polo, a mitotic mutant of *Drosophila* displaying abnormal spindle poles

CLAUDIO E. SUNKEL and DAVID M. GLOVER*

Cancer Research Campaign, Eukaryotic Molecular Genetics Research Group, Department of Biochemistry, Imperial College of Science and Technology, London SW7 2AZ, UK

*Author for correspondence

**Summary**

Neuroblast cells in larvae homozygous for mutant alleles of the locus *polo* show a high frequency of metaphases in which the chromosomes have a circular arrangement, and anaphase figures in which chromosomes appear to be randomly oriented with respect to at least one of the spindle poles. These defects appear to lead to the production of polyploid cells. Sex chromosome disjunction is affected in male meiosis, primarily in the second division, and the meiotic spindles of living cells are abnormal. One allele is a larval lethal, whereas another is semi-lethal with about 7% of homozygotes surviving as adults. Embryos from homozygous *polo* females have aberrant mitotic spindles that are highly branched and have broad poles. Immunofluorescence studies with an antibody that recognizes an antigen associated with the centrosome indicate that the organization of this organelle is disrupted in the mutant embryos.

Key words: *Drosophila*, mitotic mutant, spindle poles.

**Introduction**

Genetic approaches have been highly successful in analysing the cell cycle in yeasts, leading to a detailed pathway of the events of mitosis (see, e.g., Hartwell *et al.* 1974; Pringle & Hartwell, 1981; Beach *et al.* 1982; Nurse, 1985). However, aspects of the mechanics of mitosis differ in yeasts from those in the multicellular eukaryotes, and furthermore there are additional constraints upon cell division in higher organisms because of the need to maintain temporal and spatial control of cell proliferation.

*Drosophila* offers considerable advantages as an organism in which to study cell division. Work by Baker and his colleagues has shown that certain loci originally identified by meiotic mutants, and mutants showing increased mutagen sensitivity, encode products that function in mitosis (Baker *et al.* 1978; Baker & Smith, 1979). Additional mitotic mutants have been identified by their effects on chromosome integrity and fidelity of mitotic chromosome transmission (Baker *et al.* 1982; Smith *et al.* 1983). There are two major stages of *Drosophila* development at which one can expect to detect mutations affecting mitotic cell division. The first is during early embryogenesis, when there are 13 nuclear divisions at about 10-min intervals before individual nuclei undergo cellularization (Zalokar & Erk, 1976). During this time there is little or no zygotic gene expression, and so one might therefore expect a class of maternal effect lethal mutations that disrupt the functions of the genes encoding these components. There are then only three to four more cell divisions during embryogenesis and most of larval development involves cell growth, with the endoreduplication of DNA in the absence of mitosis. Imaginal cells, destined to form the adult organism and not themselves necessary for the survival of the larva, divide throughout larval development, as do cells of the nervous system. A second class of mitotic mutations can therefore be recognized in which the imaginal cells do not proliferate within the larva and death ensues during the late larval or early pupal stages. This late larval lethal phenotype of mutations in essential mitotic functions was first recognized in the analysis of repair-defective mutants (Baker *et al.* 1982).

We have begun to screen the embryos of flies having maternal effect mutations in order to identify aberrant mitoses during early embryogenesis. We expect that such mutations might fall into distinct categories, corresponding to: genes expressed only during
The mutation *gnu* appears to be an example of the former category. This mutation results in uncontrolled division in the imaginal and neuroblast cells of the larvae. The mutation *gnu* appears not to be affected (Freeman et al. 1986). This phenomenon also develops in unfertilized eggs, indicating that the gene plays a role in the correct establishment of zygotic development (Freeman & Glover, 1987). In this paper we describe a mutation, *polo*¹, the wild-type function of which is required both in the early embryo and in the diploid cells of the larva. Using immunofluorescence techniques we find that the mitotic spindles in polo embryos (from homozygous *polo*¹ females) are highly branched and have defective poles. Homozygous larvae also exhibit aberrant mitotic divisions in their brain cells and in addition we have observed abnormal meiosis in homozygous males. A stronger allele, *polo*², causes the death of homozygous larvae.

**Materials and methods**

**Genetic variants**

The mutation *polo*¹ was isolated by Nusslein-Volhard in a genetic screen designed to select sterile females. The chromosome used was *ru st e ca* mutagenized with ethylmethlysulphonate (EMS). *polo*¹ is a recessive mutation that maps to the left arm of chromosome 3 near the centromere at 3-46 on the basis of 112 recombinants between *Lyra* (3-40-5) and *Stubble* (58-2). The original *polo*¹ mutation is recessive and some 7% of expected homozygotes survive to eclose.

The allele, *polo*², was found among a collection of P-element-induced lethals isolated by R. Karess. All other stocks were described by Lindsley & Grell (1968). All stocks were grown at 25°C under standard culture conditions and media.

**Neuroblast preparations**

Cytological preparations were made from late third-instar larvae that had been grown at 25°C, collected and then washed in saline. They were dissected in 0.7% NaCl and the brain was transferred to a drop of 45% acetic acid for 15 s. The tissue was then placed for a further 15 s in 3% orcein dissolved in 45% acetic acid and then cleared of excess stain in 60% acetic acid. The brain was then placed in a small drop of 3% orcein in 60% acetic acid and squashed firmly. Preparations were observed under phase-contrast optics. Quantification of mitotic figures was carried out essentially as described by Gonzalez (1986). The unit used was the area of a brain-squash that can be seen under the microscope with a 63X Zeiss objective and 10X eyepieces. The number of metaphases and the number of anaphases per field was determined for at least 100 fields from 15 brains for *polo*¹/*polo*¹ or *polo*¹/+.

Preparations were also made by incubating dissected brains in 0.4% colchicine in 0.7% NaCl for 1 h before staining. After washing in saline the brains were incubated for 15 min in 30 mM-sodium citrate and then stained and squashed as before.

**Live testis squashes**

The pharate adults or recently emerged adult males were dissected in 0.7% NaCl on a siliconized slide. The whole testis was placed in a large drop of 0.7% NaCl and cut in half across the middle using dissecting needles. A small non-siliconized coverslip was placed on top and the whole preparation observed under phase-contrast with a 20X objective. The squash is performed by withdrawing excess liquid with blotting paper by capillarity. Live squashes were photographed within 10-20 min. Measurement of nuclear diameters for controls or *polo*¹ homozygotes was done by measuring at least 100 nuclear diameters from photographic prints of 10 different preparations.

**Fixation and staining of embryos**

Embryos were fixed and stained exactly as described by Freeman et al. (1986). Taxol was used to stabilize microtubules exactly as described in that paper and by Karr & Alberts (1986). We are well aware of potential artefacts that can arise from the use of taxol and were discussed at length in these two papers. In our hands the staining of wild-type embryos under these conditions gives a pattern of tubulin staining in agreement with the results of Karr & Alberts (1986).

**Antibodies**

The antibodies used in this study were the following: anti-centrosome antibody, Bx63 (Frash et al. 1987); anti-tubulin antibody, YL1/2 (Kilmartin et al. 1982); and anti-lamin antibody, T47 (Dequin et al. 1984). Rhodamine- and fluorescein-conjugated second antibodies were bought from Jackson Immunoresearch Laboratories Inc. USA.

**Results**

**The somatic phenotype**

We describe the phenotypes of two mutant alleles of a locus, *polo*, which maps close to the centromere at 46 on the third chromosome of *Drosophila melanogaster*. About 7% of flies homozygous for the original, EMS-induced allele eclose, and the eggs laid by homozygous females arrest early in development. We selected the mutation for further study since these embryos showed an abnormal distribution of nuclei, leading us to suspect a mitotic abnormality. The second, more extreme, allele (*polo*²) is a P-element-induced recessive larval lethal, which is, however, viable as a transheterozygote with *polo*¹.

We have studied the effect of *polo*¹ or *polo*² on chromosome integrity and segregation in the diploid neuroblasts of third-instar larvae. Our analysis of these cytological preparations shows that the frequency of
metaphases per field is lower in homozygous polo larvae than in wild-type larvae (see Materials and methods and legend to Fig. 1). The frequency of anaphases per field is also lower, but the relative proportion of metaphases to anaphases is roughly the same. About 70% of the metaphase figures show abnormalities (Fig. 1), which can be classified into three classes. Two classes show circular arrangements of the chromosomes. In one case there is a normal chromosomal complement (Fig. 1A). Homozygous polo larvae have about half of their mitotic figures in this configuration, compared to approximately 4% of the mitotic figures in wild-type larvae. In the second class, the nucleus is polyploid (Fig. 1B,C). Such polyploid figures are never seen in wild-type brains. In both circular configurations, the dot-like fourth chromosomes are seen in the centre of the mitotic figure with the major autosomes and sex chromosomes being arranged around them. We are not able to say categorically whether these figures are in metaphase or if they have proceeded to anaphase. We have, however, counted them as metaphases in our numerical analyses (see legend to Fig. 1). We do not observe circular mitotic figures, polyploid or otherwise, when the squashes are made in the presence of colchicine, indicating the requirement for functional microtubules for their formation (data not shown). The third class, which is much less frequent, shows aneuploid figures. Fig. 1D shows a cell that contains two extra chromosomes, a Y and a fourth. We have seen no other chromosomal defects; chromosomes are never fragmented and they appear to undergo normal condensation.

About 90% of anaphase figures seen in polo/polo larval brains are clearly abnormal. In a typical example, shown in Fig. 1E, the chromosomes attached to one of the spindle poles appear to be randomly oriented and are not all lying with their telomeres pointing towards the middle of the spindle. This arrangement is sometimes observed in wild-type larvae but at a very low frequency. The majority of wild-type anaphases resemble the one shown in Fig. 1F, in which the chromosome arms are all oriented towards the opposite spindle pole. Occasionally we have been able to observe anaphases that are trying to separate polyploid nuclei.

These cytological abnormalities are reflected in the viability of homozygous polo adults. The progeny that eclose do so on average 1-5 days later than their heterozygous siblings. The majority die either as late third instars or during pupariation. The polo allele shows a more extreme phenotype. None of the expected polo homozygotes survives, but they die as larvae during the early stages of the third instar. The cytological phenotypes of their larval neuroblasts are similar to polo homozygotes. The heterozygotes polo/polo do eclose, but with a slightly lower frequency compared to polo homozygotes. However, these trans-heterozygotes show very abnormal cuticle formation of the abdomen affecting both tergites and sternites.

The meiotic phenotype

We were interested to know whether or not the mutations also affected meiotic divisions and we chose to analyse this process in males homozygous for the original allele, which in contrast to males homozygous for polo are fertile. Not only the chromosomes, but also mitochondria, segregate to daughter nuclei upon the meiotic spindle in male germ cells. This association of the mitochondria with the meiotic spindle makes it possible to observe the spindle in living cells using phase-contrast optics. Meiotic spindles from wild-type and polo homozygous males are shown in Fig. 2. The wild-type cells undergoing meiotic division have very characteristic spindles with two clearly defined poles (Fig. 2A). However, the meiotic spindles found in polo/polo tests are generally more irregular in shape and structure. These range from almost normal to tetrapolar spindles (Fig. 2B) or even multipolar ones (Fig. 2C).

A consequence of these abnormal spindle structures would be irregular chromosome distribution amongst the resulting gametes. Perhaps the easiest stage at which abnormal chromosomal segregation can be analysed is within the cyst of spermatids referred to as the 'onion stage'. Any differences in the DNA content of the post-meiotic products found at the onion stage would be reflected by the nuclear diameters of these cells (Ripoll et al. 1985; Gonzalez, personal communication). In wild-type spermatids two round organelles are clearly visible at this stage (Fig. 3A). The clear organelle is the post-meiotic nucleus, the diameter of which is remarkably constant amongst different gametes, reflecting their equal DNA content (Fig. 3B). Our measurements of the diameters of these nuclei are in good agreement with those of Gonzalez et al. (1987) and reflect a haploid DNA content. The dark organelle, the Nebenkern, consists of mitochondria that have been distributed between daughter cells on the meiotic spindle, and which are associated with tubulin. This complex will later elongate to form the sperm tail. Before elongation starts this organelle is also very regular in size and shape in the wild type (Fig. 3A). In the post-meiotic products of polo/polo tests, most nuclei and associated Nebenkern appear abnormal (Fig. 3C). The histogram of nuclear diameters (Fig. 3D) indicates that there is a wide range of nuclear sizes. Gonzalez et al. (1987) have demonstrated that the nuclear diameter is a function of the DNA content. Comparison of the histogram in Fig. 3D with their standardized data indicates that the polo

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spermatids can contain from less than a quarter of the haploid DNA complement up to as much as a tetraploid complement. Furthermore, whilst in wild-type preparations there is always a single nucleus associated with one Nebenkern, there is no such correspondence in the mutant. The irregular size of the Nebenkern in polo* homozygotes indicates unequal partitioning of mitochondria into daughter cells on the meiotic spindles.

We have analysed male meiosis further by testing homozygote polo1 males for genetic non-disjunction of the sex chromosomes. This was carried out by crossing females carrying a Y chromosome and two X chromosomes sharing the same centromere (C(1)yc/Y and also...
referred to as $XX_yf/Y$ to males with marked $X$ and $Y$ chromosomes ($Xw/YB^*$). Meiosis comprises two divisions, during the first of which homologous chromosomes are segregated to the spindle poles, the sister chromatids not being split. In the second division, the sister chromatids are separated and the gametes receive a haploid chromosome complement. If therefore disjunction occurs correctly, the viable progeny expected from this cross would be $w$ males in which the $X$ chromosome is derived from the father and the $Y$ from the mother ($Xw/Y$), and $yf/B^*$ females, which have the attached $X$ of the mother and the marked $Y$ from the father ($XX_yf/YB^*$). Non-disjunction in the male parent during the first division results in gametes carrying both $Xw$ and $YB^*$ chromosomes (see the diagram of Table 1). Gametes carrying no sex chromosomes can result from non-disjunction in the first or second divisions. The other products of non-disjunction in the second division are gametes carrying two free $Xw$ chromosomes or two free $YB^*$ chromosomes (Table 1). Thus non-disjunction in the first mitotic division can be detected by the presence of $w B^*$ males ($Xw/YB^*/Y$), and in the second division by $w$ females ($Xw/Xw/Y$), whereas $yf$ females ($XX_yf/O$) are indicative of non-disjunction at either division.

In heterozygous $polo^{1+/+}$ males non-disjunction was observed at a frequency of about 0.07%, a level comparable to that in wild type (Sandler et al. 1968). Males homozygous for $polo$ produce two major classes of abnormal diplo-gametes (Table 1). The predominance of $XX$ gametes (17.7%) relative to $XY$ gametes (0.23%) suggests that most of the aberrant segregation is occurring in the second meiotic division. The low frequency of nullo (O) gametes within this sample is striking and contrasts with observations that have been made with most other mutants that affect meiosis in Drosophila (see review by Baker & Hall, 1976; and the Discussion). It could be explained by the selective loss of these gametes. An alternative explanation would be that directed non-disjunction is occurring, so that, in addition to having no sex chromosomes, these sperm are also either missing autosomes or have additional autosomes and so would produce inviable zygotes.

In order to test this latter idea, we have looked for the simultaneous non-disjunction of the sex chromosomes and the second chromosome by crossing $Xw/YB^*$ males to females having wild-type $X$ chromosomes and a marked pair of second chromosomes attached through a common centromere ($C(2)EN$). These females will give rise to either nullo-2 or attached diplo-2 gametes and so progeny will survive only if non-disjunction has occurred in the male in order that the eggs are fertilized by diplo-2 or nullo-2 sperm, respectively. As the second chromosomes of the male are unmarked, we cannot distinguish between non-disjunction in the first or second meiotic divisions. The cross does, however, allow one to determine whether or not non-disjunction of the second chromosome is occurring and, if so, whether or not it is independent of non-disjunction of the sex chromosomes. Simultaneous non-disjunction of the sex chromosomes in the males should give the same set of gametes as described above, and would be recognizable by $X/O$ male progeny (from nullo-gametes produced in either the first or second meiotic divisions); and $X/Xw/YB^*$ females (indicative of $XY$ gametes arising in the first meiotic division). $Xw/Xw$ gametes from the male would give triplo-$X$ zygotes, which are not considered because of their poor and irreproducible viability.

As expected, non-disjunction does not occur in heterozygous $polo^{1+}$ males, which consequently give no progeny when mated to $C(2)EN$ females (Table 2). The results with homozygous $polo^{1+}$ males indicate that non-disjunction of the second chromosome does occur (Table 2). In those cases in which second chromosome non-disjunction is independent of the sex chromosomes (to give gametes having only one sex chromosome and being either diplo-2 or nullo-2), one sees a higher recovery of diplo-2 gametes at the expense of nullo-2 gametes, indicating either their selective loss or their production at a lower frequency. Simultaneous non-disjunction of the second chromosome and the sex chromosomes occurs at a high frequency and in this cross is detected predominantly by nullo-$X$ gametes. In those cases exhibiting simultaneous non-disjunction of both the second and sex chromosomes, we recovered only one $Xw/YB^*$ gamete (0.3% of total gametes recovered) from the males, indicating as with the first
Fig. 2. Meiotic spindles in live cells during spermiogenesis. A,B. A cyst of wild-type cells undergoing the second meiotic division. C,D. A tetrapolar spindle in a homozygous polo<sup>1</sup> cell undergoing meiosis. E,F. A multipolar meiotic spindle in a homozygous polo<sup>1</sup> cell. Details of the preparation are given in Materials and methods. Bars; A, 10 μm; C,E, 10 μm.
cross that the frequency of sex chromosome non-disjunction in the first meiotic division is low. Of the nullo-X gametes indicating simultaneous non-disjunction, 95% are diplo-2 rather than nullo-2. These data could be explained if the defect in polo males directed the preferential (but not absolute) disjunction of non-homologous chromosomes. In such a case, nullo-X gametes would contain an extra complement of one of the autosomes, and so would not be detected in the first cross (Table I), whereas the second cross allows one specifically to recover gametes that are nullo-X and diplo-2. The dearth of nullo-X; nullo-2

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Table 1. Genetic non-disjunction test for the sex chromosomes in polo\(^{1}\)/polo\(^{1}\) and control males

<table>
<thead>
<tr>
<th>Gametes</th>
<th>polo(^{1})/polo(^{1})</th>
<th>polo(^{1})/+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal meiosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Y^w)</td>
<td>(Y^w)</td>
<td>(X^w)</td>
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</tbody>
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Non-disjunction Division I

<table>
<thead>
<tr>
<th>Gametes</th>
<th>polo(^{1})/polo(^{1})</th>
<th>polo(^{1})/+</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Y^w)</td>
<td>(Y^w)</td>
<td>(X^w)</td>
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</tbody>
</table>

Non-disjunction Division II

<table>
<thead>
<tr>
<th>Gametes</th>
<th>polo(^{1})/polo(^{1})</th>
<th>polo(^{1})/+</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Y^w)</td>
<td>(Y^w)</td>
<td>(X^w)</td>
</tr>
</tbody>
</table>

*These gametes were counted together.

\(\square\) nullo gametes cannot be differentiated and therefore are counted together. The numbers in parenthesis refer to percentages of total gametes recovered.

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gametes (which ought to be recoverable) could then be explained if the sex chromosome and second chromosomes were segregating into separate gametes. These possibilities are currently under investigation.

The maternal effect

The small proportion of homozygous polo\(^{1}\) females that survive to become adults lay a large number of eggs when mated with either Oregon R or polo\(^{1}\)/polo\(^{1}\) males. However, none of these 'polo' embryos hatch. Fig. 4 shows representative polo embryos that have been allowed to develop for increasing amounts of time before fixing and staining with the fluorescent dye, Hoechst 33258. DNA replication and nuclear multiplication seem to proceed in a disorganized way, resulting in polyploid nuclei. Some polyploid nuclei are already evident after about 30 min of development (Fig. 4A), and these increase in number between 1 and 2.5 h (Fig. 4B, C). The appearance of these mutant embryos contrasts with the wild-type embryo shown in Fig. 4F. This embryo, which is from roughly mid-way through this period (cell cycle 11), has regularly spaced nuclei of equal sizes. The wild-type embryo is syncytial until the 14th nuclear division cycle, at which point the individual nuclei become cellularized. The nuclei of polo embryos, on the other hand, never cellularize, and the nuclei continue both to increase in number and size.
X* marked with an asterisk were scored together. Because the progeny have low viability. Gametes of this class fathers gave no progeny as expected (see the text). The numbers polo'/polo* fathers. An analogous experiment with polo'/polo' with polo'/polo' of the text). The numbers in parenthesis refer to the percentages of total gametes recovered. NR refers to gametes that were not expected to be recovered because the progeny have low viability. Gametes of this class marked with an asterisk were scored together.

The genotypes of gametes are shown that result from a cross with polo/polo fathers gave no progeny as expected (see the text). The numbers in parenthesis refer to the percentages of total gametes recovered. NR refers to gametes that were not expected to be recovered because the progeny have low viability. Gametes of this class marked with an asterisk were scored together. 

To attain high levels of polyplody up to 6-9 h (Fig. 4D, E). Thereafter, there seems to be general deterioration of the embryo and breakdown of the nuclei.

A more detailed examination of polo embryos in the early stages of their development shows that their nuclei appear to be at comparable stages of the division cycle. Thus the majority of nuclei in any given embryo appear to have chromatin that is either undergoing condensation, or that appears in anaphase, or that is decondensing into telophase and subsequent inter-phase (data not shown). Furthermore, the nuclear envelope, as revealed by staining with an anti-lamin antibody, also seems to undergo synchronous changes in parallel with the chromatin condensation cycle as is observed of the nuclear lamin in wild-type embryos (Fuchs et al. 1983). This suggests that, although nuclei are polyploid and look very abnormal, they still undergo many of the normal cyclical aspects of mitosis.

The condensed chromatin in these early polo embryos was, however, highly disorganized and appeared as if it were not segregating correctly upon mitotic spindles. We therefore chose to examine the mitotic apparatus more closely by immuno-staining using antibodies that recognize tubulin or the centrosome. In the wild-type embryo, microtubules are nucleated by centrosomes to give asters during interphase. These split and migrate to the positions of the spindle poles during prometaphase. In metaphase, the microtubules of the spindle pole become attached to the condensed chromosomes at their kinetochores. The centrosome remains a 'focal point' for the spindle pole, and some astral microtubules still persist (see Karr & Alberts, 1986). An example of a field of prometaphase nuclei from a wild-type embryo is shown in Fig. 5G-1.

These nuclei are regularly spaced and contain condensed chromatin (G); the mitotic spindle has formed (H) and distinct centrosome staining can be seen with Bx63 antibody at the spindle poles (I). Examples of the microtubules of polo embryos are shown in panels B and E. In the first field, condensed chromatin (A) is associated with spindle-like microtubular structures that seem to be undergoing anaphase (B). Many of these spindle structures share one or both of their poles. Furthermore, many have very broad poles in contrast to wild-type spindles in which the polar microtubules converge on the dot-like centrosome. Surprisingly, there is no evidence of centrosomes associated with these highly branched structures and Bx63 staining is not apparent (C). At no stage of development were we able to observe asters of microtubules in the polo embryos in association with interphase-like nuclei, as in wild-type embryos (data not shown). Furthermore, there was no evidence of free asters in the cytoplasm as is observed with gnu embryos (Freeman et al. 1986) and with several other mitotic mutants (Leibowitz & Glover, unpublished results). In some embryos, however, we observed complex microtubular structures mainly associated with condensed chromatin, but resembling complex astral structures more than spindles (E). In this field, the Bx63 antibody illuminates scattered particulate matter in the cytoplasm (F). The absence of centrosome staining by Bx63 caused us to wonder whether the polo1 mutation might eliminate or cause a structural change to the antigen recognized by this antibody. We therefore carried out Western blotting experiments to look for this antigen within polo embryos. We were, however, unable to detect any difference in either molecular weight or abundance of the Bx63 antigen between wild-type or polo embryos (data not shown). We suspect therefore that centrosomal components are not organized into discrete structures in early polo embryos. When we examine later polo embryos using the Bx63 antibody, however, we are able to detect several well-defined punctate bodies that are associated with polyploid nuclei containing decondensed chromatin (Fig. 6), but never appear to be associated with microtubules.

**Discussion**

We have described a locus, _polo_, that is essential for mitosis in _Drosophila_, and have examined the phenotypes of _polo_ mutants in both larval and embryonic stages of development. A larva homozygous for _polo_ can survive through embryogenesis because its heterozygous mother has provided sufficient _polo* gene product for embryonic development. Most of the ensuing larval development involves cell growth, with the endoreduplication of DNA in the cells of many

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**Table 2. Genetic non-disjunction test for the sex and second chromosomes in polo*/polo' males**

<table>
<thead>
<tr>
<th>Sex chromosomes in male gametes</th>
<th>Second chromosomes in male gametes</th>
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<tbody>
<tr>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Cross: X/YY* ; 2/2; polo'/polo' × X/X ; C(2)EN, hec</td>
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</tr>
</tbody>
</table>

| X/* | 37 (14) | 74 (29) |
| YFP/YFP/YFP* | 45 (17) | 62 (24) |
| X*/YFP | 2 (0-7) | NR |
| O | 2 (0-7) | 39 (15) |

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tissues in the absence of mitosis. The diploid imaginal cells of the larva are not themselves necessary for its survival. In homozygous polo^2 larva, these cells are eventually blocked in their mitotic division, and so become unable to proliferate to form adult structures and the organism dies in the larval stage of development. In this respect, polo falls into a class of mitotic mutants that have a late larval lethal phenotype, first recognized in the analysis of repair-defective mutants (Baker et al. 1982). In the case of the polo^1 allele, however, a few homozygous animals survive to adulthood and the females can produce embryos, which begin to develop but show abnormal mitoses at a very early stage of embryogenesis. The embryo develops of the order of a hundred or so polyploid nuclei and never undergoes cellularization.

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Fig. 5. Double immunostaining of polo embryos with antibodies against tubulin and centrosomes. Embryos were incubated sequentially with Bx63 antibody (mouse anti-centrosome), rhodamine-conjugated anti-mouse IgG, YL1/2 (rat anti-tubulin), fluorescein-conjugated anti-rat IgG, and Hoechst (see Materials and methods). A–F. Stainings of two different polo¹/pol⁰ embryos; G–I, staining of a wild-type control. Bars: A, 10 μm, D, 5 μm, G, 10 μm.

In very early embryos, the nuclei are evidently undergoing cyclical changes to their nuclear membranes reflected by the patterns of lamin staining, and these appear to be synchronized with chromosome condensation, metaphase and anaphase. Whilst these cyclical aspects of mitosis are retained, at least for the early nuclear cycles, the morphology of the mitotic spindles is grossly abnormal. Virtually all the spindles are branched and multipolar and, rather than having tightly focused poles, many are broad and barrel-shaped. This could be explained by a change in the structure of the centrosome. Barrel-shaped spindles are found in plant cells, for example, which differ from most animal cells in lacking centrioles and a tightly localized spindle microtubule-nucleating centre. Another indication of defective centrosomes is the absence of asters associated with interphase nuclei in polo embryos, unlike wild-type embryos. In several other mitotic mutants, we have observed free centrosomes that are dissociated from nuclei or condensed.

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Fig. 6. Nuclear staining of a late polo embryo with Bx63 antibody. Polyploid nuclei from a late polo embryo from an 8-h collection, stained with Hoechst (A) and with the anti-centrosome antibody Bx63 (B). Bar, 5 μm.

chromatin. The most extreme example is gnu, in which there is no mitosis and yet the centrosomes replicate and nucleate asters of microtubules (Freeman et al. 1986). We can also see free asters dissociated from abnormal spindles that are found in other mutants, for example, lodestar, aurora and thule (Leibowitz & Glover, unpublished data). These mutant embryos contrast with polo embryos, in which we are unable to detect any free cytoplasmic asters.

Immunostaining of POLO embryos with the monoclonal antibody, Bx63, against an antigen associated with the centrosome indicates that POLO centrosomes are abnormal. In the wild-type embryo, the Bx63 antibody stains a large dot either at the centre of asters or at the poles of the spindle (Frasch, 1985; Frasch et al. 1986; Freeman et al. 1986). When we stain polo embryos with Bx63, we see either no distinct staining, or dispersed, very fine punctate staining with no obvious association with the spindle poles. Later in development, as the polo nuclei become obviously polyploid, the antigen appears to coalesce in large punctate aggregates apparently associated with the nuclear membranes of interphase nuclei. The Bx63 antigen is present throughout these stages, however, in levels comparable to wild-type embryos, as indicated by Western blotting experiments. Together these observations suggest that the polo mutation might affect a protein required for the correct structure and function of the centrosome, although such a protein need not necessarily be a centrosome component per se.

The abundance of tubulin within the larval brain hinders the immunocytological examination of spindles in neuroblasts, and so our observations have been made only upon stained chromosome preparations. The phenotype that we observe in larval neuroblasts could be explained by a lesion affecting the centrosome. The most common mitotic aberrations that we observe in these cells are polyploid circular mitotic figures, and anaphase figures in which the chromosomes progressing towards one of the poles are randomly oriented. Circular mitotic figures have also been observed in the mutant merry-go-round (mgr) (Gonzalez, 1986; Gonzalez et al. 1987), and the mutant lodestar (Leibowitz & Glover, unpublished data), both of which fully complement polo. They could arise if one of the spindle poles was defective with the consequent distortion of the mitotic plate as all the chromosomes are pulled to the single remaining pole. This would also explain the polyploidy that we frequently observe in these cells and in mitotic figures of this type. The abnormal anaphase structures could be a weaker manifestation of this phenotype in which one spindle pole behaves normally, and the other is only weakly affected. In the wild type, the spindle elongates during anaphase and the poles move further apart, suggesting that motive forces acting on the centrosome play an important role. One can speculate that the random orientation of the chromosomes around one of the spindle poles could be a consequence of inadequate transfer of motive force through a defective centrosome. We are unable to detect circular mitotic figures in brains from polo homozygotes treated with colchicine. Gonzalez et al.
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References


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