rough deal: A Gene Required for Proper Mitotic Segregation in Drosophila

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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 meiocyte 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.

THE faithful execution of mitosis is essential to proper organismal development. This complex process has evolved to assure that a complete euploid complement of chromosomes will be delivered to each daughter cell. While much has been learned about the role of the mitotic spindle apparatus, and its biochemical components (15, 30, 32), until recently, little attention has been given to the specific role of chromosomes in mitosis (reviewed in 35). The chromosomes, once thought to be passive vehicles on the spindle rails, are now believed to actively participate in the mitotic process. Among the required chromosome-associated functions are: accurate, tangle-free condensation from chromatin fibers, capture and stabilization of the spindle microtubules radiating from the poles, faithful separation of sister chromatids in response to the signal triggering the onset of anaphase, and migration along the spindle fibers towards the poles.

Genetic dissection of mitosis by mutational analysis in the yeasts has elucidated the roles of known components of the mitotic apparatus (3, 19, 21, 48) and has identified new ones (see, for example, 18, 38, 47). In *Drosophila*, mutations in essential mitotic functions have recently been described (10, 14, 42, 46; and reviewed in 1, 12, 41). *Drosophila* offers a unique way to identify mitotic mutations, based on a fundamental developmental distinction made during embryogenesis between the body cells of the developing larva and the imaginal cells. Larval body cells are for the most part polyploid or polytene, that is, the chromatin replicates but the cells do not divide. Since most larval cells are generated during rapid cleavage cycles in the first few hours of embryogenesis, during which time the mitotic machinery is maternally derived, there is relatively little requirement for zygotically encoded mitotic functions in larvae (1, 41). Only in the imaginal tissues, in which the cells normally remain diploid and divide more or less logarithmically throughout the larval period (39), would defects in essential mitotic functions be manifest, and then only when the imaginal disks are needed for the viability of the organism (43) during metamorphosis. Thus, a selection for mutants that live as larvae but die as pupae greatly enriches for mutations in genes encoding mitotic functions (10).

We have conducted such a screen and report here on the isolation and characterization of several mutant alleles of a gene called *rough deal* (*rod*). The predominant mitotic phenotype of *rod* mutants is aneuploidy, with many normally diploid cells becoming polysomic for one or more chromosomes. During anaphase, individual chromosomes frequently fail to move properly to the poles. The evidence suggests that the reduced fidelity of chromosome transmission is due to a failure in a mechanism involved in assuring the proper release of sister chromatids in response to the anaphase trigger.

Materials and Methods

Fly Stocks

tion of a P transposable element. However, in the case of $rod^{H4.8}$, no such association was found. The $rod^{H4.8}$ 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 $rod^{H4.8}$ stock should be considered an M' strain (23).

Mapping of rough deal

$rod^{H4.8}$ was recombined with the multiply marked chromosome-3 known as "rucuca" (27), which carries the recessive markers ru h th st cu sr e 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(3;1)48 (100B7,8; telomere), and Dp (3;1)1A (100B5,6; telomere), obtained from Dr. T. Strecker and described in references 9 and 24, completely rescue a homozygous rough deal fly, as did Dp(Y;3)L129 (100B,C; telomere) while the deletions Df(3R)tll^E (100A1,2; 100B7,C1) (reference 45), Df(3R)K-pn A (100D1;100E) (reference 3a), and FM7;T(1;2); T(2;3), a synthetic deficiency for 100DE-telomere from L. S. B. Goldstein, all fail to uncover the $rod^{H4.8}$ mutation. Thus we tentatively place the rough deal locus in 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 ^{137}Cs source, and crossed en masse to TM1, Me/TM3, 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$^{H4.8}$/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*/ca rod$^{H4.8}$ flies (homozygous for ca). From tubes producing no normal ca flies, balanced brothers of genotype rucuca*/TM3, Sb Ser were retested, and used to establish a stock. Among ∼8,000 chromosomes screened, we identified four alleles, designated rod^{X-1} through rod^{X-4}. 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 (Tb), 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^{-5} M colchicine in saline, followed by hypotonic shock for 10 min in 1% 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 lactoaceto-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 63× 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 sn^3 v /y sn^{3}v; ca rod$^{H4.8}$ /TM3, Ser × +/Y; ca rod$^{H4.8}$ /+ two classes of female offspring were examined: y sn^3 v/+; ca rod$^{H4.8}$/ca rod$^{H4.8}$ and y sn^{3}v/+; ca rod$^{H8.4}$/+. The y sn^3 v/+; +/+ controls were generated by crossing the y sn^3 v/y sn^3 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, $rod^{H4.8}$, 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 telomere, on the standard genetic map (27, 28, 28a, 28b). Cytogenetic mapping of the locus placed it in the polytene interval 100C1,2-100D1,2 (see Materials and Methods). Four additional radiation-induced alleles, rod^{X-1}-rod^{X-4}, 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 pharates, but a significant fraction surviving to produce sterile, weak adults. The exceptional allele is rod^{X-1}, homozygotes of which die uniformly as early pupae. The percentage of homozygotes surviving to adulthood at 25°C varies with the allele: rod^{X-1}, 0%; $rod^{H4.8}$, 25%; rod^{X-2}, 27%; rod^{X-3}, 49%; and rod^{X-4}, 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^{X-1} 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 polysomic 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 autosome arm is a cell-lethal event [40]). The polysomic 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 catagory 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 were acentric (Fig. 3 *E*). But fibers can often be seen spanning the gaps between the segments and the bulk of chromsomes moving towards the poles (Fig. 3, *C*, *D*, and *G*). Since gaps (reflecting breaks or local chromatin decondensation) were not seen among the metaphase chromosomes 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 telomeres.

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
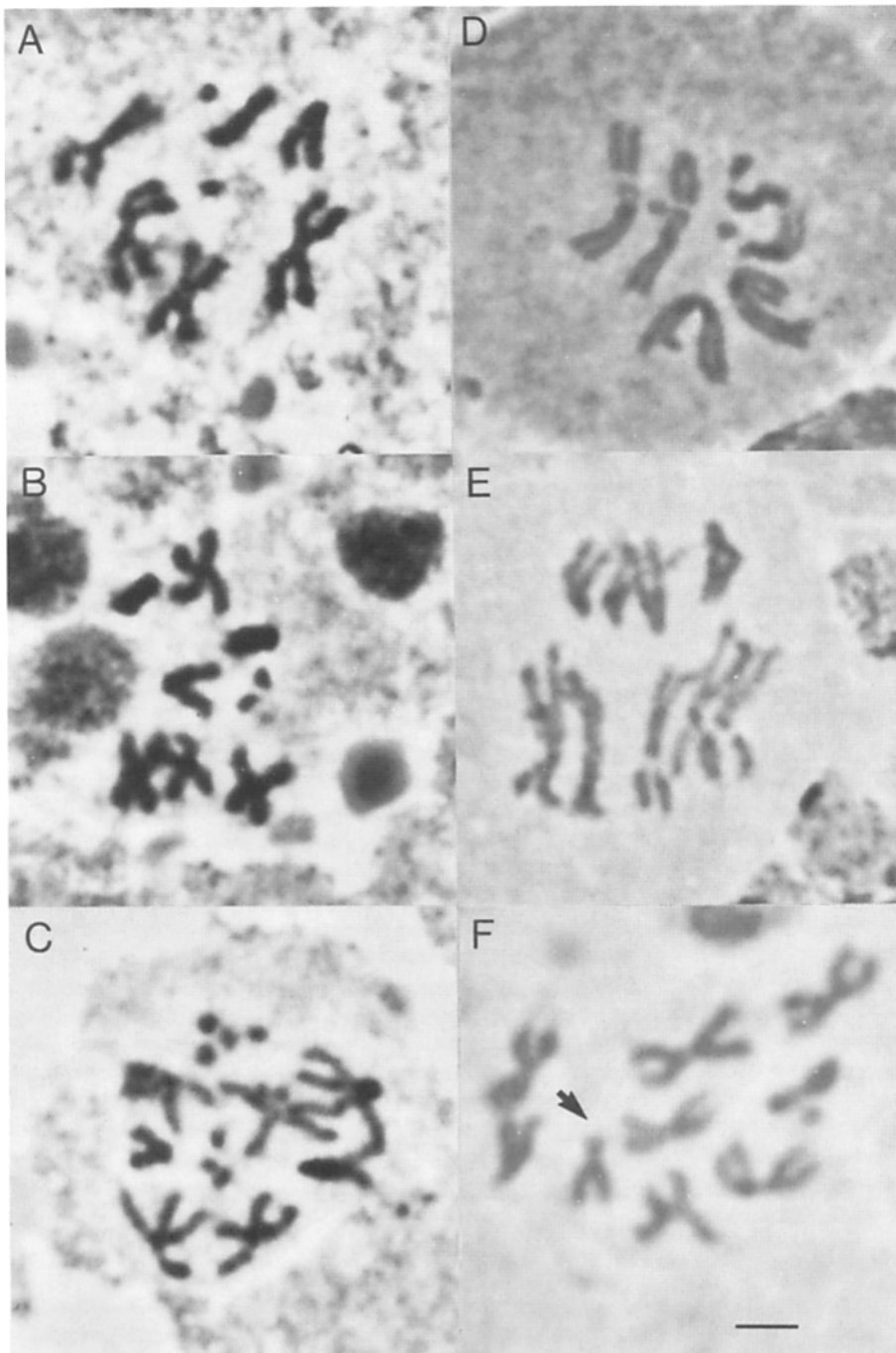
Figure 1. Metaphase figures from wild-type and *rough deal* larval neuroblasts, treated with colchicine (*A–C*, and *F*) or untreated (*D*, *E*). (*A–C*) Three different cells from the same colchicine-treated larval brain homozygous for *rod*$^{X-1}$ showing three different ploidies. (*A*) A euploid 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*$^{X-4}$ 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

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

* 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.
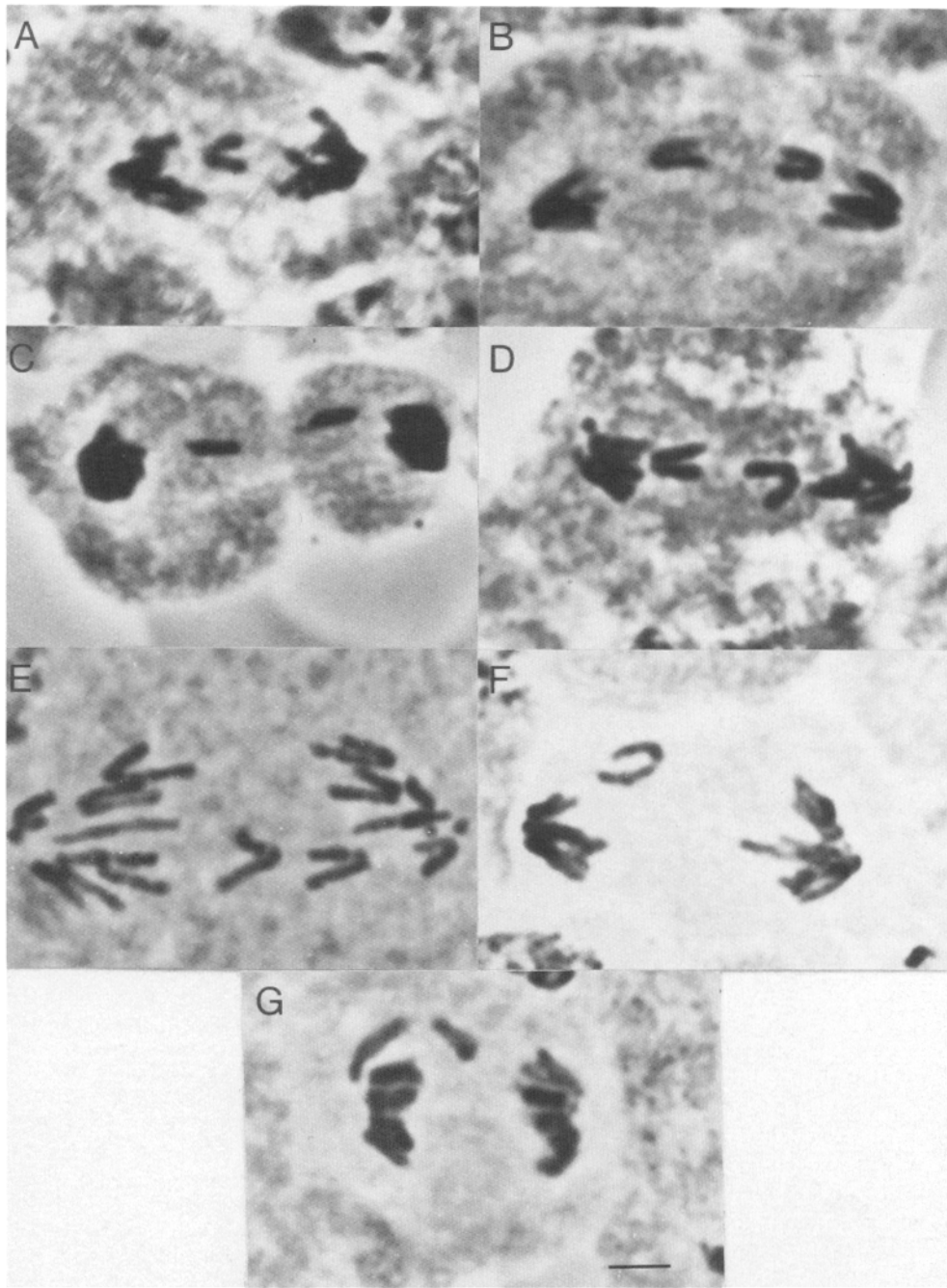
Figure 2. Abnormal anaphases from *rod* mutant cells: Lagging chromatids and chromatid pairs. (*A, B,* and *D*) $rod^{H4.8}$; (*C*) rod^{X-1} (actually a telophase, and cytokinesis is nearly complete. Note that most of the chromosomes have already begun assembling into a nucleus again, while one pair remains behind); (*E* and *G*) rod^{X-4}; (*F*) rod^{X-2}. Bar, 2 μm.

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 $rod^{H4.8}$, 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 $rod^{H4.8}$, relative to wild type. This slight dominance of $rod^{H4.8}$ may indicate a haplo-insufficiency for this locus, that is, a single wild-type dose of rod^+ is insuf-

ficient 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
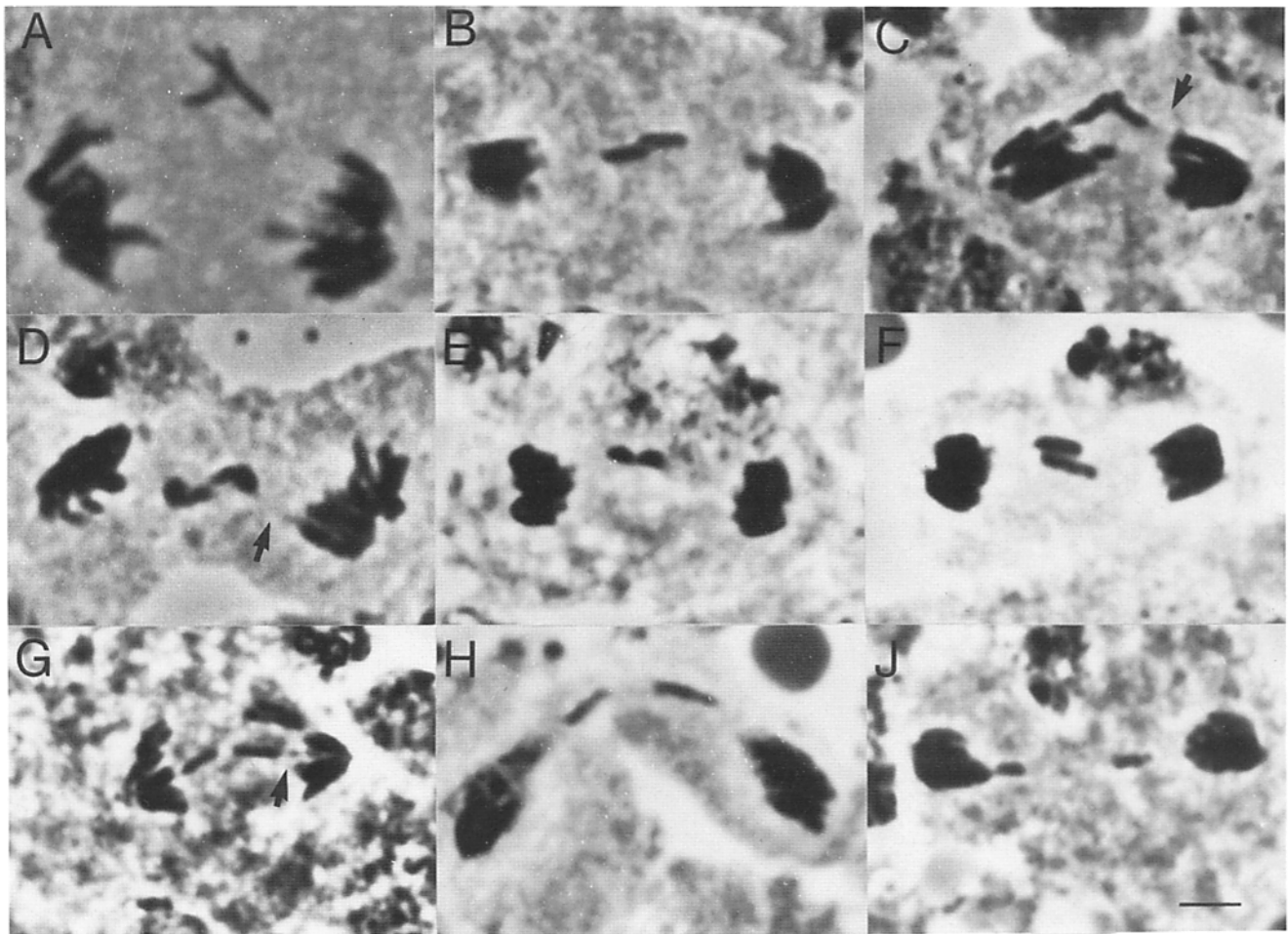
Figure 3. Abnormal anaphases from *rod* mutant cells: anaphase bridges and stretched chromatid arms. (*A–F*) Anaphase bridges; (*G–J*) stretched chromatid arms. The genotypes of the cells depicted are (*A*) rod^{X-4}; (*B*) rod^{X-2}; (*C*) $rod^{H4.8}$; (*D*) rod^{X-2}; (*E*) rod^{X-3}; (*F* and *G*) rod^{X-1}; (*H* and *J*) rod^{X-4}. Arrows indicate decondensed chromatin fibers connecting the bridge to the poles in *C*, *D*, and *G*. Bar, 2 μm.

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 testis, 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 homozgyous *rod* males are always small, and contain correspondingly fewer cycling cysts and mature sperm bundles, although cysts at each stage of spermatogene-

sis 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 *rod*^H4.8 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 rod^{X-3} spermatocytes, in which one cell appears to be improperly segregating its chromatids, while another cell in the same cyst is
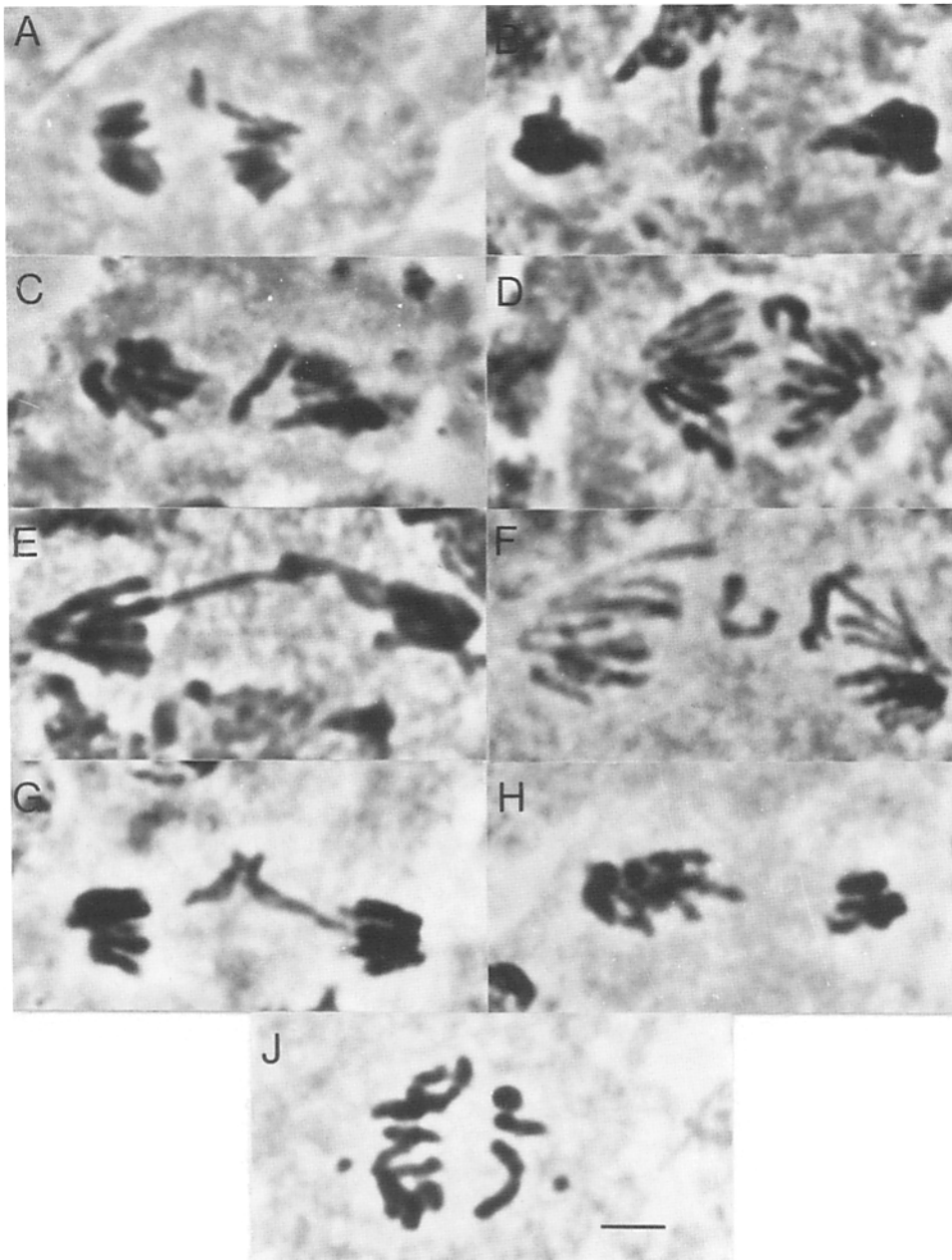
Figure 4. Abnormal anaphases from *rod* mutant cells: other unusual figures. (*A–D*) "Stuck" chromatids, or chromatid pairs, at the mitotic equator; (*E*) anaphase bridge between a single chromatid and an unseparated chromatid pair; (*F*) two lagging chromatids, or chromatid pairs; (*G*) an anaphase bridge apparently involving two pairs of chromatids; (*H* and *J*) unequal distribution of chromatids. The genotypes of the cells depicted are as follows: (*A, B, F, G*) *rod*$^{H4.8}$; (*C, H*) *rod*$^{X-1}$; (*D*) *rod*$^{X-2}$; (*E*) *rod*$^{X-3}$; (*J*) *rod*$^{X-4}$. Bar, 2 μm.

dividing properly. Fig. 6 *E* shows an abnormal anaphase II from a *rod*$^{H4.8}$ 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$^{H4.8}$

Genotype	Flies with y sn clones*	Total number of clones*
y sn^3/ + +; +/+	1/28	1
y sn^3/ + +; rod$^{H4.8}$/rod$^{H4.8}$	15/15	61
y sn^3/ + +; rod$^{H4.8}$/ +	11/56	13

* 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*$^{X-4}$ to 42.5% in *rod*$^{X-1}$. The fraction of lagging chromatids among the abnormal anaphases is similar for all alleles, except *rod*$^{X-1}$. The relative reduction of laggards in *rod*$^{X-1}$ 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
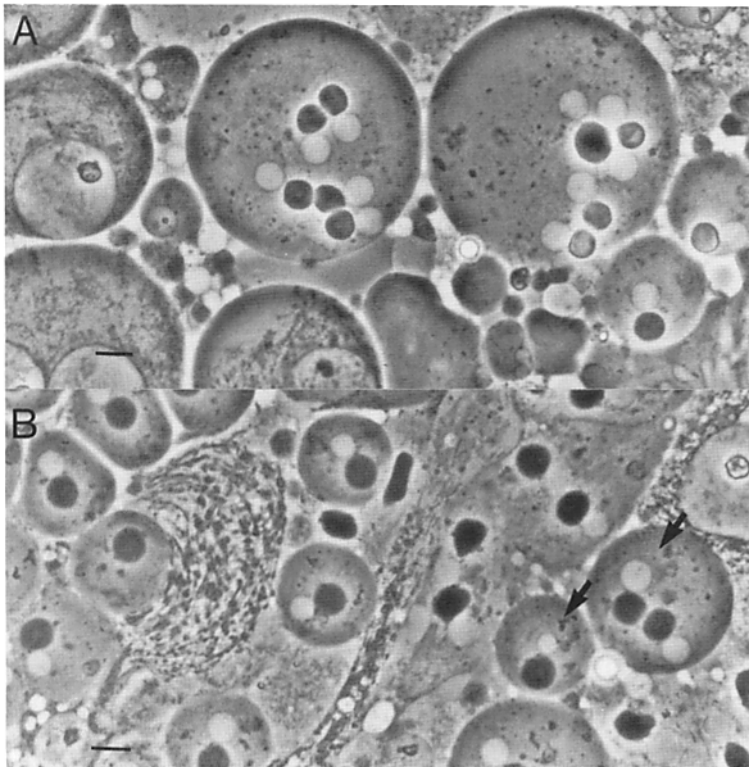
Figure 5. Saline squash of live, immature spermatids from wild-type (A) and $rod^{H4.8}$ (B) testes. The nuclei are seen as white circles and the mitochondrial derivative as black circles. The uniformity of the mitochondrial derivatives is retained in the *rod* testis, but the size of the nuclei is variable (*arrows*). Bar, 10 μm.

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
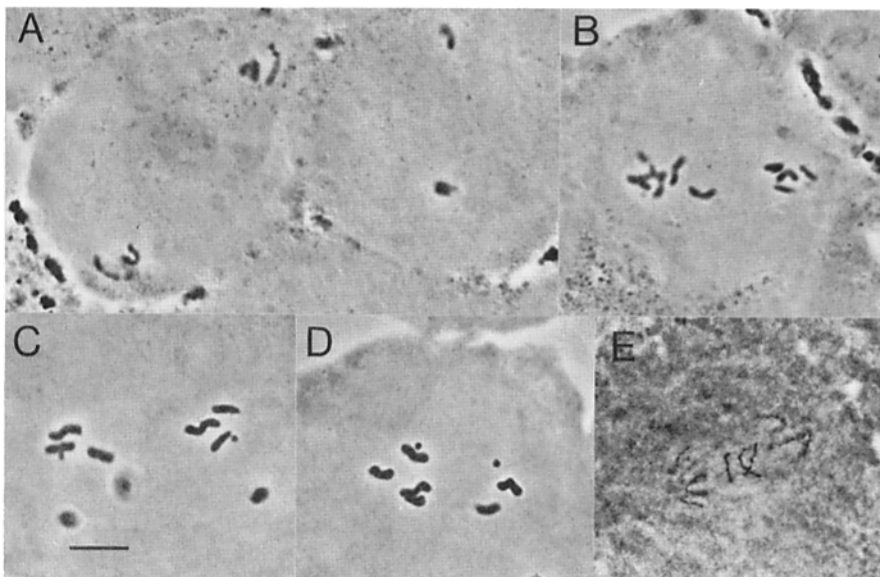

Figure 6. Euploid and aneuploid meiotic anaphase II figures from *rod* testes. (A, B) Three aneuploid spermatocytes from a single rod^{X-2} testis. In A, two hypoploid cells are undergoing a "normal" anaphase II. In B, a hyperploid cell shows unequal distribution of chromatids. The event initially producing 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^{X-3} testis, showing correct and incorrect, respectively, disjunction of a euploid chromosome set. (E) Lagging chromatids from a $rod^{H4.8}$ 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 polysomic for any of the four chromosomes. However, the polysomy in a given cell is generally limited to only a few chromosomes; polyploid 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 aneuploid) sperm are produced. In summary, the observed aneuploidy 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 aneuploidy, 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 rod^+ is producing an essential mitotic function, one might expect a null mutation to cause all anaphases to fail. That even rod^{X-1}, the only completely lethal allele in our collection, still allows many normal anaphases to proceed indicates that some residual rod-like function is being provided by the cell. It is possible that none of our exant alleles are null mutations, or that the 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 presence 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 rod resides, precludes our testing for residual activity in the rod mutants.

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

A striking feature of rough deal anaphases is that the chromosomes 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. Perhaps 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 failure of a chromosome to disjoin would result in a daughter cell trisomic for that chromosome, and this is the probable origin of the observed polyploidy in rod tissues. We will consider two mechanisms for generating the anaphase phenotype, 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 reduction 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 migrate 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 remain 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 actually defines the start of mitotic anaphase, sister chromatid release 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 described (INCENP) whose presence at the points of sister chromatid adhesion in metaphase chromosomes and subsequent loss at anaphase implicates it in this process (5). Mutations in rod might be causing the sporadic failure of sister chromatid release by interfering with the functioning or dissolution 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(l)zw10* causes a high frequency of mitotic nondisjunction, not unlike that found in *rod* mutants. However, genetic and cytological studies suggest that aneuploids in *l(l)zw10* are generated by premature separation of sister chromatids and subsequent random disjunction at anaphase (2, 44). Similarly, mutations at *ord* and *mei-S332* 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 mechansim involved in assuring the fidelity chromatid disjunction during anaphase.

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