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Supernova (*spno*), a new maternal mutant producing variable-sized cleavage nuclei in *Drosophila*

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Summary

This paper describes a new recessive maternal lethal which disrupts normal nuclear division and migration during cleavage in *Drosophila*. We have named this gene locus *supernova*. Deletion mapping and *in situ* hybridization have located this gene to 88 F9/89 A1 on the polytene chromosome map. The terminal mutant phenotype is characterized by the presence of many variable-sized nuclei scattered throughout the cytoplasm of the unhatched egg. Following fertilization, the initial cleavage divisions appear delayed and are often accompanied by the formation of ring-like association of chromosomes and/or chromosome bridges. Although the polymerization of tubulin into spindles occurs during the initial cleavage divisions, there appears to be both a spatial and temporal uncoupling of DNA replication from the formation and proper functioning of spindles. Eventually no functional spindles are formed, but nuclei continue to increase in size and number with increasing age of the embryo following fertilization.

1. Introduction

The genetic control of cleavage divisions in *Drosophila* has been the subject of a recent review (Glover, 1991). The products of some genes discussed in this review, namely p34^{cdc2} (Jimenez *et al.* 1990; Lehner & O'Farrell, 1990*b*), cyclins A and B (Lehner & O'Farrell, 1989, 1990*a*; Whitfield *et al.* 1990), and *string* (Edgar & O'Farrell, 1989, 1990), form components of, or are associated with, the universal mitotic oscillator. In addition to these gene loci which have a direct control over nuclear cycling, Glover describes a number of other genes which do not control entry into S or M phases directly, but their products are necessary for normal cleavage in *Drosophila*. Thus, *fs(1)Ya*, is essential for the fusion of the male and femal pronuclei (Lin & Wolfner, 1991), whilst the normal alleles of *gnu* (Freeman & Glover, 1986) *mh* (Edgar *et al.* 1986) *plu* and *png* (Shamarski & Orr-Weaver, 1991) appear to be involved in suppressing DNA replication in oocytes until after fertilization. Another group of genes appear to be involved in the functioning of the mitotic spindle during early cleavage divisions. *abc* (Vessey *et al.* 1991), and some alleles of *polo* (Sunkel & Glover, 1988) and *asp* (Gonzalez *et al.* 1990) all have a role in the formation of the spindle apparatus and/or its interaction with the kinetochore to facilitate normal chromosome segregation. Mutants in these genes seem to uncouple DNA synthesis from centrosome division and the organization and functioning of the spindle apparatus. Thus they are characterised by having abnormal spindles which are often irregularly distributed with respect to both the nuclear material and the centrosomes.

This paper describes a new maternally expressed gene, *supernova* (*spno*), which is located at 88F9/89 A1 on the polytene chromosome map. Mutants in this gene appear to initially cause a delay in DNA replication, which becomes uncoupled from the formation of the spindle apparatus leading to the occurrence of chromosome bridges, tripolar spindles and spindles which fuse due to the failure of nuclei to separate during an earlier division. Eventually microtubular polymerization ceases. However, DNA synthesis continues to occur, albeit at a slower rate, to form the terminal phenotype of the mutant which is characterized by many variable-sized nuclei distributed throughout the egg cytoplasm.

2. Materials and Methods

(i) *Genetic stocks*

The mutant, *fs(3)2-210*, described here was produced by P-element mutagenesis and obtained from Dr R. Saint. Preliminary observations made during deletion mapping of a number of maternal lethals indicated

$$\frac{Birm}{Birm};\frac{ry}{ry}\ \text{♀♀} \times \frac{+}{+};\frac{\Delta 2\text{–}3}{\Delta 2\text{–}3}\ \text{♂♂}$$

$$\downarrow$$

$$\frac{+}{Birm};\frac{ry}{\Delta 2\text{–}3}\ \text{♂♂} \times \frac{+}{+};\frac{+}{TM3\ Sb\ ry}\ \text{♀♀}$$

$$\downarrow$$

$$\frac{+}{+};\frac{Df(3R)Sbd^{105}\ ry\ sr\ e}{LVM}\ \text{♂♂} \times \frac{+}{+};\frac{ry}{TM3\ Sb\ ry}\ \text{♀♀}$$

$$\downarrow$$

Select $\frac{+}{+};\frac{ry}{Df(3R)sbd^{105}\ ry\ sr\ e}$ ♀♀ × wild-type ♂♂ for fertility

If sterile, generate stock using $\frac{+}{+};\frac{ry}{Df(3R)sbd^{105}\ ry\ sr\ e}$ ♂♂

Fig. 1. Crosses used to generate P-element mutants.

that the mutant was overlapped by the *stubbleoid* deficiency *Df(3R)sbd*^{105} (Moretti, unpublished). The deficiency stocks *Df(3R)bxd*^{100}, *bxd*^{100}*/TM1* (Struhl, 1982), which deletes bands 89 B 5/6 to 89 E2/3, and *Df(3R)ea*^{831RPJ}*/TM3* (Chasan & Anderson, 1989), which deletes bands 88 E7/13 to 89 A1, were used to generate *trans*-heterozygotes with *fs(3)2–210*, thereby facilitating a more definitive location for the mutant.

In addition, other alleles of *fs(3)2–210* have been isolated. One, *A42*, has been produced by ethyl methane sulphonate mutagenesis, whilst three other alleles (*P66*, *P76* and *P82*) have been isolated using the P element *P*[*ry*$^+$ *Δ2–3 (99B)* described by Robertson *et al.* (1988). In these experiments, the *Birmingham* strain, which contains seventeen non-autonomous P-elements, was crossed to *CyO/bw*^{V1}*;* *ry*^{506} *Sb P*{*ry*$^+$*Δ2–3*} *(99B)*/TM6 *osw*^{k}, which contains transposase activity. Maternal lethals generated by P-element mobilization and which overlapped *Df(3)sbd*^{105} were selected and tested for allelism with *fs(3)2–210*. Details of the crossing procedures are shown in Fig. 1. Other genetic strains used in these studies are as described in Lindsley & Grell (1968) and Lindsley & Zimm (1987).

(ii) *Histochemical staining of embryos*

Eggs laid by strains homozygous for the various *supernova* alleles as well as heterozygotes of each of these alleles in *trans* with *Df(3R)sbd*^{105} or *Df(3R)ea*^{831RPJ} were collected and aged for either 4, 6 or 24 h periods on standard *Drosophila* medium, dechorionated in half-strength commercial bleach and fixed using a modified protocol of Mitchison & Sedat (1983). After dechorionation, embryos were rinsed and placed in a 1:1 solution of heptane/90% methanol in PBS containing 1 mM-EGTA and shaken continuously for 10 min. The heptane phase was then removed together with any embryos at the interface, and the embryos in the methanol phase were transferred to 90% methanol/PBS containing 1 mM-EGTA and 1 mM-GTP, which stabilizes microtubules (Axton *et al.* 1990), until they were ready for microscopic examination. Subsequent washing and staining with the antibody YL$\frac{1}{2}$ (Seralab) was carried out as described by Warn & Warn (1986), except that the second antibody was biotinylated anti-rat IgG and the detection system avidin Texas-red (Amersham). DAPI staining was as described by Warn & Warn (1986). Embryos were examined with a Leitz Dialux fluorescent microscope using appropriate filter blocks.

(iii) In situ *hybridization*

Chromosomes from salivary glands were prepared according to the procedure described by Pliley, Farmer & Jeffery (1986). The probe Pπ25.1 (Δ2–3)w.c. was obtained from Dr R. Saint and biotin-labelled using the BRL Bionick system. Prehybridization treatments and hybribidization protocols were as described by Whiting, Farmer & Jeffery (1987). Colour detection used the BRL Bluegene kit and was carried out according to the manufacturer's instructions.

3. Results and Discussion

Preliminary screening of a number of maternal mutants (Moretti, unpublished) has indicated that *fs(3)2–210* affected cleavage in *Drosophila* and was a mutant in a previously undescribed gene which we have named *supernova* (*spno*). We suspected that this mutation was produced by a P-element insertion and this report describes experiments designed to map the gene and characterize its phenotype.

(i) *Location of the* spno *gene*

When *fs(3)2–210/fs(3)2–210* females were mated to wild-type males the frequency of unhatched eggs was about 95%. This frequency was also obtained if *fs(3)2–210/Df(3R)sbd*^{105} females were mated either with males of the same genotype or wild-type males. This indicated that *fs(3)2–210* was indeed a maternal effect mutant overlapped by the deficiency *Df(3R)sbd*^{105} and was therefore located between 88 F9/89 A1 and 89 B9/10 on the polytene chromosome map. To facilitate the localization of the mutant to a smaller region, crosses to other deletions in the region, *Df(3R)ea*^{831RJP} (88 E 7/13–89 A1) and *Df(3R)bxd*^{100} (89 B 5/6–89 E2/3), were made in order to produce mutant/deficiency heterozygotes which were then brother-sister mated to determine whether *fs(3)2–210* overlapped or complemented each deficiency. These experiments were carried out at 21 °C to minimise any hybrid dysgenesis. If overlap occurred then none of the eggs laid should hatch, compared with the result expected when the mutant complements the deficiency, in which case hatch rate should approach 75% (it will not be 100% since the homozygous deficiency is an embryonic lethal). The results showed that *fs(3)2–210* does not overlap the $Df(3R)bxd^{100}$ deficiency (59·4% eggs hatched), but does appear to partially overlap or interact with the *easter* deficiency (29·4% eggs hatched), which suggests that the *spno* gene is located at 88 F9/89 A1.

The other mutants produced by P-element transposition (i.e. *P66*, *P76* and *P82*) overlap $Df(3R)sbd^{105}$ and appear to behave as weak maternal lethals (between 50 and 80% of eggs fail to hatch) when crossed to wild-type males. They are also allelic in that heterozygotes in all possible combinations have about the same frequency of eggs failing to hatch. The phenotypes produced are also similar to that produced by *fs(3)2–210* (see below), suggesting that all these mutants are alleles at the *spno* locus. *In situ* hybridization experiments confirm the localization of *spno* to 88 F9/89 A1. When salivary gland chromosomes from *P82* homozygotes or *P82*/+ heterozygotes were probed with Pπ25.1 (Δ2–3)w.c., a prominent band was present at 89 A proximal to the *Ubx* site. Although up to 17 regions of hybridization were seen, only three other sites were on the right arm of chromosome III and none of these were near the region overlapped by $Df(3)sbd^{105}$. The band at 89 A was also seen when chromosomes from *fs(3)2–210* were probed.

When *trans*-heterozygotes are made between the various P alleles and *fs(3)2–210*, about 80–90% of eggs hatch. This high frequency is probably generated by P-element mobilization. This is consistent with the model recently presented by Engels *et al.* (1990) where P-element transpositions are increased up to 100-fold when a wild-type base sequence is opposite the P-insertion site. On the basis of this model we would expect that *fs(3)2–210/fs(3)2–210* and $fs(3)2\text{–}210/Df(3R)sbd^{105}$ females should have a zero frequency of transposition, whereas *fs(3)2–210 trans*-heterozygotes with the various P alleles will have a much higher mobilisation frequency due to the fact that these alleles probably do not occupy exactly the same site as *fs(3)2–210*. Consequently, they will revert to wild-type with a much higher frequency as a result of the transposase associated with strain *fs(3)2–210*. Loss of the P-element from site 89 A in these cases has been confirmed by *in situ* hybridization. Also consistent with the model of P-element mobilization is the apparent partial overlap of *fs(3)2–210* with $Df(3R)ea^{831RPJ}$ referred to above. There still remains the question of why about 5% of eggs deposited by *fs(3)2–210* homozygotes or heterozygotes with $Df(3R)sbd^{105}$ hatch. One possible interpretation is that the P-element is present in a

$$w;\frac{+}{CyO};\frac{\Delta 2\text{–}3Sb}{fs(3)2\text{–}210\ e\ ro}\ ♂♂ \times \frac{+}{+};\frac{ry\ Pr\ ro}{TM3\ Sb\ ry}\ ♀♀$$

$$\downarrow$$

$$\frac{Df(3R)sbd^{105}\ e\ sr\ p^{p}}{LVM}\ ♀♀ \times \frac{+}{CyO};\frac{fs(3)2\text{–}210^{rev}\ e\ ro}{TM3\ Sb\ ry}\ ♂♂$$

$$\downarrow$$

Select $\frac{+}{+};\frac{fs(3)2\text{–}210^{rev}}{Df(3R)sbd^{105}\ e\ sr\ p^{p}}$ ♀♀ and test for fertility

Select $\frac{+}{+};\frac{fs(3)2\text{–}210^{rev}}{LVM}$ flies to maintain stock

Fig. 2. Details of crosses used to generate revertants.

regulatory part of the *spno* gene, thereby affecting its functioning rather than abolishing activity as would be characteristic of a null mutant. Indeed, Kelley *et al.* (1987) have found that all thirteen P-element induced mutants of *Notch* insert near the start sequence in the regulatory region of the locus, but they do not all occupy exactly the same molecular site. An alternative explanation for this low frequency of hatched eggs is that when *fs(3)2–210* is homozygous or overlapped by $Df(3R)sbd^{105}$, ectopic pairing between the P-insert and another insert elsewhere in the genome could initiate mobilization and therefore reversion of *fs(3)2–210* to wild-type. This latter interpretation appears to be supported by the *in situ* hybridization studies, which show absence of the P-element at 88 F9/89 A1.

In order to obtain revertants of *fs(3)2–210*, this strain was crossed to one carrying the Δ2–3 chromosome as shown in Fig. 2. As shown by Robertson *et al.* (1988), this chromosome, by providing transposase activity, will facilitate the 'jumping' of the numerous P-elements – in excess of 30 autonomous and non-autonomous as detected by *in situ* hydribization – in strain *fs(3)2–210*. If the P-element, which is specifically associated with *fs(3)2–210*, transposes to another location, then this chromosome should be viable and fertile in *trans* with $Df(3R)sbd^{105}$, provided the loss of the P-element has not produced a lethal in another gene or generated a deficiency containing other genes overlapped by $Df(3R)sbd^{105}$. Eight presumptive revertants, *e ro* $fs(3)2\text{–}210^{rev}/LVM$, were selected. Males from these stocks were crossed again to $Df(3)sbd^{105}\ e/LVM$ females to confirm that they were revertants. In each case, the ebony females, *e ro* $fs(3)2\text{–}210^{rev}/e\ Df(3)sbd^{105}$, were crossed to wild-type males to test for fertility and determine the phenotype of any unhatched eggs (see below). Four of the eight revertants were fertile, whilst the other four produced eggs which failed to hatch, except for a few rare exceptions. Loss of the P-element from 89 A in these

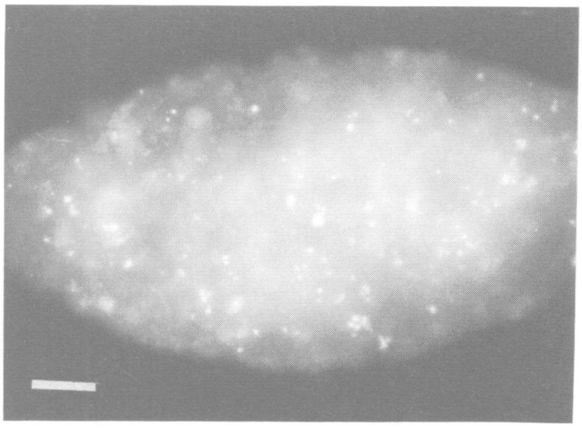

Fig. 3. DAPI stained nuclei of a *spno* embryo [*fs(3)2–210/Df(3R)sbd*^{105}] with less than 100 variable-sized nuclei. Approximate age after fertilization is 20 h. Anterior to the left and dorsal uppermost. Bar, 50 μm.

lines has been confirmed by *in situ* hybridization. It is likely that the fertile revertants resulted from precise excision of the P-element restoring normal base sequence. On the other hand, the revertants which yielded only unhatched eggs probably resulted from faulty excision of the P–element producing a deficiency. Examination of the polytene chromosomes in these four revertants failed to show any discernable cytological deletion, indicating that it is probably very small. The following observation is consistent with the deficiency hypothesis. When 1 of the 4 revertants producing unhatched eggs (*line 6*), was crossed to the allele *A42* it was found that the *A42/fs(3)2–210*^{rev} heterozygote was about 90% sterile, suggesting that the deficiency generated by P-element mobilization in *line 6* was small and either overlapped or interacted with that part of the gene occupied by allele *A42*. The few eggs hatching are due to the allele *A42* not being fully penetrant. On the other hand, the *A42/fs(3)2–210*^{rev} *trans*-heterozygotes produced by the other three revertants were fully fertile, suggesting that the genetic change generated by P-element mobilization fully complemented the *A42* mutant site. Since in each case *fs(3)2–210*^{rev}*/Df(3R)sbd*^{105} were fully viable, the deficiency generated by P-element loss could not have extended into neighbouring genes overlapped by *Df(3R)sbd*^{105}. This hypothesis will need to be confirmed by restriction mapping and sequencing.

(ii) *Phenotypic studies*

Eggs laid by *fs(3)2–210* homozygotes or *fs(3)2–210/Df(3R)sbd*^{105} heterozygotes were collected over a 3 h period and aged for 24 h prior to fixing and staining with DAPI to detect changes in the organization of nuclei. Homozygotes and heterozygotes showed no obvious differences. Similarly heterozygotes for *fs(3)2–210/Df(3R)ea*^{831RPJ} and *trans*-heterozygotes of the various P alleles showed no differences in the nuclear phenotype amongst those embryos which failed to hatch. In each case, embyros had a variable number of nuclei randomly distributed throughout the cytoplasm. Some of the nuclei were quite large and either polyploid or polytene. Chromatin bridges were sometimes apparent in these embryos. An embryo with a typical phenotype is shown in Fig. 3. If the embryo is not fertilized then the pronucleus does not divide. This was shown following an examination of 84 mutant embryos which were not fertilized and in no case did any of them show division of the female pronucleus. This clearly distinguishes *spno* from *gnu* (Freeman & Glover, 1987), and *plutonium* and *pan gu* (Shamarski & Orr-Weaver, 1991).

Younger embryos (up to 4 h old) were collected from mutant/deficiency heterozygotes for alleles *fs(3)2–210, P66, P76* and *P82* and stained with DAPI and the cytoskeletal component tubulin. Fig. 4(*a*, *b*) shows a normal wild-type embryo at the sixth cleavage stained with DAPI (*A*) and the antibody YL$\frac{1}{2}$ (*B*) which detects tubulin. There is a normal arrangement of dividing nuclei and spindles are associated with them as would be expected. On the other hand, Fig. 4*c* shows a mutant embryo (*fs(3)P66/Df(3)sbd*^{105}) which is about 4 h old. This appears to be during the fifth or sixth division so cleavage is very much delayed in these mutants. In addition, there is asynchrony of division as well as an irregular distribution of nuclei, which are not all in the same plane of focus as would normally be expected at this stage. The corresponding figure for spindle organization is shown in Fig. 4*d*. Spindles also show irregular orientation and organization. There is a coalescence of microtubules (arrowhead) for two nuclei very close together which may be due to a partial nuclear fusion resulting from incomplete separation during the previous division. In some cases tripolar spindles were seen (result not shown). It appears that these irregular divisions begin very early, perhaps as early as the first cleavage, and become more abnormal during subsequent divisions.

Figure 4(*e*, *f*) shows a slightly older embryo, but still in the sixth cleavage, where cytoskeletal organization appears to be degenerating. Spindles are clearly not normal and in some cases (arrowhead) there appears to be no polymerization of spindles associated with nuclear material which is condensed and appears to be attempting division. After about the sixth or seventh cleavage, microtubules no longer apear to be polymerized and therefore there is no orderly distribution of chromosal material. However, the nuclear material appears to continue replication, giving rise to large nuclei some of which fragment to produce the many variable-sized nuclei characteristic of 20 h-old embryos.

The terminal phenotype of unhatched eggs laid by *e2–210*^{rev}*(line 6)/A42* revertants is much weaker than that produced by the *fs(3)2–210/Df(3)sbd*^{105} heterozygotes. In this case most embryos reach germ

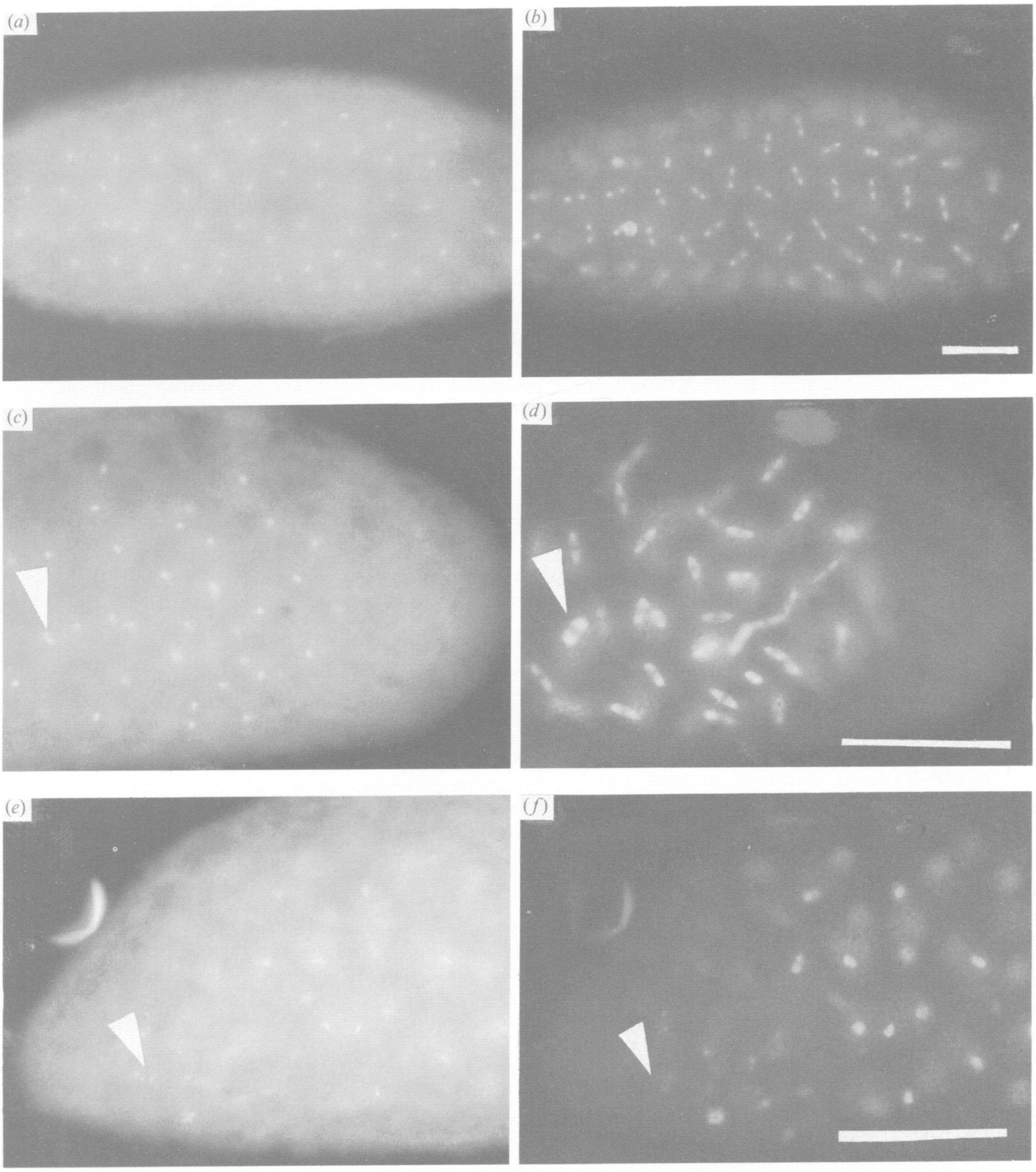

Fig. 4. DAPI (left hand panel) and tubulin (right hand panel) stained embryos. (*a*, *b*) Wild-type (6th cleavage), showing a regular arrangement of dividing nuclei and corresponding spindle apparatus. (*c*–*f*)—*fs(3) P76/Df(3R)sbd*^{105} mutant embryos, age about 4 h. (*c*, *d*) shows portion of an embryo with about 30 dividing nuclei. These nuclei are randomly distributed in various planes of focus. Two nuclei have only partially separated during the previous division and have generated a double spindle at the next division (arrowhead). (*e*, *f*) Another embryo of about the same age which shows about 30 nuclei dividing asynchronously. Some nuclei are fragmenting (arrowhead) and spindles do not appear normal in shape or size and in some cases have not formed at all (arrowhead). For tubulin staining the antibody YL$\frac{1}{2}$ was used. This was reacted with biotinylated anti-rat IgG which in turn was detected with avidin Texas-red. During fixation spindles were stabilized with 1 mM-GTP. (*a*, *b*) Bar, 20 μM; (*c*–*f*), bar, 80 μm.

band shortening before development ceases. However, all embryos show an an abnormal distribution of nuclei with some regions being almost devoid of nuclear material.

The *supernova* locus described here has certain features in common with *polo* (Sunkel & Glover, 1988), *asp* (Gonzalez *et al.* 1990), and *abc* (Vessey *et al.* 1191) in that it possesses certain phenotypic features which partially overlap with each of them. Thus DNA synthesis appears to continue in *polo* and *asp* giving rise to polyploid nuclei, but it does not take place in unfertilized eggs except in the case of some *asp* mutants (Gonzalez *et al.* 1990). It is also different from *abc*, because in this case DNA replication and cleavage division appear to cease soon after the sixth cleavage (Vessey *et al.* 1991). A complete spindle apparatus appears to form during early cleavages in *spno*, which is similar to the situation in *asp* and *abc*. However, in each of these cases spindles progressively become irregular in their spatial distribution as well as abnormal in that they often form monopolar or tripolar spindles. Eventually microtubles cease to be polymerized into spindles. In the case of *abc* and *spno* chromatin bridge formation also appears to occur, whilst *spno* has some ring-like associations of chromosomal material similar to that found in *polo* (Sunkel & Glover).

Clearly, normal cleavage requires the integration of DNA synthesis, centrosome division and migration, microtubule polymerization and organization into the spindle apparatus and the association of chromosomes with kinetochores facilitating their subsequent segregation. McIntosh & Koonce (1989) suggest that the stability of the spindle is dependent on a complex interaction between its initiation at the centrosome, kinetochore association and the importance of several other microtubule associated proteins (MAPs). Such proteins have been described in *Drosophila* by Kellogg *et al.* (1989) who have isolated antibodies to about 20 different proteins (MAP's) which are associated with the centrosomes at some stage during mitosis, whilst Compton *et al.* (1991) have identified at least four novel centromere/kinetochore associated proteins in humans. Furthermore, Yasuda *et al.* (1991) have suggested that centrosomes and DNA have separate roles during early divisions and in fact cycle independently, functioning together to regulate the early synchronized divisions in the syncytial embryo. Indeed, DNA controls the assembly of nuclear laminae and organization of the nuclear membrane, whilst centrosomes appear to be correlated with the formation of microtubules. Consistent with this idea of separate cycling is the observation of Raff & Glover (1989) that the behaviour of centrosomes appears to be independent of DNA synthesis in the formation of pole cells.

In the light of our results as well as these other observations, there appear to be two alternative possibilities as to the type of protein coded for by the *spno* gene. Firstly, it may be a protein which is associated with the centromere/kinetochore and enables the capture of free microtubules to form spindle arrays, as proposed by Mitchison & Kirschner (1985). Secondly, it may be a protein which facilitates the movement of chromosomes along the spindle fibre (Gorbsky *et al.* 1987; Reider *et al.* 1990). Yamamoto *et al.* (1989) have shown that the mutant *claret non-disjunctional* (ca^{nd}) affects chromatid segregation in larval neuroblasts as well as chromosome segregation during meiosis I in females. Subsequent studies by this group (Endow *et al.* 1990) have suggested that the gene product associated with ca^{nd} corresponds to the heavy chain of kinesin, which is important to mediate attachment of chromosomes to the spindle (Yang *et al.* 1989). More recently Komma *et al.* (1991) have shown that the ca^{nd} gene product has two functions, one affecting chromosome segregation during meiosis and the other mitotic segregation. We are currently investigating whether *spno* has any zygotic function, particularly in regard to initiating non-disjunction events which may be associated with the formation of chromosome bridges and abnormal spindle organization noted in the embryo.

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