (Fig 3A), indicating that this peculiar subcellular localization of Polo is independent of BicD. However, GFP-Polo dots were found in all the cells of the cysts in region 2a, (Fig 3A), and, they became undetectable in regions 2b and 3, instead of accumulating in one cell of the cyst.

We also analyzed GFP-Polo localization and meiotic progression in the hypomorphic mutant $BicD^{PA66}$. The resulting mutant BicD protein retains some function, but fails to localize and accumulate in the presumptive oocyte, leading to the formation of cysts containing 16 nurse cells (Suter and Steward, 1991) (Fig 6A). In region 2a of $BicD^{PA66}$ germaria, meiosis initiated properly in 2 to 4 cells per cyst indicating that a detectable active transport of BicD protein is not required for this process (Fig 3B). Then, in region 2b, the number of SC-positive cells varied from 0 to 2 depending on the cyst, but we rarely observed cysts with only one meiotic cell (2/31). Cysts positive for SCs in region 3 were an exception (see below). This strongly suggests that BicD is required in the presumptive oocyte for the normal restriction of meiosis to this cell. In the $BicD^{PA66}$ mutant, GFP-Polo dots failed to properly accumulate in the central part of the cysts in regions 2a and 2b, and were not found in the presumptive pro-oocyte. Polo was not detected in region 3 of $BicD^{PA66}$ germaria. Among 186 analyzed $BicD^{PA66}$ only one contained GFP-Polo dots in a cyst of region 3 and it was also the only one that had SC-positive cells (data not shown).

BicD function during oogenesis is also dependent on Egl and the Dynein complex (Mach and Lehmann, 1997 ; Bolivar et al., 2001). To confirm that Polo localization depends on BicD function in polarized transport, we also investigated Polo localization in an egl null background. Polo localization and meiotic progression always showed the same defects as in BicD null mutants (Fig. 3C). We also generated germline clones for a null mutant of the Dynein complex component dynamitin (dmn) (Januschke et al., 2002). Similarly to BicD and egl loss-of-function mutants, in the absence of Dmn, Polo invariably failed to accumulate in one cell of the cyst (Fig. 3D). In most of the dmn clones, meiosis started normally in four cells and was then restricted to the two pro-oocytes in region 2a. However, dmn cysts in region 2b contained 0, 1 or 2 C(3)G-positive cells, similar to $BicD^{PA66}$ ovaries (Fig. 3D). Meiosis was never observed in region 3, and cysts systematically failed to form an oocyte. Together, these results show that Polo localization and the restriction of meiosis to the oocyte are progressive processes throughout region 2a, and that both are dependent on a polarized transport to the oocyte.

polo mutants affect meiosis progression and BicD-dependent transport

As changes in Polo localization were correlated with meiosis progression in wild type conditions as well as in different mutant backgrounds, we asked whether Polo could influence this process. As polo is required for cell viability and division, we took advantage of the hypomorphic allele $polo^{1}$ to study the effect of its loss of function during oogenesis (Sunkel and Glover, 1988). This allele was associated in trans with a chromosomal deficiency covering the polo locus (Df(3L)rdgc-cos2) or the strong alleles $polo^{9}$ or $polo^{16\text{-}1}$ (Donaldson et al., 2001). These three different genotypes gave identical phenotypes that were completely rescued by one GFP-Polo transgene. In polo cysts, C(3)G staining was usually found only in few spots in each cyst in region 2a (Fig 4B, B′). In contrast, cysts in region 2b contained two to four cells with normal SCs reaching the pachytene, which in wild type is typical for region 2a, indicating a significant delay in meiosis entry and in the restriction to one cell. C(3)G was still present in at least two cells in region 3, thus confirming the delay of meiosis restriction to one cell (Fig 4B, B′). However, the staining intensity for C(3)G is reduced in region 3 compared to wild type, and the protein was only found in a few small dots per nucleus. Surprisingly, in later stages, meiosis was restricted to the oocyte and the SCs appeared normal. In conclusion, partial loss of Polo function led to two distinct phenotypes during the first steps of meiosis. On one hand, it is involved in the initiation of SC formation and in their maintenance in the oocyte. On the other hand, it is also involved in the restriction of meiosis to the oocyte.

We also investigated oocyte differentiation in polo mutants using the BicD protein itself as a reporter. In wild type conditions, BicD starts to accumulate in the pro-oocytes as early as region 2a, and is globally restricted to the future oocyte when the cysts enter region 2b (Fig 4A, A″). When the cysts progressed into region 3, BicD migrated from the anterior to the posterior margin of the oocyte, indicating its antero-posterior polarization. In cysts with a partial loss of Polo function, BicD failed to accumulate in pro-oocytes of region 2a (Fig 4B, B ″). However, the accumulation of BicD was only delayed as it started to accumulate properly in region 2b. In region 3, BicD remains at the anterior of the oocyte but this polarization defect was corrected in later stages, indicating that it corresponds to a delay in oocyte differentiation. We did not observed important changes in microtubule organization in polo mutant cysts suggesting that Polo does not act through a direct effect on the microtubule network. However, DNA and BicD staining revealed that, in less than 1% of cases, hypomorphic polo mutations led to cyst without an oocyte and with 16 endoreplicative nurse cells, confirming that polo is involved in meiosis and oocyte differentiation (data not shown).

We produced germline clones for the null allele $polo^{9}$. Unfortunately, we did not find germline cysts in which all cells were homozygous mutant, probably due to the function of Polo during mitotic division. Thus, we could not test the effect of a complete depletion of Polo activity on meiosis progression and oocyte determination. We observed 48 mosaic cysts among them ten contained a single mutant cell and all of them present the same phenotype (Fig 4C). DNA staining indicates that the endoreplication has occurred normally in this single polo mutant nurse cell. However, BicD was present in this cell at higher level than in neighboring cells though its anterior position indicated that it was not one of the four initial meiotic cells. This strongly suggests that Polo is autonomously required in each cell of the cyst for transport of the BicD protein to the oocyte independently of its possible role in meiosis.

Polo overexpression affects meiosis progression and oocyte differentiation

Since loss of Polo function seems to indicate that it is required for meiosis, we wondered whether it might act as a trigger for meiosis when overexpressed. Polo overexpression in the germline was obtained in two different ways. On one hand, we used flies homozygous for the GFP-Polo transgene in a wild-type context for endogenous polo . On the other hand, we produced flies in which a UAS-polo construct was specifically expressed in the germline. Similar phenotypes were observed in both lines. First, in region 2a we observed that approximately half of the cysts had more than 4 cells containing SC with, generally, 6 to 8 cells in meiosis (Fig 5A). Thus, Polo overexpression can induce more cells of a cyst to enter meiosis than is seen in wild-type. Furthermore, in regions 2b and 3, cysts always contained at least two cells with SC. In some cases, cysts in region 3 still contained 4 C(3)G-positive cells (Fig 5). The restriction of meiosis to one cell eventually occurs during stages 3–5. Observation of Polo distribution itself gave further insight into this phenotype. Intense spots of GFP-Polo were observed in more than one cell per cyst, even in regions 2b and 3 (Fig 5B, B‴). Moreover, the presence of intense GFP-Polo spots correlated with the presence of C(3)G-positive cells (Fig 5B′, B‴). Finally, Polo became restricted to the oocyte at the same time as meiosis during vitellogenic stages. Surprisingly, Polo gain-of-function led to similar defects as partial loss of function on oocyte differentiation, with a delay in the accumulation of BicD in the oocyte and in the early polarization of the oocyte (Fig5B‴, B‴). These results show that Polo overexpression leads to a delay in its own localization to the oocyte, probably because its overabundance exhausts the process leading to its asymmetric distribution. Polo overexpression also induced defects in the initiation and restriction of meiosis, and these defects correlated with Polo localization. As in the case of partial polo loss of function, these data strongly suggest that Polo is involved in the initiation, maintenance and restriction to one cell of meiosis. Our data suggest that meiosis is controlled by the level of the Polo protein in each cell of the cyst, and that the specific localization of Polo to the oocyte is required for meiosis restriction.

Overexpression of Polo restores $BicD^{PA66}$ localization and function during oocyte differentiation

We reasoned that if Polo contributes to the activation of BicD-dependent transport early in oogenesis, the overexpression of Polo might rescue $BicD^{PA66}$ mutants. As described previously, $BicD^{PA66}$ is a hypomorphic allele that does not interfere with the initiation of meiosis in 4 cells, but blocks oocyte differentiation and the restriction of meiosis to one cell, leading to a 16 polyploid nurse cell terminal phenotype. Moreover, the $BicD^{PA66}$ protein does not localize to the oocyte but remains diffuse in all cells of a cyst (Fig 6A and Suter and Steward, 1991). $BicD^{PA66}$ shows a decrease level of phosphorylation, which is likly responsible for its reduced functional activity. When we overexpressed a UAS-Polo in a $BicD^{PA66}$ mutant germline, we observed that the follicles contained an oocyte at their posterior (Figs 6B and 6C) and that SCs were present in every cyst from region 2 of the germarium until stage 6 follicles (Fig 6B). All of the control $BicD^{PA66}$ follicles examined (n=224) had 16 nurse cells, whereas 98% of $BicD^{PA66}$ follicles overexpressing Polo (n=238) had an identifiable oocyte. BicD localized preferentially to the posterior of the oocyte in these follicles (Fig 6B), although not as well as in wild-type follicles. Eventually, this posterior localization of BicD was not maintained beyond stages 3 or 4 of oogenesis, and germ cells of these cysts degenerated at around stage 8 of oogenesis (Fig 6D). Since BicD localization is dependent on its own function in polarized transport, these data indicate that overexpression of Polo in the germ cells is able to suppress the early phenotypes of $BicD^{PA66}$ and to restore its ability to mediate polarized transport to the oocyte. This confirms that Polo has a direct role in regulating the polarized transport in germline cells and suggests that this function is mediated by BicD.

DISCUSSION

Recent studies on oocyte determination and on the control of meiosis have pointed out that both mechanisms are closely linked, although the nature of these links remains unknown. Our analysis of the physical and functional interaction between BicD and Polo reveals a new function for Polo, and contributes to a better understanding of meiosis control, meiosis restriction and oocyte differentiation. Our data allow us to provide a model for explaining the links between meiosis and oocyte differentiation.

Polo localization to the oocyte requires BicD-dependent polarized transport

This paper describes the localization of the Polo protein and its genetic control in the Drosophila germline during early oogenesis. Polo has a peculiar subcellular localization in cytoplasmic dots that do not correspond to any well-known structures of germline cysts or microtubule minus-ends where BicD accumulates. Polo has previously been described to co-localize with several subcellular structures depending on cell cycle phase, but none of these correspond to the localization observed here (Barr et al., 2004). Similar cytoplasmic dots

were observed in the primordial germline cells of the Drosophila embryo as soon as they are formed suggests that this unusual localization could be a specific feature of the germline (Moutinho-Santos et al., 1999).

From region 2a onward, Polo dots are present mostly in the cells containing SCs. This is the first report of a cell cycle regulator whose localization is spatially and temporally correlated with meiotic progression during early oogenesis. Moreover this correlation is still conserved in mutants that affect polarized transport and the restriction and maintenance of meiosis. This indicates that Polo localization is dependent on polarized transport. One possibility is that Polo itself is directly transported to the oocyte. This hypothesis is reinforced by the physical interaction between BicD and Polo proteins, according to the proposed function of BicD as adapter for Dynein cargos. However, the BicD-dependent localization of Polo is not sufficient to explain its expression profile. Polo is strongly expressed in region 1 of the germarium, and the overall amount of the protein in the cyst progressively decreases, becoming undetectable after stage 2. This degradation seems to be compensated in meiotic cells and then in the oocyte by the polarized transport. The progressive degradation of Polo is also observed in egl and BicD null mutants. Degradation in association with a complete absence of Polo transport may explain why all the cells of a cyst enter into meiosis in these mutants (all the cells contain the same amount of Polo) and then exit meiosis simultaneously (none of the cells preferentially accumulates enough Polo). Alternatively to a direct transport of Polo to the oocyte, its asymmetric distribution in the cyst could be due to a differential control of its stability between nurse cells and oocyte, under the control of the BicD dependent polarized transport.

The initial restriction of meiosis requires a dynein-independent BicD function

BicD and egl null mutants, showed a very similar phenotype, in which all 16 cells of a cyst first enter in meiosis but subsequently lose the SCs. This phenotype cannot be compared with null mutants of the dhc, as Dynein is required at earlier steps of cyst formation. The human homolog of BicD interacts directly with Dynamitin, and this interaction is thought to mediate the interaction of BicD with the Dynein complex (Hoogenraad et al., 2001). In contrast to BicD, Dynamitin is not involved in the initial restriction of meiosis, showing that the interaction of BicD with Dynamitin, and thus probably Dynein, is not required for the initial restriction of meiosis. In a similar way, LC8 null mutants or egl mutants that specifically block the interaction between Egl and LC8 do not interfere with the initiation of meiosis in only four cells (Navarro et al., 2004). We found that a transport of the BicD protein between the cyst cells is apparently not required for this first step, as the $BicD^{PA66}$ allele or drug-induced microtubule depolymerization do not affect this initial restriction although BicD is diffuse throughout the entire cyst (see results and Huynh and St Johnston, 2000). Finally, null mutant for the plakin shot, which has been proposed to be an essential upstream component of the Dynein function in centrosome migration, exhibits variable meiotic phenotypes but allows a normal initial restriction of meiosis to four cells (Roper and Brown, 2004). These data are consistent with a function of BicD and Egl independent of Dynein in the initial restriction of meiosis.

Polo is involved in the control of meiosis

Polo is involved in many crucial steps of the cell cycle, including the G2/M transition of mitosis and meiosis processes (reviewed in Barr et al., 2004). Here, we show that hypomorphic polo alleles lead to a delay in meiotic entry, and that Polo overexpression can trigger meiosis in more than four cells per cyst in region 2a. These phenotypes could be related to the function of Polo in the G2/M transition. In vertebrates, Polo is an activator of the String/CDC25 phosphatase, and it has also been proposed that Polo can repress the kinases Myt1 and Wee1. String is the main activator of the cyclinB/CDC2 complex whose activity triggers the G2/M transition, whereas Myt1 and Wee are repressors of this complex. However, the role of the cyclin B and CDC25 in meiosis in Drosophila oogenesis is not yet well understood, since, for example, CDC25 seems to act as a negative regulator of meiotic oocyte cell fate (Mata et al., 2000). Further investigations will be needed to determine how Polo triggers meiotic entry during early oogenesis.

We have shown that in partial polo loss of function mutants, SCs start to disassemble in region 3 but are well formed again in stage 2/3 before disappearing in the following stages. One possible hypothesis to explain how meiosis is finally properly maintained in polo hypomorphic mutants is that the repression of cyclin E by Dacapo during stage 2/3 represses endoreplication, and thus allows meiotic progression (Hong et al., 2003). This is consistent with the finding that the specific localization of Dacapo to the oocyte and its requirement for meiosis maintenance begins only in region 3. Moreover, null mutations of dacapo do not lead to a fully penetrant 16 nurse cell phenotype, confirming the existence of a partially redundant control. Therefore, we propose that the balance in favor of meiosis is initially due to the localized activation of meiosis by Polo, and later to the localized inhibition of endoreplication by Dacapo, and that both mechanisms partially overlap.

Polo is involved in polarized transport and oocyte determination

We also observed that Polo is required for the normal restriction of meiosis. Moreover, the defects in the restriction of meiosis caused by both loss- and gain-of-function of polo are correlated with defects in oocyte determination. As described previously, meiosis restriction and oocyte specification both depend on the Dynein complex and BicD polarized transport system. Thus, we assume that these Polo phenotypes indicate that Polo is involved in polarized transport. This role may be indirect and thus reveal the influence of meiosis and cell cycle control on oocyte differentiation. Such influence has been observed in case of the activation of the meiotic checkpoint due to a

failure in DNA double-stand break repair (Gonzalez-Reyes et al., 1997; Ghabrial et al., 1998). However, at least two results argue for a direct role of Polo in polarized transport, independently of its meiotic function. First, in mosaic germline cysts, non meiotic cells mutant for polo retain BicDprotein. Thus, this phenotype cannot be due to the activation of the meiotic checkpoint. This strongly suggests that Polo is required in each cell of the cyst to initiate BicD-dependent transport to the presumptive oocyte. Second, the overexpression of Polo is able to restore the localization and therefore the function of $BicD^{PA66}$ protein. Interestingly, this mutant allele is due to a single amino-acid substitution (A40V) which leads to a hypophosphorylation of BicD, and genetic evidence indicates that this phosphorylation is crucial for its function (Suter and Steward, 1991). Polo overexpression might restore a functional level of $BicD^{PA66}$ phosphorylation. Therefore, even if we failed to observe significant change in the gel mobility of BicD in polo hypomorph mutants, it is tempting to propose that the function of Polo in the polarized transport could be, to activate, directly or indirectly, BicD by phosphorylation,

A model for oocyte determination and meiosis control

Together, our results allow to propose a model that can explain a reciprocal requirement between the control of meiosis and oocyte specification (Fig 6C). This model is based on four major points. First, as previously described, BicD is required for the Dynein-dependent polarized transport of oocyte determinants. Second, BicD is also required for the progressive localization of Polo to the oocyte. Third, Polo appears to trigger meiosis in the germarium. Fourth, Polo is required to activate the BicD and Dynein-dependent polarized transport. Together, this leads to a positive feedback loop between Polo and BicD proteins, and therefore between oocyte specification and meiosis.

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Figure 1

Interaction between BicD and Polo in two-hybrid and in vivo

A) Yeast two-hybrid interaction between Polo and the N-terminal part of BicD (amino acids120–350). The whole Polo-Box domain (Polo-Boxes 1 and 2 (red) and a short helix represented in grey) but not the kinase domain (green) are required for the interaction. B) Immunoprecipitation using anti-GFP antibodies (IP-GFP) from ovary extracts from wild-type (lane 1), GFP-Polo (lane 2) and Ubi-NlsGFP (lane 3) flies, showing BicD specifically coprecipitates with GFP-Polo (lane 2, IP-GFP).

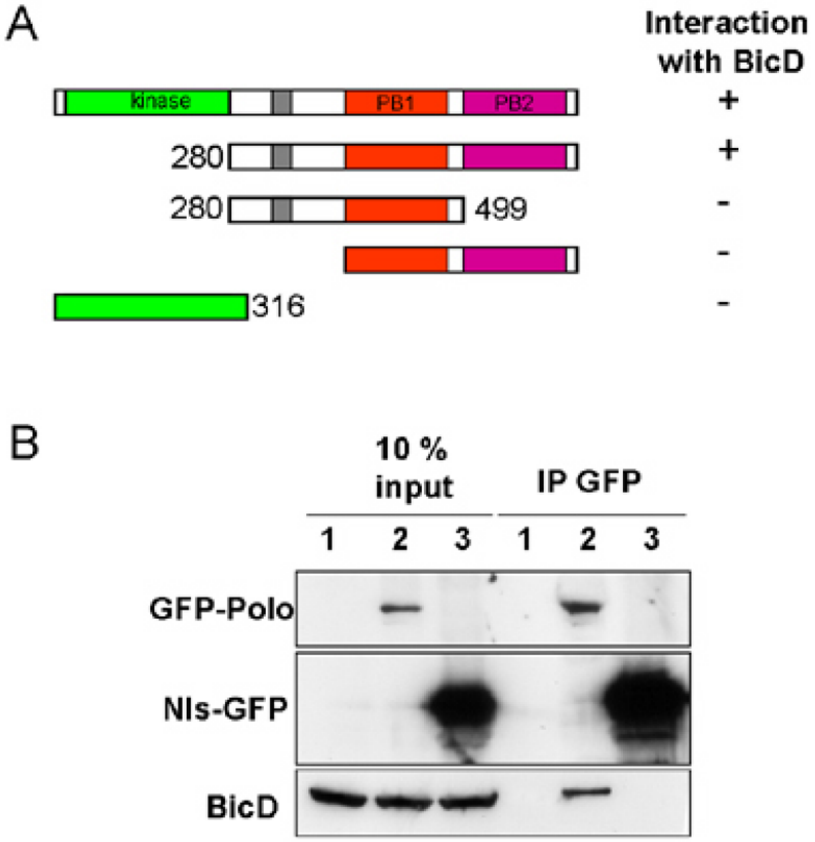

Figure 2

Polo localization during the early steps of oogenesis

A) Schematic representation of a Drosophila germarium focusing on oocyte determination and meiosis progression. B) General view of GFP-Polo expression (green) in a germarium and a stage 2 follicle. Arrowheads indicate Polo spots in region 2 and 3. C, D and E are enlargements of the B picture. C) In region 1, Polo is found in many dots in each interphase germline cell, but is not colocalized with centrosomes (CP309, red). D) In region 2a, Polo dots are progressively restricted to the pro-oocytes but do not show colocalization with BicD (blue) or centrosomes (red). E) In region 3, Polo is found at the anterior or lateral part of the oocyte whereas BicD and centrosomes are in the posterior region. F) and G) A germarium expressing GFP-Polo (green) stained for SCs (C(3)G, red) and fusome and follicular cells (Hts, blue). Polo concentrates in meiotic cells from region 2a (F) to region 3/stage 1 follicle (G). H) Single confocal section of cysts in region 2 showing that GFP-Polo (green) is not localized on the remnant fusome (red). I) and J) Endogenous Polo protein (green) co-stained with SCs (red) shows a similar dynamic localization to GFP-Polo in regions 1 (I), 2 and 3 (J) of the germarium. K) polo mRNA in situ hybridization. Strong expression of polo is detected in the germline in region 1 of germarium (arrow) and in follicle cells in region 2a (arrowhead).

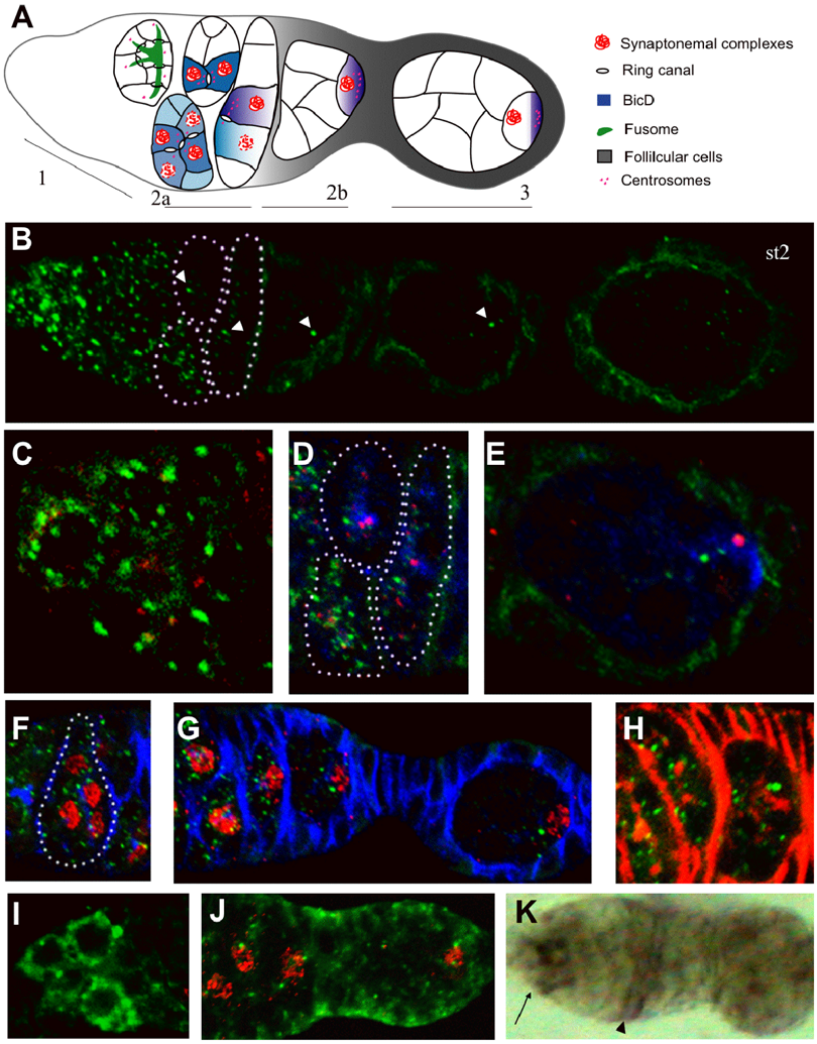

Figure 3

Polo localization is dependent on BicD, egl and the Dynein complex

A) BicD^{r5} germline clones marked by the absence of NlsGFP. B) BicD^{PA66} homozygous germarium. C) egr$^{u50/RC12}$ ovariole D) dmn^{K16109} germline clones marked by the absence of GFP. In A to D, the first images show SCs (red, white in the second picture), GFP-Polo (green dots, white in the third picture) plus NlsGFP (green nuclei, white nuclei in the third picture) and Hts (blue) in A and D. Polo localization is not restricted in any of these different genotypes, and the meiosis is not maintained in any cell of the cyst (at least 50 mutant cystes were scored for each genotype). Note that in BicD (A) and egl (C) null mutants, meiosis starts in all the cells in region 2a whereas in BicD^{PA66} (B) and dmn (D) mutants normal initiation of meiosis in two to four cells is observed.

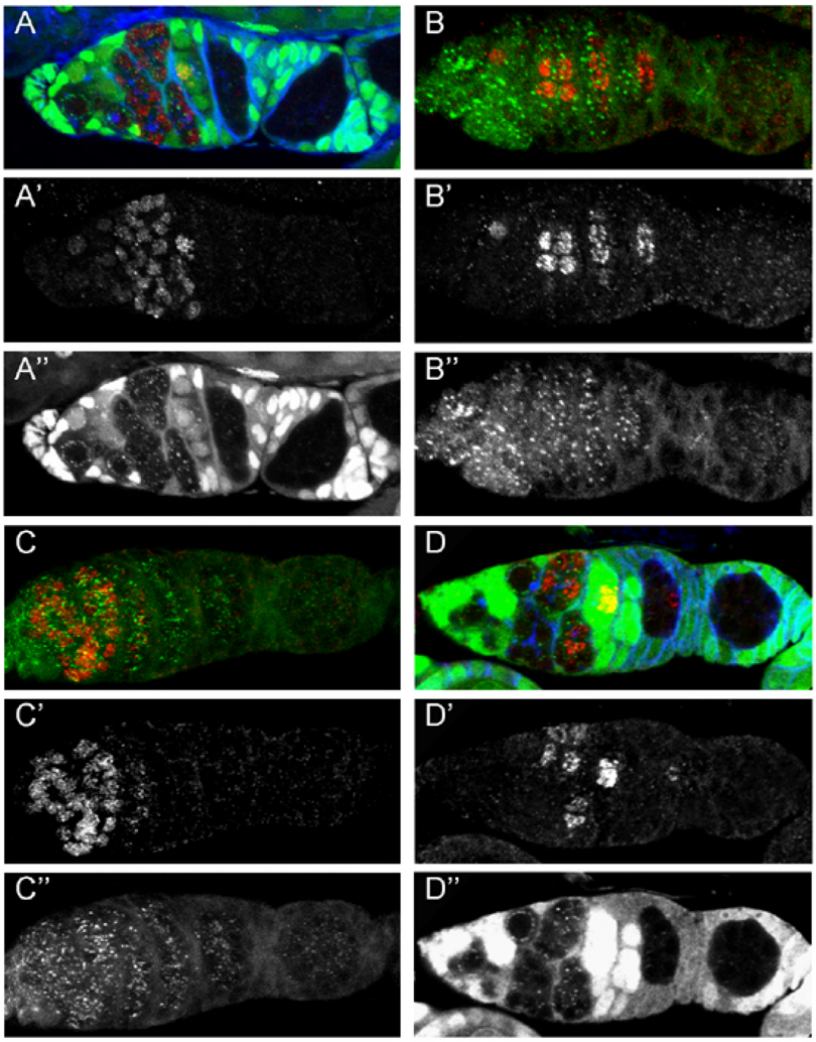

Figure 4

Loss of function of Polo affects meiotic progression and BicD polarized transport

A) A wild-type germarium stained for SCs (red, white in A′) and BicD (green, white in A″). SC formation is initiated in two to four cells in region 2a, then is restricted to one cell in region 2b. BicD accumulation in meiotic cells begins in region 2a. Through region 2b, BicD redistributes to the posterior of the oocyte. B) A $polo^{1}$ /$polo^{9}$ germarium. Meiosis does not initiate properly in region 2a. Cysts often contain abnormal SCs, and meiosis restriction to one cell is delayed. BicD starts to accumulate in pro-oocytes only in region 2b. In region 3, BicD is still at the anterior of the oocyte. C) A mosaic $polo^{9}$ follicle at stage 7 with a single mutant cell (arrow) marked by the absence of nuclear GFP (green, white in C′). In this nurse cell, the amount of BicD protein (red, white in C″) is higher than in the neighboring wild-type nurse cells, but lower than in the oocyte (arrowhead). DNA is shown in blue.

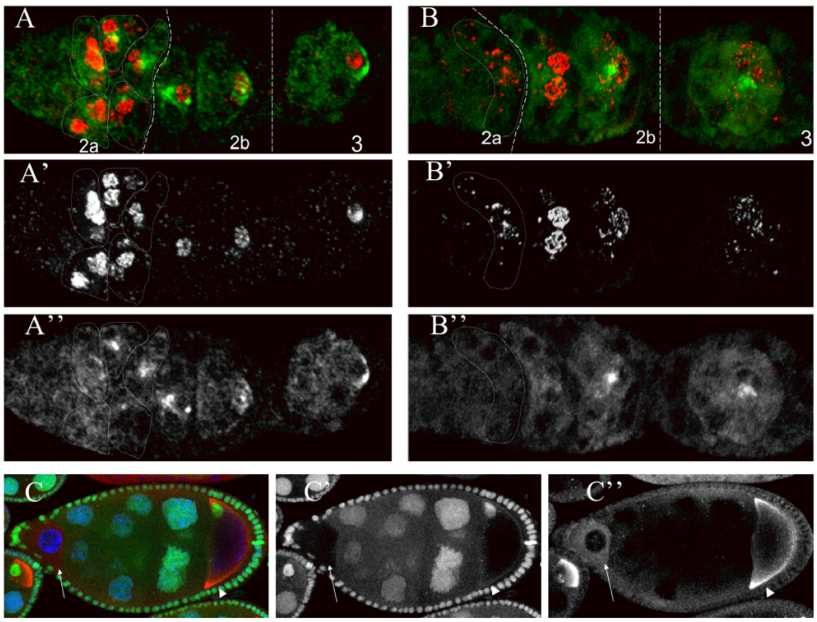

Figure 5

Polo overexpression affects meiosis progression and oocyte differentiation

A) UAS-Polo overexpression with a germline-specific driver leads to the formation of SCs (white) in more than 4 cells per cyst in region 2a, and to a delay in meiotic restriction in region 2b. B) Overexpression of GFP-Polo. C(3)G (red, white in B′), BicD (blue, white in B″), and GFP-Polo (green, white in B‴). B′) Meiosis can initiate in more than 4 cells and cysts in region 3 still contain 4 cells in meiosis, thus indicating a strong delay in meiosis restriction to one cell. B″) Polo overexpression leads to a delay in BicD accumulation in the oocyte and localization to the posterior of the oocyte. B‴) Cysts from region 2a to region 3 contain an unusual high number of Polo dots that are not restricted to one cell. Note the correlation between cells containing the strongest Polo speckles and meiotic cells in the cyst in region 3.

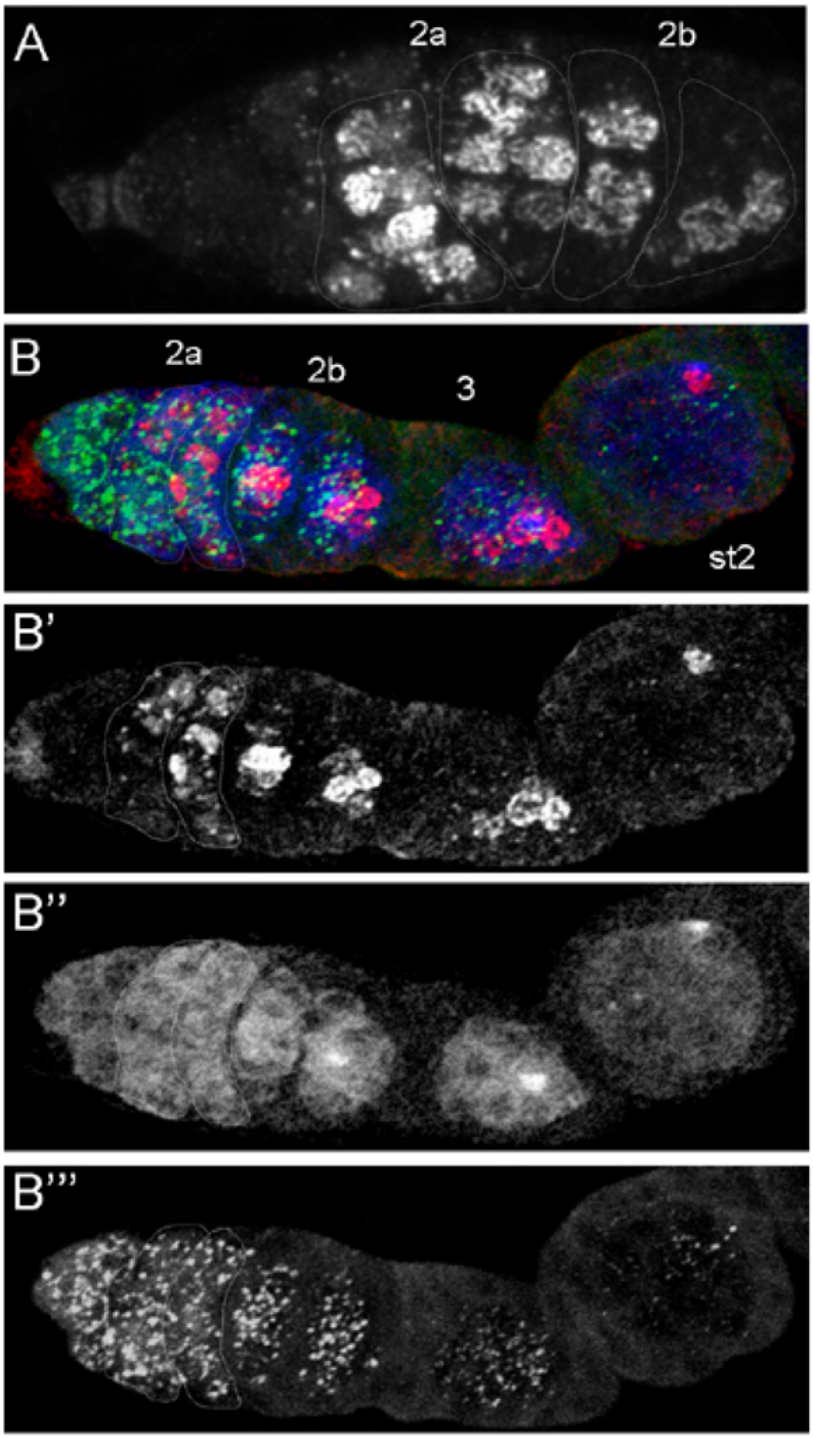

Figure 6

Polo overexpression restores BicD^{PA66} protein function during early oogenesis

A) and B) C(3)G is in red and BicD in green. A) BicD^{FA66} ovariole with no cell in meiosis from stage 2b of the germarium, and diffuse localization of BicD. B) BicD^{PA66} ovariole overexpressing Polo. Each follicle contains an oocyte in meiosis which accumulates BicD. C) DNA staining of later stage follicles in a BicD^{PA66} ovariole overexpressing Polo. Stage 4 and stage 6 follicles contain an oocyte marked by the presence of the condensed DNA (arrows). The follicle at stage 9 is degenerating. D) Model of a positive feedback loop between Polo and BicD proteins during meiosis restriction and oocyte differentiation. The Polo kinase is required to trigger meiosis and to activate BicD-dependent transport. In turn, BicD is required for the transport of oocyte determinants and for Polo localization.