**Abstract.** We describe a genetic locus *rough deal* (*rod*) in *Drosophila melanogaster*, identified by mutations that interfere with the faithful transmission of chromosomes to daughter cells during mitosis. Five mutant alleles were isolated, each associated with a similar set of mitotic abnormalities in the dividing neuroblasts of homozygous mutant larvae: high frequencies of aneuploid cells and abnormal anaphase figures, in which chromatids may lag, form bridges, or completely fail to separate. Surviving homozygous adults are sterile, and show cuticular defects associated with cell death, i.e., roughened eyes, sparse abdominal bristles, and notched wing margins. The morphological process of spermatogenesis is largely unaffected and motile sperm are produced, but meiotic aneuploidy is common. The nature of the observed abnormalities in mitotic cells suggests that the reduced fidelity of chromosome transmission to the daughter cells is due to a failure in a mechanism involved in assuring the proper release of sister chromatids.

**Materials and Methods**

**Fly Stocks**

All stocks were maintained on standard *Drosophila* media of cornmeal, yeast extract, molasses, and agar. Gene and chromosome symbols are described in references 27 and 28, 28a, 28b. Unless otherwise indicated, the stocks employed were initially obtained from the Mid-America *Drosophila* Stock Center, Bowling Green, OH. All crosses were performed at 25°C.

The original mutant allele *rod* was identified in a collection of larval and pupal lethal mutations induced on chromosome-3, in a screen for mutants specifically affecting mitotic chromosome structure or behavior. The mutations in this collection were generated by subjecting wild-type flies carrying an isogenic *red e* chromosome-3 to the mutagenic effects of P-M hybrid dysgenesis (23). Such mutations usually are associated with the insert-
tion of a P transposable element. However, in the case of rod<sup>u4.8</sup>, no such association was found. The rod<sup>u4.8</sup> stock was repeatedly crossed to an M strain, until all autonomous P elements had been removed. A few defective P elements remain, and thus the rod<sup>u4.8</sup> stock should be considered an M' strain (23).

**Mapping of rough deal**

rod<sup>u4.8</sup> was recombined with the multiply marked chromosome-3 known as "rucuca" (27), which carries the recessive markers ru h ch st cu h st ca. A preliminary mapping placed rod distal to ca. Based on 28 recombination events between ca and rod among 582 chromosomes scored, rod was mapped to position 3-105.1, or 4.8 map units to the right of ca, on the standard genetic map (27).

A series of deficiency and duplication chromosomes were used to determine the cytogenetic location of rod. The terminal duplications Dp(3L)48 (100B7; telomer); and Dp(3;1)A (100B5,6; telomer), obtained from Dr. T. Strecker and described in references 9 and 24, completely rescue a homozygous rough deal by, as did Dp(Y;3)1L29 (100B,C; telomer) while the deletions Df(3R)ve8 (100A1,2; 100B7;1C) (reference 45), Df(3R)K-pn A (100D1;100E) (reference 3a), and FMT7;1(2,3); T(2,3), a synthetic deficiency for 100DE-telomer from L. S. B. Goodstein, all fail to uncover the rod<sup>u4.8</sup> mutation. Thus, we tentatively place the rough deal locus in the polytene bands 100C1,2-100D1,2.

**Isolation of Radiation-induced Alleles of rough deal**

Male flies, 1–5 d old, isogenic for the rucuca chromosome were irradiated with 4,000 rads from a 137Cs source, and crossed en masse to TM1, M(3)TM, Ser virgin females at 25°C (~50 males and 200 females per bottle). After 5 d the males were discarded, and the females transferred to fresh bottles. The heterozygous rucuca/TM1, Me or rucuca/TM3, Ser male offspring were mated individually to ca rod<sup>u4.8</sup>/TM3, Sb Ser females, in 10-cm culture tubes (indicates a potentially mutagenized chromosome). The offspring of each cross were screened for abnormal or absent rucuca genes. From tubes producing normal ca flies, balanced brothers of genotype rucuca<sup>+</sup>/TM3, Sb Ser were retetted, and used to establish a stock. Among ~800 chromosomes screened, we identified four alleles, designated rod<sup>x-1</sup> through rod<sup>x-4</sup>. None of the four alleles was associated with any rearrangement of the polytene chromosome map.

To reduce the possible effect of modifiers present in the stocks, the other chromosomes in flies bearing the radiation-induced alleles were replaced by standard Canton-S chromosomes. The rucuca rod chromosomes were recombined with a wild-type chromosome from Canton-S, selecting only for the retention of ca and rough deal. From each original mutant line, two or three ca rod sublines were maintained, and used for each of the studies reported here. As no differences were seen among the sublines of a given allele, the results have been pooled.

**Preparation of Mitotic Figures from Larval Brain**

All of the rod alleles were maintained over the balancer chromosome TM6b, Tb e ca (6) which carries the dominant larval marker Tubby (7b), and which thus enabled us to identify homozygous (rod/rod) larvae among their heterozygous siblings. The mitotic figures were examined in aceto-orcein squashes in third instar larval brains, by a modification of the procedure described (11). Homozygous larvae were selected, washed in water, and their brains dissected in a drop of isotonic saline. The tissue was fixed by transferring it to a drop of 45% acetic acid for 15 s, followed by 60% acetic acid for an additional 15 s. Finally the brains were stained in a drop of aceto-orcein (2% solution in 60% acetic acid) on a scrupulously clean siliconized coverslip, for 1–2 min. The coverslip was then picked up with a clean microscope slide and the tissue squashed with pressure applied from a thumb over the coverslip area.

For colchicine-treated mitoses, the brains were first incubated for 30–60 min in 10% M colchicine in saline, followed by hypotonic shock for 10 min in 1.5% sodium citrate. Finally the brain was fixed and stained as above. All cytological examinations used a Nikon microphot phase-contrast microscope and a Zeiss 63X plan apochromat phase-contrast objective.

**Examination of Spermatogenesis**

Male meiotic chromosomes were prepared from testes dissected from either late pupae or young adults, as described (25), fixed 30–60 s in 45% acetic acid, and stained 3–4 min with aceto-orcein (2% solution in 60% acetic acid). After transfer to a drop of 60% acetic acid on a siliconized coverslip, the testes were cut in half, and the contents allowed to ooze out. A drop of lacto-aceto-orcein was added, and the tissue picked up with a clean slide. Live testis squashes were prepared from young adult males, as described (16). Dissected testes were cut in half in a drop of saline on a siliconized coverslip. A clean slide picked up the specimen without squashing, and the excess liquid was slowly removed with a corner of absorbent paper, while the testis contents was being examined under the microscope. Once the cells were sufficiently flattened, the absorbent paper was removed.

**Quantitation of Mitotic Defects**

Brains were examined for all metaphases and anaphases, and any aberrations were noted. Mitotic index was determined as described (14), defining it as the number of cells with visible mitotic chromosomes per microscopic field. The field was based on a Nikon Microphot microscope, with the 63X Zeiss plan-apochromat phase-contrast objective, and 10× eyepieces. To minimize the variations among individuals, ~10–20 fields were sampled from each of 13 or more brains.

**Somatic Sector Analysis**

From a cross of y snl<sup>+</sup>v y snl<sup>+</sup>; ca rod<sup>u4.8</sup>/TM3, Ser × +/Y; ca rod<sup>u4.8</sup>/+ two classes of female offspring were examined: y snl<sup>+</sup>v<sup>+</sup>; ca rod<sup>u4.8</sup>/ca and y snl<sup>+</sup>v<sup>+</sup>; ca rod<sup>u4.8</sup>/+. The y snl<sup>+</sup>v<sup>+</sup>; +/- controls were generated by crossing the y snl<sup>+</sup>v y snl<sup>+</sup>v stock to standard wild-type (canton S) males. The flies were examined under a dissecting microscope at 50× for y sn bristles on their thoracies and abdominal tergites.

**Results**

**Identification and Mapping of rough deal**

The original allele, rod<sup>u4.8</sup>, was identified in a search among pupal lethal mutations for cytologically visible mitotic phenotypes, as described in Materials and Methods. Based on 28 recombination events between ca and rod among 582 chromosomes scored, rod was mapped to position 3-105.1, or 4.8 map units to the right of claret, near the telomer, on the standard genetic map (27, 28, 28a, 28b). Cytogenetic mapping of the locus placed it in the polytene interval 100CI.2-100DI.2 (see Materials and Methods). Four additional radiation-induced alleles, rod<sup>x-1</sup>–rod<sup>x-4</sup>, were subsequently isolated, and were mapped to the same cytogenetic interval. As no other mutations with an associated mitotic phenotype have been described in this region of the genome, rough deal mutations appear to define a new mitotic gene.

**Morphological Phenotype**

Four of the five mutant alleles are semilethal, the homozygotes dying as late pupae, containing fully developed pharate, but a significant fraction surviving to produce sterile, weak adults. The exceptional allele is rod<sup>x-1</sup>, homozygotes of which die uniformly as early pupae. The percentage of homozygotes surviving to adulthood at 25°C varies with the allele: rod<sup>x-1</sup>, 0%; rod<sup>x-4</sup>, 25%; rod<sup>x-3</sup>, 27%; rod<sup>x-2</sup>, 49%; and rod<sup>x-4</sup>, 73%. If they survive to adulthood, the flies possess numerous cuticular defects, the most characteristic of which are the small, rough eyes with irregularly formed ommatidia, and reduced numbers of abdominal bristles, usually in disarray. Other common cuticular defects include missing or thin thoracic bristles, and occasional notched wing margins. Such a constellation of cuticular aberrations is often found in genetically hyperploid flies (29), and is usually taken to reflect the occurrence of significant cell death.
Surviving females homozygous for any rod allele are completely sterile. They lay a few eggs, but these fail to develop, although some do apparently begin somewhat abnormal cleavage divisions (data not shown). More than 90% of the surviving mutant males are sterile. However, the remainder will sire a few (<20) offspring when presented with wild-type females.

The imaginal disks of third instar larvae homozygous for the semilethal alleles are normal in size and morphology, with the exception of the eye disks, which in some individuals may be very small. By contrast, rod<sup>X-1</sup> larvae consistently develop only small rudimentary imaginal disks. During larval development, homozygous individuals grow at the same rate as their heterozygous siblings, and the polytene tissues, such as salivary glands, are normal.

**Larval Neuroblasts of rough deal Mutants Contain Many Aneuploid Cells**

We observed mitotic figures in squashed, stained preparations of third instar larval brains, which, being largely imaginal structures (preparing to become the central nervous system of the adult fly), are full of dividing neuroblasts. Aneuploid cells are common among the dividing cells of homozygous rod individuals regardless of the allele examined. Between 5 and 40% of the identifiable mitotic figures in a given brain may be polyploid for one or more chromosomes. In contrast, polysomies in wild-type mitotic figures are rarely found (<0.1%; reference 10; this study, data not shown). (Hypoploid cells were not counted in these studies, since artifactual loss of a chromosome during the preparation of a squash is difficult to avoid. Moreover, the loss of an entire major autosomal arm is a cell-lethal event [40]). The polysomy cells are not intact multiples of diploidy (which might indicate a total failure of disjunction). Rather, they usually contain one or more extra chromosomes (Fig. 1). In Fig. 1, A–C, mitotic cells from a single colchicine-treated brain show three different states of ploidy: one diploid, one XYY, and one with five major autosomes and several fourth chromosomes. Fig. 1 E depicts a hyperploid cell (four X-chromosomes and five major autosomes) without colchicine treatment, in which the "pairing" of the homologs is still evident, regardless of their number. The polysomy can involve any of the four chromosomes.

**Anaphases Are Abnormal in rough deal Cells**

Cells caught in anaphase yielded more information about the nature of the defect in homozygous rod individuals. Abnormalities could be found in 14–42% of the rod anaphase figures, depending on the mutant allele, while in wild-type cells, abnormal anaphases were rare (3.4%). The most common abnormalities seen are lagging chromatids, anaphase bridges, and stretched chromatid arms. More complicated defects occur as well (Table I, and Figs. 2–4). Except where noted, all of the following observations apply to each of the mutant rod alleles.

Of the observed types of abnormal anaphases, those with lagging chromatids (Table I and Fig. 2) are the most common, representing about half of the total abnormal figures. A chromatid pair or a single chromatid lags behind on the spindle, while most of the chromosomes move synchronously to the poles. Among wild-type mitotic figures such asynchrony of chromosome movement is seen in <1% of all anaphases. Occasionally lagging chromatids in rod cells are found on the "wrong" side of the spindle equator, oriented towards the pole on the other side (e.g., Fig. 2, F and G). These may well result in two aneuploid daughter cells.

Anaphase bridges and stretched chromatid arms (Table I and Fig. 3) each represent ~20–25% of the abnormal figures. Within this category we are defining bridges as abnormal associations of chromatid arms appearing to be stressed by the force of the spindle. The bridges so defined are not the classical bridges seen when dicentric chromosomes enter mitosis (Fig. 3, B and C, and see also Fig. 4, E and G). The chromatid arms involved in the bridges can appear incomplete, as if they wereacentric (Fig. 3 E). But fibers can often be seen spanning the gaps between the segments and the bulk of chromosomes moving towards the poles (Fig. 3, C, D, and G). Since gaps (reflecting breaks or local chromatin decondensation) were not seen among the metaphase chromosomal of rod mutants, these are probably regions of chromatin that have been stretched, tethering the bridge to the centromere as it is pulled by the spindle to the poles (33, 34). Pairs of unlinked, stretched chromatid arms in rod anaphases (Fig. 3, G, H, and J) are also common (Table I). These excessively long and often gapped arms may result from the dissolution of a bridge. The disruption of an anaphase bridge by breakage of one chromatid arm (as a consequence of the spindle pulling, or by cytokinetic cleavage) should produce a centric and acentric fragment. At the next metaphase of the daughter cells, the centric fragment could produce a chromosome with a pair of equally shortened sister chromatid arms. Such broken chromosomes were sometimes found among rod metaphase figures (e.g., Fig. 1 F).

Other abnormal anaphase figures (Fig. 4 and Table I) include abnormalities not easily fitting a single category, and more rarely found defects. Anaphases in which unseparated chromatid pairs remain at the metaphase plate, while the other chromosomes migrate towards the poles (Fig. 4, A–D and F), represent 2–3% of all abnormalities. These stuck chromosomes presumably have properly attached to the spindle since they were able to congress to the spindle equator. Severely unbalanced anaphase figures, about to generate two aneuploid daughter cells, are also observed (Fig. 4, H and J). In Fig. 4 E an anaphase bridge has formed between a chromatid arm and an unseparated chromatid pair. Fig. 4 G shows a bridge between two unseparated chromatid pairs. Note that the bridge junction is near, but not at, the telo-

**Chromosome Instability Occurs in Most if Not All Dividing Tissues**

To determine whether homozygous rod flies displayed any manifestations of chromosome instability in the imaginal cells generating adult cuticle, we used somatic clone analysis, a method first applied by Baker and colleagues to the analysis of mitotic mutants (2). If a fly is heterozygous for recessive cell-autonomous markers of the adult cuticle, it is possible to detect certain abnormal mitotic events by examining the generation of somatic clones displaying those recessive phenotypes. The presence of a spot of cuticle bearing recessive markers on an otherwise wild-type cuticle means
Figure 1. Metaphase figures from wild-type and rough deal larval neuroblasts, treated with colchicine (A-C, and F) or untreated (D, E). (A-C) Three different cells from the same colchicine-treated larval brain homozygous for rod^x-1^ showing three different ploidies. (A) A euploid cell, with an X, a Y, two pairs of major metacentric autosomes (chromosomes 2 and 3 are of similar size and cannot easily be distinguished), and the pair of tiny fourth chromosomes. (B) A cell with two Y chromosomes. (C) A cell containing five major autosomes and at least seven fourth chromosomes. (D) A euploid cell from a wild-type larval brain. (E) Mitotic figure from rod^a4.s^ homozygous female larva, with four X chromosomes and five major autosomes. (F) A colchicine-treated mitotic figure from rod^H4.8^ homozygote, with six intact metacentric chromosomes, and a broken metacentric (arrow), missing two sister chromatid arms. Bar, 2 μm.

Table I. Quantitative Effect on Mitosis of rough deal Alleles

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Anaphases scored</th>
<th>Abnormal anaphases*</th>
<th>Lagging chromatids (percent)†</th>
<th>Stretched chromatid arms (percent)†</th>
<th>Anaphase bridges (percent)†</th>
<th>Other (percent)†</th>
<th>Mitotic index‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canton-S</td>
<td>1,130 (13)</td>
<td>38 (3.4)</td>
<td>9 (24)</td>
<td>7 (18)</td>
<td>9 (24)</td>
<td>13 (34)</td>
<td>9.9 ± 1.9 (20)</td>
</tr>
<tr>
<td>rod^H4.8^</td>
<td>840 (7)</td>
<td>170 (20.2)</td>
<td>80 (47)</td>
<td>31 (18)</td>
<td>38 (22)</td>
<td>21 (12)</td>
<td>7.4 ± 3.4 (19)</td>
</tr>
<tr>
<td>rod^x-1^</td>
<td>574 (11)</td>
<td>244 (42.5)</td>
<td>81 (33)</td>
<td>50 (20)</td>
<td>56 (23)</td>
<td>57 (23)</td>
<td>8.8 ± 1.7 (21)</td>
</tr>
<tr>
<td>rod^x-2^</td>
<td>736 (12)</td>
<td>152 (20.7)</td>
<td>73 (48)</td>
<td>29 (19)</td>
<td>34 (22)</td>
<td>16 (10)</td>
<td>10.0 ± 2.9 (19)</td>
</tr>
<tr>
<td>rod^x-3^</td>
<td>827 (12)</td>
<td>124 (15.0)</td>
<td>67 (55)</td>
<td>25 (20)</td>
<td>29 (23)</td>
<td>3 (2)</td>
<td>9.9 ± 3.0 (13)</td>
</tr>
<tr>
<td>rod^x-4^</td>
<td>2,221 (24)</td>
<td>308 (13.9)</td>
<td>147 (48)</td>
<td>78 (25)</td>
<td>74 (24)</td>
<td>9 (3)</td>
<td>11.4 ± 1.7 (19)</td>
</tr>
</tbody>
</table>

* Percent of all anaphases showing any abnormality.
† Percent of abnormal anaphases with the indicated defect.
‡ As determined by Gonzalez et al. (14).
§ Number of brains examined to score anaphases is shown in parentheses.
¶ Number of brains examined to determine the mitotic index is shown in parentheses. 10 or more fields were sampled per brain. See Materials and Methods.
that the dominant (wild-type) alleles have been lost from those cells displaying the recessive phenotype. Such spots can be the result of mitotic instability (chromosome breakage or loss, nondisjunction, mitotic crossing over, or mutation). Table II shows results of such a study of rod [mu], using the recessive cuticular markers yellow and singed (which affect the color and morphology, respectively, of the cuticular bristles). Surviving adult flies of the genotype y sn/++; rod/rod produce significantly more y sn spots than do flies heterozygous for rod or homozygous for the wild-type allele. Every rod fly contained at least one, and usually more than one somatic spot, on the thorax or abdominal tergites. (A small elevation in the number of somatic spots is apparent in flies heterozygous for rod [mu], relative to wild type. This slight dominance of rod [mu] may indicate a haplo-insufficiency for this locus, that is, a single wild-type dose of rod is insufficient to maintain wild-type levels of mitotic fidelity. However, since no small deletions of rod are available, this possibility could not be pursued.)

Thus, in homozygous rod flies, mitotic instability is evident in the cells destined to become the thoracic and abdominal cuticle. This combined with the cytological evidence from larval neuroblasts and testes (see below) makes it likely that all types of normally dividing cells of homozygous rough deal flies suffer from high levels of chromosome disjunctional failure.

**Some Aspects of Male Meiosis Are Affected by Mutations in rod**

A number of mutations affecting the process of meiosis also affect the behavior of mitotic chromosomes (2). We wished...
to ascertain whether rod+ was necessary for male meiosis. Adult males homozygous for the semilethal rod alleles are sterile when mated with wild-type females, although motile sperm can be seen when the testes are dissected in saline. In live, saline squashes of wild-type testes, cysts composed of 64 immature spermatids are found. These are the descendants of a single primary spermatocyte, after four mitotic and two meiotic divisions. At the so-called onion stage, the immature spermatids are seen as pairs of black and white circles, the mitochondrial derivatives and the haploid nuclei, respectively. The mitochondrial derivative subsequently elongates into the sperm tail, and the nucleus compacts and elongates to become the sperm head (16, 22). While in a preparation from wild-type testes, both nuclei and mitochondrial derivatives are each uniformly sized (e.g., Fig. 5 A), several mutations known to affect spindle function (14, 22, 46) produce heterogeneously sized nuclei and mitochondrial derivatives within a single cyst. The diameters of the nuclei have been shown to reflect the number of major chromosomes within (14). This supports the argument that both chromosome and mitochondrion are sequestered into daughter spermatids by spindle-dependent functions.

The testes of homozygous rod males are always small, and contain correspondingly fewer cycling cysts and mature sperm bundles, although cysts at each stage of spermatogenesis can be found. In every cyst of onion stage immature spermatids from rod testes, nuclei of varying diameters are seen (Fig. 5 B), while the mitochondrial derivatives are unaffected. This suggests that at least those microtubule-based functions required for cytokinesis and sperm shaping are still normal in rod flies, yet aneuploid spermatids are generated. But it does not address the origin of the spermatid aneuploidy, which could be a consequence of either meiotic or premeiotic disjunctional failure. For this reason we examined stained chromosome squashes of rod meiocyte cysts.

In favorable preparations where the number of chromosomes can be determined, aneuploid nuclei are evident among both pre- and postmeiosis I spermatocytes from homozygous rod adult testes (e.g., Fig. 6, A and B). For example, in one cyst from a rodX4 testis, 9 of 15 postmeiosis I cells were visibly aneuploid. However, we were unable to obtain interpretable squashes of the first meiotic anaphase, and therefore we cannot say that the observed aneuploidies are ever a consequence of meiosis I disjunctional failure, in addition to mitotic nondisjunction. Nevertheless, irregularities in chromosome behavior during the second meiotic division are often found. Fig. 6, C and D show two apparently euploid cells from a single anaphase II cyst of rodX3 spermatocytes, in which one cell appears to be improperly segregating its chromatids, while another cell in the same cyst is
dividing properly. Fig. 6 E shows an abnormal anaphase II from a rod<sup>HI.8</sup> cyst, closely resembling the kinds described earlier in mitotic cells. Abnormal meiosis II anaphases are found in all four semilethal alleles of rough deal. At least one clearly abnormal anaphase could be found in every meiosis II cyst examined (13 total). Thus, at least in the second meiotic division, mutations in rough deal can interfere with normal chromosome behavior.

Table II. Generation of Somatic Sectors by rod<sup>HI.8</sup>

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Flies with y sn clones*</th>
<th>Total number of clones*</th>
</tr>
</thead>
<tbody>
<tr>
<td>y sn&lt;sup&gt;1&lt;/sup&gt;/ +; +/+</td>
<td>1/28</td>
<td>1</td>
</tr>
<tr>
<td>y sn&lt;sup&gt;1&lt;/sup&gt;/ +; rod&lt;sup&gt;HI.8&lt;/sup&gt;/rod&lt;sup&gt;HI.8&lt;/sup&gt;</td>
<td>15/15</td>
<td>61</td>
</tr>
<tr>
<td>y sn&lt;sup&gt;1&lt;/sup&gt;/ +; rod&lt;sup&gt;HI.8&lt;/sup&gt;/ +</td>
<td>11/56</td>
<td>13</td>
</tr>
</tbody>
</table>

* Thorax and tergites only.

Quantitative Comparison of rough deal Alleles

The mitotic phenotypes associated with the five mutant alleles of rough deal are qualitatively indistinguishable. High frequencies of aneuploidy are observed in larval brain and the whole range of abnormal anaphases are found, regardless of the allele. Since an aneuploid cell can produce aneuploid daughters at each division, the number of such cells does not reflect the frequency of abnormal events. We therefore used the observed frequency of abnormal anaphases as a quantitative measure of the rough deal mitotic phenotype (Table I). The percentage of abnormal anaphases ranged from 13.9% for rod<sup>X-4</sup> to 42.5% in rod<sup>X-1</sup>. The fraction of lagging chromatids among the abnormal anaphases is similar for all alleles, except rod<sup>X-1</sup>. The relative reduction of laggards in rod<sup>X-1</sup> corresponds to an increase in more complicated figures, involving more than one chromosome. The five alleles can thus be crudely ordered into an allelic series, with X-1...
the most severe and X-4 the least. This ordering correlates with the observed relative viability of the homozygotes. Therefore cumulative damage caused by mitotic failure appears the most likely cause of death. All heteroallelic combinations were constructed, but no interallelic complementation generating wild-type cuticular morphology was observed. Regardless of the allele, or heteroallelic combination, each surviving fly clearly had the rough deal phenotype of roughened eyes and poorly formed tergites.

A parameter we call “mitotic index” (the average number of cells with condensed chromosomes observed per field of view) was not greatly affected by the choice of mutant allele, averaging around 10 dividing cells per field (Table I), in good agreement with the wild-type determinations of others (14, 46). This supports the argument that the lesion in rod is not associated with arrested or retarded progression through the mitotic cycle (10).

Discussion

We have described the mitotic phenotype of mutations in a Drosophila gene called rough deal. The larval neuroblasts of individuals homozygous for any of the mutant alleles of rod display high frequencies of aneuploidy and abnormal

Figure 5. Saline squash of live, immature spermatids from wild-type (A) and rod^{lo1} (B) testes. The nuclei are seen as white circles and the mitochondrial derivative as black circles. The uniformity of the mitochondrial derivatives is retained in the rod testis, but the size of the nuclei is variable (arrows). Bar, 10 μm.

Figure 6. Euploid and aneuploid meiotic anaphase II figures from rod testes. (A, B) Three aneuploid spermatocytes from a single rod^{X2} testis. In A, two hypoploid cells are undergoing a “normal” anaphase II. In B, a hypoploid cell shows unequal distribution of chromatids. The event initially producing these three aneuploid cells could have occurred either premeiotically or in meiosis I. (C and D) Two anaphases from the same cyst of a rod^{X3} testis, showing correct and incorrect, respectively, disjunction of a euploid chromosome set. (E) Lagging chromatids from a rod^{X1} testis. Bar, 5 μm.
anaphases. Cells containing extra chromosomes represent from 5 to 40% of all the scoreable mitotic cells within a larval brain, and can be polysemic for any of the four chromosomes. However, the polysemic in a given cell is generally limited to only a few chromosomes; polysemic cells containing multiples of the haploid complement are not found. Abnormal anaphases occur from 14 to 40% of the time, depending on the allele. Lagging chromatids are the most common abnormalities seen, followed by anaphase bridges and stretched chromatid arms. The anaphase defects most often involved only a single chromatid or pair of chromatids in a cell, even in the most extreme allele rod\(^{x-1}\). Chromosome instability, detected by the generation of cuticular clones bearing recessive markers, occurs in dividing cells other than neuroblasts, such as imaginal disk cells and abdominal histoblasts. Male meiosis is also affected by mutations in rough deal, as chromosomes in the second meiotic anaphase display abnormal behavior similar to that seen in mitotic cells. However, other aspects of spermatogenesis proceed normally, and motile (though presumably aneu- plloid) sperm are produced. In summary, the observed aneu- ploidy in rod mutants appears to be a consequence of a failure of sister chromatids to migrate faithfully to the poles, and the reduced fidelity of chromosomal disjunction is a general property of all dividing cells. The phenotypes of poorly formed adult cuticle, mitotic aneuplidy, and abnormal meiotic and mitotic anaphases are inseparable by recombination, and are associated with each of the four subvital mutant alleles examined. Because the mitotic phenotypes are locus specific, and not allele specific, it is most likely that the mutations are all lesions at a single genetic locus, and the rough deal is specifying an essential mitotic function.

Nevertheless, while abnormal anaphases are common in rod mutants, the majority of anaphases, and the majority of chromosomes within an affected cell, behave normally. If \( \text{rod}^+ \) is producing an essential mitotic function, one might expect a null mutation to cause all anaphases to fail. That even \( \text{rod}^{x-1} \), the only completely lethal allele in our collection, still allows many normal anaphases to proceed indicates that some residual \( \text{rod} \)-like function is being provided by the cell. It is possible that none of our exant alleles are null muta- tions, or that the \( \text{rod} \) function is partially redundant with that of another gene function, or finally that the mutant phenotype is tempered to some extent by the lingering pres- ence of wild-type gene product from the maternally derived cytoplasm of the egg, a phenomenon known as perdurance. We cannot yet distinguish among these possibilities. The absence of viable heterozygous deletions for the chromosomal region 100C, in which \( \text{rod} \) resides, precludes our testing for residual activity in the \( \text{rod} \) mutants.

Several characteristics of other Drosophila mitotic mu- tants (1, 10, 12, 41) such as elevated mitotic index, unipolar or multipolar spindles, polysemic cells containing multiples of the diploid set, highly condensed metaphase figures (resembling the morphology of cells treated with colchicine), are not found among the \( \text{rod} \) larval neuroblasts. This suggests that \( \text{rod} \) mutants do not suffer from spindle dysfunction, but rather from a defect in a chromosome-dependent process. More recently we have examined \( \text{rod} \) mitotic spin- dles by immunofluorescent staining with anti-tubulin ant- isera, and find that they are not distinguishable from wild type (data not shown).

A striking feature of rough deal anaphases is that the chromo- somes in rough deal cells are not uniformly affected even within a single cell; they behave independently with regard to their failure to migrate synchronously to the poles. Per- haps the affected chromatids are only inefficiently executing some process that normally occurs synchronously during anaphase. The lagging chromatids, the anaphase bridges, and the stuck chromosomes of rough deal mutants can be thought of as part of a continuum of defects with a common origin, namely, an inability of chromatids to fully separate in response to the signal triggering anaphase. Complete fail- ure of a chromosome to disjoin would result in a daughter cell trisomic for that chromosome, and this is the probable origin of the observed polyplody in rod tissues. We will con- sider two mechanisms for generating the anaphase pheno- type, a weakened traction force pulling those chromatids to the poles, and an inefficient release of sister chromatids at the onset of anaphase.

Some current models of anaphase view the kinetochore as the site of the anaphase motor (15, 32). A reduction in the number of microtubules bound to the kinetochore, or a reduction in the strength with which the spindle fibers pull an individual chromosome could cause chromosome lagging during anaphase. Nicklas (34, 36), using micromanipulative techniques on normal grasshopper spermatocytes, has estimated that wild-type microtubules are capable of generating a traction force 10\(^4\)-fold greater than what is normally necessary to pull a chromosome to the pole. Thus any reduc- tion in force needed to explain the laggards in rough deal cells would have to be of a similar magnitude. However, it is apparent from Fig. 2 that forces are still pulling the lagging chromosomes in rod as evidenced by the V shape conformation of the lagging chromatid arms. Moreover, failure to mi- grate to the spindle equator during prometaphase, a process that also depends on proper functioning of microtubules and kinetochore, is not observed in rod mutants. Finally, it is not clear how a weakened traction force, or impaired spindle function in general, would produce anaphase bridges as well as laggards.

Mitotic and meiotic sister chromatids and homologues re- main closely apposed up to the end of metaphase, not just at the centromere, but along their entire length. Little is known about the nature of this affinity. While the release of sister chromatids along the arms and at the centromere actu- ally defines the start of mitotic anaphase, sister chromatid re- lease is not a spindle-dependent process. Even acentric sister chromatid fragments remain paired during prometaphase, and are released in synchrony with the intact chromatids at the onset of anaphase (4). Recently a protein has been de- scribed (INCENP) whose presence at the points of sister chromatid adhesion in metaphase chromosomes and subse- quent loss at anaphase implicates it in this process (5). Muta- tions in \( \text{rod} \) might be causing the sporadic failure of sister chromatid release by interfering with the functioning or dis- solution of the natural chromatid glue.

Tardy release of sister chromatids, due to a prolonged or inappropriate “stickiness” of chromatids for each other, could account for the observed phenotypes. If the adhesion is relieved belatedly after the onset of anaphase, one would expect the affected chromatid pair to lag. If it persists along one chromatid arm, this should lead to a bridge or stretched arms. Complete failure to alleviate the adhesion could pro-
duce unseparated chromosomes balanced on the anaphase spindle equator of the type depicted in Fig. 4, A–D.

Lagging chromatids are among the aberrations found when cultured cells recover from transient exposure to mitotic poisons such as colchicine (20). It has been proposed that simple prolongation of the mitotic cycle will reduce the fidelity of chromosome transmission (17, 20), and that the chromosomal proteins sustain structural damage by prolonged exposure to the cytoplasmic environment during colchicine arrest (20). The damage to kinetochore or inner centromere would then be responsible for the observed abnormal anaphases. There is no evidence for prolongation of mitosis in rod mutants, since the mitotic index is essentially normal (Table II), but the mutant rod function itself may be responsible for damage and dysfunction of kinetochore or centromere. Some types of inappropriate chromosome stickiness may be due to the tangling of chromatin fibers during chromosome condensation (31). This suggestion is supported by the observation that topoisomerase II is important both for the structure (8) and proper behavior (7, 19, 48) of mitotic chromosomes. By reducing the ability of topoisomerase-like functions to untangle the intertwined chromatin fibers of individual chromatids, rough deal mutants might thus effect the observed mitotic abnormalities (Drosophila topoisomerase II itself has been cloned, and maps on a different chromosome from rod (37)).

Whatever the cause of the retarded separation of chromatids in rod cells, the fact that delayed separation of occasional chromatid pairs does occur argues that a chromosome-associated mechanism is actively proceeding, albeit inefficiently, even after the rest of the chromosomes have received the signal to separate and begin anaphase. Thus if tangling of chromatin fibers is the cause, then it is tangling which can resolve itself during the time the chromosomes are migrating in anaphase. If the timely release of the natural sister chromatid glue is impaired, then it still must eventually be dissolved fully to generate the laggards.

Three other mutations have been identified in Drosophila that appear to suffer sporadic nondisjunctional failure. The pupal lethal (l)2zw0 causes a high frequency of mitotic nondisjunction, not unlike that found in rod mutants. However, genetic and cytological studies suggest that aneuploids in (l)2zw0 are generated by premature separation of sister chromatids and subsequent random disjunction at anaphase (2, 44). Similarly, mutations at ord and mei-5332 have been interpreted as defects in processes necessary for maintaining appropriate sister chromatid adhesion (13, 26) during the first and second meiotic divisions in spermatocytes, while in mitotic cells elevated levels of chromosome instability are observed by somatic sector analysis (2). Even though these mutants appear to suffer from a problem the opposite of that seen in rough deal, it is possible that their wild-type products and rod* all contribute to a mechanism involved in assuring the fidelity chromatid disjunction during anaphase.

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References


