Abnormal meiotic spindles cause a cascade of defects during spermatogenesis in *asp* males of *Drosophila*

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Summary

Since spermatogenesis in *Drosophila* is a series of interconnected and interdependent steps and most of the spermatogenic events take place in the absence of transcription, failures in a given stage can give rise to a cascade of defects later on. The *asp* locus of *Drosophila melanogaster* codes for a non-tubulin component implicated in proper spindle structure and/or function (Ripoll *et al.* 1985). Homozygous *asp* males exhibit abnormal meiotic spindles giving rise to altered segregation of chromosomes and mitochondria and failures in cytokinesis. Postmeiotic spermatogenic stages of *asp* males show a series of alterations that we interpret as due to the previously occurring defective meiosis because

meiotic spindles are the only microtubular structure altered in mutant testes. The most conspicuous alterations are: (i) variable size of nuclei and nebenkerns of early spermatids, which are also multinucleate instead of having single and uniformly sized nuclei; (ii) elongating spermatids in which abnormal-sized mitochondrial derivatives elongate alongside more than one axoneme; (iii) failures in the individualization process, where abnormal spermatids remain syncytial, and seem to be eliminated during the coiling stage.

Key words: *Drosophila*, *asp*, spermatogenesis, abnormal spindles.

Introduction

Spermatogenesis is a well-known model system for both morphological and genetic studies of developmental phenomena in higher eukaryotes (see Hennig, 1987). A sophisticated control system must underlie the progressive series of dramatic changes that accompany the transformation of stem cells into specialized mature spermatozoa. The morphology of spermatogenesis in *Drosophila melanogaster* is a well-studied process and most of the stages have been described both at the cytological and at the ultrastructural levels (see Lindsley and Tokuyasu, 1980 for a detailed review). Spermatogenesis in *Drosophila* is a nearly isolated system that occurs almost independently of somatic development (Marsh and Wieschaus, 1978). Moreover, transcriptional activity is absent during the time when the most conspicuous morphological changes take place (Gould-Somero and Holland, 1974), so these changes have to be programmed in advance. One logical conclusion of this feature is that one mistake in the process can lead to a cascade of defects later on, making classical genetic analysis very difficult (Hardy *et al.* 1981).

We know of a large number of loci that seem to be involved in *Drosophila* spermatogenesis (Lindsley and

Lifschytz, 1972; Lifschytz and Hareven, 1977; Lifschytz and Meyer, 1977; Kempfues *et al.* 1979; Lindsley and Tokuyasu, 1980; Goldstein *et al.* 1982). By studying the alterations produced by mutations in these genes many authors have attempted to deduce the step of spermatogenesis for which the normal gene function is necessary (Kiefer, 1966; Wilkinson *et al.* 1974). However, the fact that there can be pleiotropic effects makes it difficult to know whether the defects seen in one step are due to the lack of normal gene function at that time as opposed to the indirect effects of a dysfunction occurring during some previous step. The best approach is to find the first detectable defects (see Lindsley and Tokuyasu, 1980). However, even this can be misleading: identification of the earliest defects does not necessarily mean that the normal gene product is not needed earlier nor does it mean that all defects are secondary consequences of failures in a previous step rather than the requirement of the normal product during different stages of spermatogenesis. On the other hand, if the step where the gene product is needed can be unequivocally identified, the phenotypic consequences during subsequent development can give information about the dependence of later steps on the affected one.

The aim of this work is to present the effects on

spermatogenesis of the mutation in the *abnormal spindle* locus (*asp*), a gene known to be needed for the proper function of both the mitotic and meiotic spindles (Ripoll *et al.* 1985). The spindle is a microtubular structure responsible for the segregation of chromosomes during cell division (McIntosh, 1982). The major components of microtubules are α and β tubulin (Ludueno, 1979). They also contain microtubule-associated proteins (Olmsted, 1986). A preliminary biochemical characterization of extracts obtained from *asp* larvae showed that tubulins were normal (Ripoll *et al.* 1985), so the *asp* product probably codes for a non-tubulin component that is therefore implicated in the function of the spindle. In this report, we present additional evidence indicating that *asp* specifically affects the spindle and not other microtubular structures. Consequently, we have used *asp* as a tool to examine downstream effects following spindle malfunction.

Materials and methods

Males of the y/y^+Y ; *red asp/TM2* stock used for this work were reared on standard *Drosophila* medium. Homozygous *asp* flies were recognized by *red*, which gives the eyes a brownish coloration. *asp* (3-85.2: 96A20-25;96B1-10) was isolated in a screen of EMS-mutagenized third chromosomes for late lethals or semilethals (Ripoll *et al.* 1985). Since *asp* is fully recessive (Ripoll *et al.* 1985; Gonzalez *et al.* 1989b), their heterozygous siblings were used as control individuals. See Lindsley and Grell (1968) for a description of mutations and balancer chromosomes.

For EM observation of spermatogenesis, young males were dissected in 0.1 M-Pipes pH 7.2, 5% glutaraldehyde (Fluka EM grade), 2% tannic acid. The testes were fixed in the same solution for 1 h, rinsed with cold buffer and postfixed for 2 h with 1% OsO_4 in the same buffer. After being dehydrated in successive concentrations of acetone, the testes were embedded in Vestopal resin. The polymerization reaction was carried out at 65°C for 24 h. Ultrathin sections were cut with an LKB ultramicrotome, stained with 2% uranyl acetate, and examined in a JEOL 100 B microscope.

Acetic-orcein staining and observation of unfixed cells were carried out according to Lifschytz and Hareven (1977). Immunostaining of meiotic apparatuses was carried out following a method that will be described in detail elsewhere (Casal *et al.* 1990). Briefly, the testis was dissected on a slide in 10 mM-Tris-HCl pH 6.8, 183 mM-KCl, 47 mM-NaCl, 10 μM -taxol and gently squashed under a siliconized coverslip. After removing the coverslip in liquid nitrogen, the testis was fixed in 3.7% formaldehyde in PBS (1.15 mM- KH_2PO_4 , 6.5 mM- Na_2HPO_4 pH 7.0, 137 mM-NaCl, 2.7 mM-KCl), washed in the same buffer and incubated in a moist chamber with YL1/2 ascites fluid (Kilmartin *et al.* 1982) diluted 1:200 in PBS. After washing in PBS, the testis was incubated with an anti-rat FITC-conjugated IgG. Chromatin was stained with Hoechst 33342. Diameters of the onion-stage nuclei were measured on photographic negatives following Gonzalez *et al.* (1988, 1989a).

Results

Individuals homozygous for *asp* show a large number of alterations (Ripoll *et al.* 1985; Gonzalez *et al.* 1989b)

such as rough and reduced eyes, nicked wings and a reduced viability. These phenotypic traits are thought to be the consequence of the mitotic abnormalities (mitotic arrest and a high number of aneuploid cells) produced by *asp*. Homozygous *asp* males show a high frequency of exceptional sperm for all the chromosomal complement, while females homozygous for *asp* are sterile (Ripoll *et al.* 1985). All these traits are stronger at 18 than at 25°C (Ripoll *et al.* 1985).

Male fertility

Individuals homozygous for *asp* show highly reduced viability (0.6% at 18°C and 7% at 25°C, Gonzalez, 1986). Homozygous mutant males grown at 18°C are always sterile. The sterility is due to abnormal spermatogenesis and not to failures in testicular development: the testes are filled with abundant debris except at the apical end, where normal spermatogonia and primary spermatocytes are seen. However, meiotic stages are difficult to recognize, postmeiotic stages are scarce and disorganized, and most early spermatids appear multinucleated. Considerable elongation of some cysts is observed, but most of these elongated cysts seem to degenerate before individualization. We have never found coiled spermatid bundles in males raised at 18°C. The seminal vesicles are filled with debris and mature sperm were never seen.

Crosses *en masse* of *asp* males with wild-type females give rise to viable progeny when they are reared at 25°C. However, when these males are tested singly only one fourth of them produce progeny. The sterility of the remaining three fourths does not seem to be due to failures in either testicular development or spermatogenesis. Instead, our observations suggest that an abnormal mating behavior is the basis of this apparent sterility. Although they do not give rise to progeny, these males have their seminal vesicles as distended and filled with abundant motile sperm as wild-type males kept away from females. Moreover, the males that gave rise to progeny were never able to inseminate more than one female, even though they were always kept with at least two females. Adult mutant individuals show severe abnormalities in brain morphology as observed in histological sections (Gonzalez, 1986), which can explain their mating behavior. When mutant males grown at 25°C are transferred to 18°C they do not mate due to a decrease in vigour.

Even the fertile *asp* males have reduced fecundity (around 20% that of their heterozygous siblings). Since motile sperm are abundant in the seminal vesicles of fertile homozygous males, their reduced fecundity is probably a direct consequence of the production of aneuploid sperm. This is supported by the observation that, in all fertile crosses, necrotic eggs, indicative of abortive embryonic development, are found.

The sterility shown by mutant males grown at 18°C is reversible: some of these males are able to produce offspring if they are transferred to 25°C. We have monitored the recovery of fertility by observing the appearance of motile sperm in seminal vesicles of males that developed at 18°C and were then held without

Fig. 1. Recovery of fertility in homozygous *asp* males. Homozygous *asp* males were reared at 18°C, transferred to 25°C and kept away from females for different periods. Their seminal vesicles were then dissected and the presence of motile sperm was scored. Each measurement is based on twenty seminal vesicles (ten males).

females at 25°C for different lengths of time. The response of the testes to the temperature shift is gradual (Fig. 1). Motile sperm are first seen in small amounts and in only a few seminal vesicles during the third day at 25°C. By the sixth day one half of the seminal vesicles show motile sperm in almost normal amounts. After ten days the storage of sperm causes the vesicles to swell. The time needed for half of the testes to produce sperm after the temperature shift is only slightly longer than the average time needed at 25°C for germ cells in wild-type males to proceed from the onset of meiosis to mature sperm (120 h, see Lindsley and Tokuyasu, 1980). This is a strong indication that at 18°C spermatogenesis is frequently blocked prior to meiosis or that meiosis has to take place at 25°C in order to result in functional spermatozoa.

Even though the frequency of exceptional gametes produced by *asp* males grown at 25°C is already very high, there are mutants in which this frequency is even higher (Peacock *et al.* 1975; McKee, 1984). Therefore, since all the phenotypic traits are stronger in individuals grown at 18°C than in those grown at 25°C (Ripoll *et al.* 1985; Gonzalez, 1986), the frequency of exceptional gametes in *asp* males is expected to be higher when meiosis takes place at the lower temperature. The analysis of the production of exceptional sperm for the sex chromosomes in *asp* males reared at different temperatures are shown in Table 1. The data show either that this trait is not cold sensitive or that meiosis has to take place at the semirestrictive temperature in order to result in functional sperm. The complementary experiment, males grown at 25°C and crossed at 18°C, does not provide information since homozygous *asp* males do not mate at 18°C and consequently they store the sperm produced at 25°C. Nevertheless, transfer of *asp* males from 25 to 18°C has served to establish that, unlike other phenotypic traits, the motility of their sperm is not affected by the temperature shift: up to ten

days after transfer to 18°C the sperm stored in the seminal vesicles remain fully motile.

In summary: (i) spermatogenesis in *asp* males grown at 18°C is so disorganized that it prevents further examination; (ii) this abnormal spermatogenesis can, at least preliminarily, be traced back to defective meiosis and (iii) males grown at 25°C produce exceptional progeny (also indicative of defective meiosis) but have apparently normal testes. To know how meiosis is affected and if these defective meiosis also alter spermiogenesis, we have carried out a cytological analysis of testes of mutants grown at 25°C.

Meiosis

Observation of unfixed *asp* cells under phase-contrast optics does not reveal defects during spermatogonial divisions and, under the electron microscope, all primary spermatocytes are morphologically normal prior to meiosis (data not shown).

The first obvious and consistent defects are seen during meiosis. Immunostaining of spindles with an anti-tubulin antibody (Fig. 2) reveals departures from the wild-type spindle. Whereas the meiotic spindles in wild-type individuals show a typical two-cup-shaped structure (Fig. 2A,B, and Casal *et al.* 1990) with microtubules that run from each pole to an unstained region in the equator of the cell, *asp* spermatocytes contain bundles of long microtubules together with a more diffuse microtubular network (Fig. 2C,D). A more detailed description of abnormal microtubular structures in *asp* individuals during male meiotic divisions and embryonic and neuroblast mitoses will be presented elsewhere (Gonzalez *et al.* unpublished).

When meiotic cells are squashed and stained with acetic-orcein, the most conspicuous defect can be seen during metaphase. In wild-type males, during prometaphase I the bivalents orientate and congress to the metaphase plate (Goldstein, 1980; Church and Lin, 1982, 1985). We scored 153 meiosis I cells in *asp* males. Around 30% of them were expected to be in metaphase I (Goldstein, 1980, and our observations). However, we found no cells in which bivalents were arranged on a metaphase plate. This lack of arrangement is also evident during the second meiotic division. 110 meiosis II cells were scored in *asp* males, and again we observed no cells resembling a metaphase II (we expected at least 10 to be in metaphase II). The chromosomes always appear scattered in the nucleus. Secondary spermatocytes are frequently aneuploid (Fig. 2E–G, and Ripoll *et al.* 1985) most probably due to irregular distribution of chromosomes during the first meiotic division.

Spermiogenesis

The first postmeiotic defects found in *asp* males are seen during the onion stage. Each cyst of early spermatids in wild-type males contains the same number of nuclei as nebenkerns. This is not the case in *asp* males where cells with more than one nucleus can be seen (Fig. 3). Of a sample of 487 nebenkerns, we counted 843 nuclei associated with them, i.e. there were 1.7 times more nuclei than nebenkerns. The size of the nuclei is

Table 1. Sex chromosome recovery in *asp* males

Male genotype	Temperature of growth (°C)	Number of male gametes (frequency)				Total progeny	Progeny per male
		X	Y	XY	0		
M*	18	352 (0.47)	148 (0.20)	30 (0.04)	225 (0.30)	755	7.6
M†	18	269 (0.48)	101 (0.18)	11 (0.02)	176 (0.32)	557	7.0
M*	25	496 (0.46)	165 (0.15)	26 (0.02)	385 (0.36)	1072	10.7
M‡	25	1526 (0.47)	627 (0.19)	157 (0.05)	943 (0.29)	3253	ns§
T*	18	538 (0.54)	455 (0.46)	1 (<0.01)	0 (0.00)	994	49.7
T‡	25	4772 (0.53)	4063 (0.46)	5 (<0.01)	11 (<0.01)	8751	ns§

Homozygous or heterozygous *asp* males were grown at 18°C or 25°C and mated at 25°C.

* 100 *y/y⁺Y*; *red asp* (M) and 20 *y/y⁺Y*; *red asp/TM2* (T) males grown at 18°C were individually mated to two *y f B* females for ten days. Their offspring were counted for nine days after emergence of the first adults.

† 80 M males grown at 18°C were held without females at 25°C for five days and then mated to females as above.

‡ Data from Ripoll *et al.* (1985).

§ Not scored.

variable in *asp* males (compare Fig. 3A with 3B). A plot of the distribution of nuclear diameters in *asp* and control onion-stage spermatids is shown in Fig. 3C. Whereas the wild-type nuclei show a narrow distribution of diameters, indicating a uniform chromosomal content (Gonzalez *et al.* 1988; 1989a) *asp* nuclei show a rather broad distribution. The distribution of nuclear diameters shows that their chromosomal content ranges from a single chromosome to three times the normal haploid complement. The size of the nebenkern is also variable in *asp* males. Besides the variations in size, no morphological alterations in mutant nuclei or nebenkerns were seen either under phase-contrast optics or in EM sections (data not shown).

Fig. 4 shows that elongating cysts of spermatids from *asp* males differ from their wild-type counterparts (Tokuyasu, 1974, 1975). A large number of spermatids containing more than one axoneme can be seen in all cysts. Although the structure of most mitochondrial derivatives is normal, large differences in the size and number are observed. Instead of the normal pair of derivatives (one 'major' and one 'minor'), spermatids with two small derivatives or spermatids in which the 'minor' is larger than the 'major' are found. Due to the differences in size, we have defined as major mitochondrial derivatives those that have at least one paracrystalline body (i.e. an area of dense crystalline material). The number of mitochondrial derivatives per spermatid is also variable. Occasionally, the structure of the mitochondrial derivatives is altered: we have found several sections of elongating spermatids in which some mitochondrial derivatives have cytoplasmic inclusions containing microtubules. These abnormal mitochondrial derivatives are always exceedingly large. The association between axonemes and mitochondrial derivatives is frequently also abnormal. Whereas in wild type each mitochondrial derivative is associated only with a single axoneme, a number of spermatids in *asp* males contain derivatives associated with a pair of axonemes. In all cases, each mitochondrial derivative is associated with no more than two axonemes, and no axoneme has been found associated with more than two mitochondrial derivatives. In those cases where two

axonemes are associated with a 'major' derivative, two paracrystalline bodies are formed. The amount of paracrystalline material deposited in the 'major' derivative seems to be independent of the size of the mitochondrial derivative. Even those elongating flagella with the normal complement (two mitochondrial derivatives and one axoneme) rarely present the arrangement found in wild-type males, where the angles between the mitochondrial derivatives and the axoneme are fixed (Tokuyasu, 1974).

Although there are a large number of alterations found during the elongation stage, axoneme structure in *asp* individuals is always completely normal. A majority of the cysts have 64 axonemes, although there are cases of cysts with a slightly lower number of axonemes as it happens sometimes in wild type (Hardy, 1975). Axonemes from wild-type and *asp* spermatids are indistinguishable (Fig. 5). In both genotypes, the typical axoneme structure, one pair of central tubules surrounded by nine pairs, is found. In later stages, accessory tubules, dynein arms and other typical structures are also seen both in wild-type and *asp* axonemes. Other microtubular arrays such as perinuclear microtubules seem to be normal, both in number and distribution, during nuclear transformation (data not shown).

Failures during the individualization stage are seen in *asp* males. Almost every cyst undergoing individualization contains several spermatids that remain syncytial (Fig. 5C). These non-individualized spermatids seem to be eliminated during the coiling stage since an excess of debris can always be seen in the basal end of *asp* testes.

Discussion

The observations reported in the present work suggest that the defects caused by mutation in *asp* are spindle-specific. We have shown by immunofluorescence that meiotic spindles are abnormal in mutant males. However, cytoplasmic and perinuclear microtubules as well as axonemes do not show any morphological abnormalities in *asp* males. Some of these structures must be

Fig. 2. Meiosis I in control and homozygous *asp* males. Double staining of meiotic cysts of heterozygous, y/y^+Y ; *red asp*/*TM2*, (A-B) and homozygous, y/y^+Y ; *red asp*, males (C,D); an anti-tubulin antibody was used to visualize the meiotic spindles (B and D) and the dye Hoechst 33342 was employed to stain DNA (A and C). The stage of meiosis was determined by the degree of chromatin condensation (Kremer *et al.* 1986). Spindles in *asp* are disorganized and never show the typical wild-type two-cup shape. (E-G) Squashes of secondary spermatocytes stained with acetic-orcein to visualize their chromosomal content. Instead of having the normal complement (E), secondary spermatocytes in *asp* males are frequently aneuploid (F,G). Bar, 5 μm .

Fig. 3. Early effects of meiotic dysfunctions in homozygous *asp* males. (A,B) Unstained phase contrast view of early spermatids during the 'onion stage'. Wild-type spermatids (A) show nuclei (arrow) and nebenkerns (arrowhead) of uniform size. Spermatids of homozygous *asp* males (B) show variations in the size of the nuclei and nebenkerns. (Arrowhead) small nucleus; (arrow) multinucleated spermatid. Bars, 10 μm. (C) Plot of the distribution of nuclear diameter in 'onion stage' spermatids of *asp* males (shadow) and wild-type males (open).

functional in order to give rise to viable zygotes, namely cytoplasmic and perinuclear microtubules, thought to play a fundamental role in spermatid elongation and nuclear shaping (Wilkinson *et al.* 1974; Fuller *et al.* 1987), and sperm flagella, which are fully motile. In addition, our data suggest that, in order to give rise to functional sperm, meiosis has to take place at 25°C, indicating that the cold-sensitive structure responsible for male sterility at 18°C has to be functional during meiosis. In contrast the sperm flagella are not affected by the temperature shift since sperm remain motile after several days at 18°C.

If the defects caused by the mutant allele we have studied are specific to the meiotic spindle, it follows that any postmeiotic abnormality present in *asp* males should be either a direct consequence of an abnormal meiotic spindle, a result of a cascade of events originated during meiosis, or the reflection of some property of normal structures facing circumstances unlikely to occur during normal spermatogenesis.

Since the segregation of the genetic material during both meiotic divisions is mediated by the spindle, a consequence of the existence of defective spindles in *asp* males must be the production of aneuploid post-meiotic nuclei. The variable size attained by early

spermatid nuclei is a direct reflection of their differences in chromosome content (Gonzalez *et al.* 1989a). Variations in nuclear size and multinucleated spermatids are also found in males homozygous for mutations in other loci related with spindle function such as *B2t⁸* (Fuller *et al.* 1987, 1988), *polo* (Sunkel and Glover, 1988) and *hay^{nc2}* (Regan and Fuller, 1988), mutations resulting in male sterility (Lifschytz and Hareven, 1977; Lifschytz and Meyer, 1977) as well as in males carrying rearrangements that disrupt the normal pairing between homologous chromosomes (Hardy, 1975; Gonzalez *et al.* 1989a).

Multinucleated spermatids in which nuclei have different sizes are due to failures in chromosomal segregation and cytokinesis resulting from spindle malfunction and not to absence of spindles. In testes of *B2t^D* (Kemphues *et al.* 1980, 1982) and *mgr* (Gonzalez *et al.* 1988) males, meiotic spindles are not formed and consequently cytokinesis does not take place. These males exhibit neither multinucleated spermatids nor variations in size among their nuclei (Kemphues *et al.* 1980; 1982; Gonzalez *et al.*, 1988). Failures in one or both meiotic cytokineses also must occur in *asp* males in order to account for the existence of spermatids containing more than one axoneme. Although we did not

Fig. 4. Elongating cysts of *asp* males. (A) Axonemes (ax), 'major' (M) and 'minor' (m) mitochondrial derivatives. Note the lack of the normal arrangement between mitochondrial derivatives and axonemes. Several spermatids show more than one axoneme (thick arrows and arrowheads) and some of them also show a number of mitochondrial derivatives higher than normal (thick arrows). Cytoplasmic inclusions are sometimes present in large mitochondrial derivatives (thick arrows). Cytoplasmic microtubules are presented both inside and outside of the mitochondrial derivatives (thin arrows). (B) Elongating cyst in an advanced stage. The paracrystalline material (p) deposited in the 'major' mitochondrial derivatives show an uniform size among spermatids. Large (thick arrow) and small (arrowhead) 'major' derivatives. Occasionally, spermatids with a 'minor' derivative bigger than the 'major' are observed (thin arrow). Bar, 0.5 μ m.

Fig. 5. (A,B) The axoneme (arrows) in wild-type (A) and *asp* (B) males show identical structure. Thin arrows point to cytoplasmic microtubules. Note that the orientation of both axonemes in B is identical. Bar, 0.1 μ m. (C) Individualizing cyst. Whereas most spermatids are already wrapped by individual membranes (arrowhead), some spermatids remain syncytial (arrow). Bar, 0.5 μ m.

examine elements of the cytoskeleton implicated in cytokinesis other than microtubules, failures in spindle formation and/or function could give rise to failures in cytokinesis (Rappaport, 1986).

Even though we never found alterations in the shape of the nebenkerns of early spermatids in *asp* males, we do see abnormalities in the number, size and, occasionally, morphology of the mitochondrial derivatives during the elongation stage. In *asp* males, postmeiotic defects appear to be the consequence of defective meiosis. The abnormal size attained by mitochondrial derivatives in spermatids of *asp* is probably the product

of an altered segregation of mitochondria during meiosis, since variations in the size of early spermatid nebenkerns can be observed. On the other hand, the abnormal number of mitochondrial derivatives in some *asp* spermatids is likely a secondary effect of the existence in these spermatids of more than one elongating axoneme. As these axonemes elongate, the unique nebenkern may unfold giving rise to several mitochondrial derivatives that elongate alongside the axonemes in a process similar to the one taking place in wild-type males (Tokuyasu, 1975).

In spite of the defects seen during spermiogenesis in

asp males, these males are still capable of producing many functional spermatozoa. Spermatids with drastic defects are probably eliminated. In fact, spermatogenesis in *Drosophila* seems to be provided with mechanisms to eliminate those spermatids that suffered mistakes in their formation. These mechanisms take place mainly during the individualization and coiling stages (Tokuyasu *et al.* 1972a, 1972b). Hardy (1975) described the elimination of multinucleated spermatids resulting from failures in the pairing of homologous chromosomes during meiosis. These spermatids failed to be wrapped by individual membranes, so that they remained as a cyst and were eliminated when the whole cyst was coiled. Elimination of spermatids has been also described in males showing meiotic drive and it is thought to be the cause of the differences in the recovery between gametic classes (Peacock *et al.* 1975; Tokuyasu *et al.* 1977; Ivy, 1981). Syncytial spermatids are observed in individualizing spermatids of *asp* males and an excess of debris is seen in the basal end of the *asp* testis, likely the product of the elimination and degeneration of abnormal spermatids.

All the phenotypical traits discussed above strongly point towards defective meiosis as the primary cause of the alterations seen in *asp* spermatogenesis. As we pointed out in the Introduction, a defect in a given stage can give rise to a cascade of abnormalities later on due to the programming of different developmental events in the absence of transcription. Hopefully, the study of stage-specific loci such as *asp* will help us to interpret the role of genes involved in the morphogenesis of the sperm, as well as the different processes taking place in an orderly fashion during spermatogenesis in wild type.

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