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Mutations of the *fizzy* locus cause metaphase arrest in *Drosophila melanogaster* embryos

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SUMMARY

We describe the effects of mutations in the *fizzy* gene of *Drosophila melanogaster* and show that *fizzy* mutations cause cells in mitosis to arrest at metaphase. We show that maternally supplied *fizzy* activity is required for normal nuclear division in the preblastoderm embryo and, during later embryogenesis, that zygotic *fizzy* activity is required for the development of the ventrally derived epidermis and the central and peripheral nervous systems. In *fizzy* embryos, dividing cells in these tissues arrest at metaphase, fail to differentiate and ultimately die. In the ventral epidermis, if cells are prevented from entering mitosis by using a *string* mutation, cell death is prevented and the ability to differentiate ventral epidermis is restored in *fizzy*;*string* double mutant embryos. These results demonstrate that *fizzy* is a cell cycle mutation and that the normal function of the *fizzy* gene is required for dividing cells to exit metaphase and complete mitosis.

Key words: *Drosophila*, *fizzy* gene, cell cycle, mitosis, metaphase arrest

INTRODUCTION

In recent years, the combination of genetic, molecular and cell biological approaches has led to a considerable advance in our knowledge of the cell cycle and mitosis. Many of the proteins that either regulate progression through the cell cycle or constitute the structural components of the mitotic apparatus have now been identified and studied in a variety of eukaryotic cells (see reviews by Nurse, 1990; Sawin and Scholey, 1991; Brinkley et al., 1992).

On the regulatory side, one crucial cell cycle regulator is the $p34^{cdc2}$ kinase of the fission yeast *Schizosaccharomyces pombe* and its homologues in other species (Simanis and Nurse, 1986; Lee and Nurse, 1987; Dunphey et al., 1988; Gautier et al., 1988; Jimenez et al., 1990; Lehner and O'Farrell, 1990). These proteins have been shown to function as part of a complex, equivalent to MPF (maturation-promoting factor) of *Xenopus* (Dunphey et al., 1988; Gautier et al., 1988), which plays a key role in controlling entry into and exit from mitosis (reviewed by Dunphy and Newport, 1988; Lee and Nurse, 1988). The activity of the $p34^{cdc2}$ kinase is regulated in part by its association with various cofactors, such as the cyclins, and in part by changes in the phosphorylation state of the $p34^{cdc2}$ kinase complex (Booher et al., 1989; Draetta et al., 1988, 1989; Dunphy and Newport, 1989; Gautier et al., 1989). In *S. pombe*, a regulatory network of kinases and phosphatases initiate mitosis by dephosphorylation of the $p34^{cdc2}$ protein, which activates the kinase activity of the MPF complex (Gould and Nurse, 1989; Morla et al., 1989). In contrast to the regulated dephosphorylation that initiates mitosis, exit from mitosis is achieved by destroying the MPF complex (reviewed by Hunt, 1991). In active MPF, the cdc2 kinase is one half of a heterodimer, the other component of which is one of the mitotic cyclins, cyclin A or cyclin B (Draetta et al., 1989; Labbé et al., 1989). Destruction of cyclins A and B occurs at different times during mitosis: in the post-blastoderm embryonic cell cycles of *Drosophila*, cyclin A levels peak during prophase and decline prior to metaphase, whereas degradation of cyclin B does not occur until the metaphase-anaphase transition (Whitfield et al., 1990). The destruction of cyclin B is essential for the cell to exit mitosis; failure to do so appropriately has been shown to result in mitotic arrest in *Xenopus* oocytes and the budding yeast *Saccharomyces cerevisiae* (Murray et al., 1989; Ghiara et al., 1991) and, in *Xenopus* oocyte extracts, the rapid destruction of cyclin B at this time has been shown to depend on ubiquitin-dependent proteolysis (Glotzer et al., 1991).

Whilst inactivation of MPF kinase activity is a crucial

step involved in exiting mitosis, in *S. pombe* and *Aspergillus nidulans* exit from mitosis has also been shown to require the activity of type 1 protein phosphatases encoded by the *dis*2 and *bim*G genes, respectively (Ohkura et al., 1989; Kinoshita et al., 1990; Doonan and Morris, 1989). This is perhaps not surprising; since high levels of MPF kinase activity maintain cells at metaphase, one might expect that the activity of at least one phosphatase, in addition to loss of MPF activity, would consequently be required to drive anaphase events. Furthermore, this suggests that the activities of the cellular proteins that form the structural components of the mitotic apparatus (e.g. spindle fibers, kinetochores etc.), and are required to carry out the physical events of late mitosis (anaphase chromosome movement, cytokinesis etc.), may be activated at least in part by protein dephosphorylation reactions. One recent and dramatic example where this appears to be the case is the finding that the direction of chromosome movement along microtubules in vitro depends on the phosphorylation state of a component of the kinetochore and that dephosphorylation of a kinetochore component is required for anaphase-like movement (Hyman and Mitchison, 1991).

This also illustrates the point that, in addition to regulatory proteins that govern progress through mitosis, the normal function of the mitotic apparatus, and the structural proteins of which it is comprised, are also, obviously, necessary for normal anaphase events to proceed. Biochemical and genetic data demonstrate that the integrity of structures such as the spindle fibers and the kinetochore are essential for normal anaphase. For instance, disruption of spindle function by microtubule poisons (Sluder, 1979; Hoyt et al., 1991; Li and Murray, 1991) can result in metaphase arrest. Similarly, injections of antibodies against a kinetochore-associated protein, CENP-E can block or delay progress into anaphase (Yen et al., 1991). In addition, the *Drosophila* kinesin-like protein *ncd* (Endow et al., 1990; Walker et al., 1990; McDonald et al., 1990) and cytoplasmic dynein (Paschal and Vallee, 1987; Pfarr et al., 1990; Steuer et al., 1990) are believed to act as motor proteins that could be involved in anaphase chromosome movement along the spindle fibers.

Lastly, the regulation of MPF kinase inactivation is thought to constitute a checkpoint for exit from mitosis (reviewed by Nishimoto et al., 1992). Some form of feedback loop is thought to exist such that destruction of MPF and commitment to exit from mitosis only occurs when all the earlier mitotic events necessary for anaphase to proceed correctly, such as proper spindle formation, have themselves occurred normally. Treatment of *S. cerevisiae* with microtubule poisons, which destabilize or destroy the mitotic spindle, results in metaphase-arrested cells, which have high levels of MPF histone H1 kinase activity (Hoyt et al., 1991; Li and Murray, 1991). These authors have isolated mutations that are insensitive to such treatments, fail to maintain high histone H1 kinase activity and exit mitosis even though their spindles are abnormal; demonstrating that such feedback control, at least between MPF activity and spindle function, exists.

We have outlined above a brief summary of what is currently known about various factors, both structural and regulatory, that are required for cells to exit mitosis. In this report, we describe the affects of mutations of the *fizzy* (*fzy*) locus (Nüsslein-Volhard et al., 1984) on the development of *Drosophila* embryos. We show that *fzy* is a novel cell cycle gene in this organism, mutations of which result in mitotic cells arresting at metaphase. Consequently, we propose that the wild-type function of the *fzy* gene is required for cells to successfully exit mitosis and discuss our results with regard to the data described above.

MATERIALS AND METHODS

Fly stocks and genetics

Other than *fzy* alleles, which we describe in this paper, details of the other stocks used can be found either in Lindsley and Zimm (1992) or Ashburner et al. (1990). The β-gal enhancer trap lines used are described by Ghysen and O'Kane (1989) and Vaessin et al. (1991).

We obtained *fzy* embryos from balanced stocks of fzy^- / CyO flies. By utilizing a CyO balancer chromosome that carried a *ftz*::β-gal insert, we could unambiguously identify *fzy* embryos by their lack of β-gal staining prior to and/or independently from the appearance of any morphological abnormalities.

To determine the effects of the maternal dose of fzy^+ on the development of *fzy* embryos, we compared embryos derived by crossing fzy^- / CyO males with (1) fzy^- / + ; + / TM3 females and (2) fzy^- / + ; Dp(2;3) osp^3, fzy^+ / + females for both the fzy^1 and fzy^3 alleles. In each case, lethal embryos with a fzy^- phenotype were recovered at the expected frequencies of approximately 25% and 12.5% for crosses (1) and (2) respectively.

fzy; *stg* double mutant embryos were obtained by crossing fzy^1 / fzy^3; Dp(2;3)osp^3, fzy^+ / stg^2 males and females. Since Dp(2;3)osp^3 is closely linked to stg^+ (less than 5 map units apart) the duplication chromosome usually segregates as fzy^+ stg^+ and the stg^2 chromosome as fzy^- stg^-. This cross produces 25% lethal embryos of which approximately 95% are *fzy*; *stg* double mutants.

Temperature-shift experiments

For the temperature-shift experiments, cages of fzy^4 / CyO flies were set up at 25°C and 18°C for 24 hours and 48 hours, respectively, prior to beginning egg collections. Eggs were collected on yeasted apple juice plates for periods of 2 hours at 25°C and 4 hours at 18°C, maintained at the collection temperature for the desired period of time and then shifted up or down as appropriate. After sufficient time to allow wild-type embryos to hatch, all unhatched embryos were mounted to examine their cuticles. At least 20 fzy^- embryos were examined at each time point and at least 40 fzy^- embryos were examined for time points found to be within the temperature-sensitive period. We have taken embryonic development at 18°C be twice as long as at 25°C for use as a correction factor when compiling the data.

Immunohistochemistry, histology and cuticle preparations

Antibody labeling of whole-mount embryos was essentially according to the protocol of Mitchison and Sedat (1983). Antibodies used were anti-Ubx, mAb FP.3.38 (White and Wilcox, 1984); mAb 22.C.10 (Zipursky et al., 1984); mouse monoclonal anti-β-gal (Promega); and fluorescein-conjugated anti-HRP antibodies (Cappel). Except for anti-HRP, antibody staining was visualized by the peroxidase/DAB method using the Vectastain Elite Kit (Vector Labs.). For double labeling, fuchsin staining was performed after antibody labeling reactions. Embryos were stained with basic fuchsin according to the protocol of Wieschaus and Nüsslein-Volhard (1986), except that embryos were fixed in 4%

paraformaldehyde in PBS over heptane and devitellinised en mass with methanol. Embryonic cuticle preparations were made according to the method of Van der Meer (1977).

Confocal and scanning electron microscopy

Confocal images of fuschin-stained nuclei were collected on a Bio-Rad MRC 600 system, using the yellow filter normally used for propidium iodide, attached to a Zeiss Axiovert compound microscope. Optical sections were combined using the PROJECT program. For photo reproduction, image files were transferred to a Macintosh IIfx computer via an ethernet link, converted to PICT format using Adobe Photoshop (Adobe Photosystems, Inc.) and printed out on T-Max100 film (Eastman Kodak) using a Matrix SliderWriter.

For scanning electron microscopy, embryos from fzy^{-} / CyO, *ftz*::β-gal stocks were fixed and stained with anti-β-gal antibodies to identify fzy^{-} embryos. fzy^{+} and fzy^{-} embryos were then separated and postfixed with 3% glutaraldehyde overnight at 4°C, dehydrated through a graded ethanol series, critical point dried, sputter coated with gold-palladium and examined on a ISI SS40 scanning electron microscope.

RESULTS

Genetic characterization of alleles at the *fzy* locus

The *fzy* locus was discovered and initially characterized by Nüsslein-Volhard et al. (1984) in their screen for embryonic lethal loci on the second chromosome. Subsequently, further *fzy* alleles were identified in a screen for lethal loci in the 35F interval by Ashburner et al. (1990) and we have fortuitously identified a further allele on the Df(2L)Sco^{R+16} chromosome (I. Dawson, unpublished observations and this work). We have examined the properties and phenotypes of the currently extant *fzy* alleles and summarize this data in Table 1. This collection of *fzy* mutations forms an allelic series ranging in severity from strong alleles such as fzy^{1}, which is recessive embryonic lethal, through to weak alleles such as fzy^{6}, which is homozygous viable but female sterile when raised at 18°C. The two strongest alleles we have analyzed, fzy^{1} and fzy^{3}, are amorphic (i.e. null) by the genetic criteria that their cuticle and mitotic arrest phenotypes are the same whether each is homozygous or hemizygous over either Df(2L)RN2 or Df(2L)r10, both of which deficiencies completely remove the *fzy* locus (I. Dawson and S. Roth, unpublished observations).

Maternal *fzy* product is required for preblastoderm nuclear cleavage division in the early embryo

Most of the available alleles of *fzy* are recessive lethals; however, combinations of weak hypomorphic alleles, such as fzy^{6} homozygous or fzy^{6}/fzy^{7} when raised at 18°C give rise to viable, morphologically normal adults. These are male fertile but female sterile. Female fertility is restored by the addition of a fzy^{+} duplication to these genotypes consistent with this sterility being due to lesions at the *fzy* locus.

Both homozygous fzy^{6} and fzy^{6}/fzy^{7} females lay approximately normal numbers of morphologically normal eggs. Examination of dechorionated eggs from these females under a dissecting microscope showed no indication of any embryonic development. Thus the female sterility is due to a requirement for maternal fzy^{+} activity very early during embryonic development. We refer to the embryos derived from homozygous fzy^{6} or fzy^{6}/fzy^{7} mothers as $fzy^{\text{maternal (mat)}}$ embryos.

To analyze the fzy^{mat} phenotype in greater detail, we examined fixed, fuchsin-stained fzy^{mat} embryos to determine if the early syncitial nuclear cleavage divisions were occurring. During the first 2 hours of development of wild-type embryos, the zygotic nucleus divides syncitially 13 times; during the later rounds of division the majority of nuclei migrate to the periphery of the embryo where they then cellularise (Campos-Ortega and Hartenstein, 1985). Thus by 2 hours of development the wild-type embryo has formed a syncitial blastoderm consisting primarily of a monolayer of approximately 5000 nuclei tightly packed around the periphery of the oocyte. In contrast, in the 2 to 3 hour fzy^{mat} embryos that we examined (n=79), we found only 2, 3 or 4 internal diploid nuclei plus the polar bodies (Fig. 1A). Fur-

Table 1. A summary of the properties and phenotypes of the available *fzy* alleles

$Allele^{\text{no. (synonym)}}$	Classification	Homozygous phenotype*
fzy^{1} (*IB115*)	Amorph	Embryonic lethal: ventral epidermis absent, CNS degenerate, PNS absent.
fzy^{2} (*IH108*)	Amorph	Embryonic lethal: as fzy^{1}.
fzy^{3} (*X4*)	Amorph	Embryonic lethal: as fzy^{1}.
fzy^{4} (*AB155*)	Strong hypomorph (Temperature sensitive)	25°C - Embryonic lethal: as fzy^{1}. 18°C - Embryonic lethal: holes in ventral epidermis, CNS degenerate.
fzy^{5} (*AR84*)	Intermediate hypomorph (Temperature sensitive)	25°C - Larval/Pupal Lethal: not examined. 18°C - Pharate adult lethal: macrochaete missing, abdominal cuticle absent.
fzy^{6} (*AS14*)	Weak hypomorph (Temperature sensitive)	25°C - Pharate adult lethal: macrochaete missing, abdominal cuticle absent. 18°C - Viable, female sterile: eggs normal, embryos fail to develop.
fzy^{7}	Weak hypomorph (Temperature sensitive)	25°C - Pharate adult lethal: as fzy^{6} at 25°C. 18°C - Viable, female sterile: as fzy^{6} at 18°C.

*Except for fzy^{7}, for which the heterozygous fzy^{6} / Df(2L)Sco^{R+16}, fzy^{7} phenotype is described.

The fzy^{1} and fzy^{2} alleles were recovered by Nüsslein-Volhard et al. (1984) and are EMS induced. We have not been able to obtain a copy of the fzy^{2} stock to examine its phenotype but it is reported to be similar to fzy^{1} (Nüsslein-Volhard et al., 1984). The fzy^{3} to fzy^{6} alleles are also EMS induced and were recovered by Ashburner et al. (1990). The fzy^{7} allele was found by us on the Df(2L)Sco^{R+16} chromosome. It is probably X-ray induced as it appears to have arisen simultaneously with, but independent from, the Sco^{R+16} deficiency to which it is tightly linked (Ashburner et al., 1983, 1990; I. Dawson, unpublished observations).

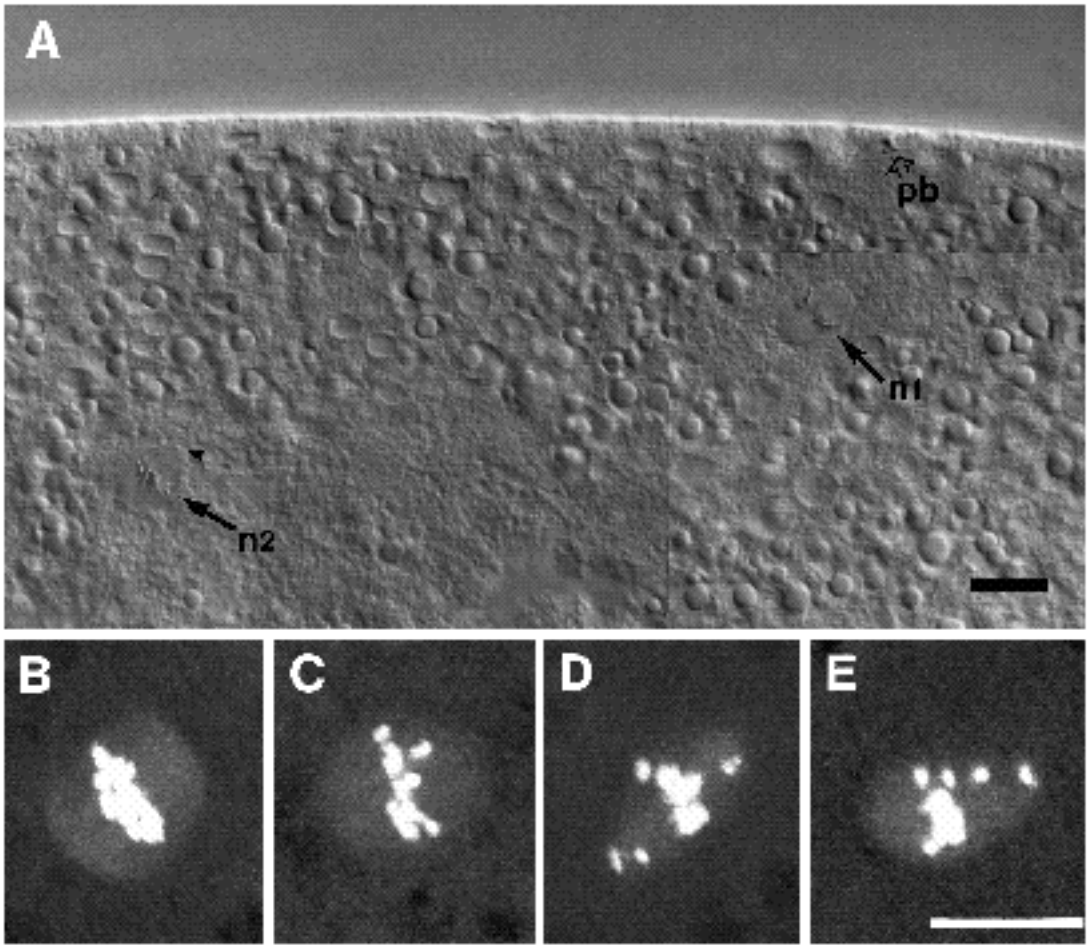

Fig. 1. The *fzy*^{mat} phenotype. (A) A montage of optical sections through a 2 to 3 hour old *fzy*^{mat} embryo stained with fuschin and viewed with DIC optics. This particular embryo contained only two internal diploid embryonic nuclei (n1 and n2, arrows) plus two polar bodies, only one of which is visible in the focal planes shown (pb, open arrow). All the chromosomes of nuclei 1 are arranged on a metaphase plate: nuclei 2 is a chimeric metaphase/anaphase nucleus, most chromosomes are arrayed on a metaphase plate but one pair of chromatids has separated and migrated to the spindle poles, only one of these is in focus (arrowhead). (B-E) Examples of fuschin-stained nuclei from *fzy*^{mat} embryos reconstructed as a set of projected images from confocal micrographs to show chromosome distribution. (B) An example of a typical nuclei found in *fzy*^{mat} embryos with condensed chromosomes arranged on a metaphase plate, metaphase nuclei make up approximately 75% of the nuclei in these embryos. (C-E) Examples of chimeric metaphase/anaphase nuclei found in *fzy*^{mat} embryos with most chromosomes arranged on a metaphase plate but with others having begun to migrate towards the spindle poles. Note that anaphase-like separation in *fzy*^{mat} embryos is frequently abnormal, in C, only one of the pair of chromatids appears to have left the metaphase plate, in D, two chromatids have migrated to the lower pole but only one is visible at the upper, in E, two pairs of chromatids appear to have separated, but only the set on the right has made substantial progress towards the pole. Scale bars are approximately 10 μm.

thermore, the majority of these nuclei (approximately 75%) in *fzy*^{mat} embryos were at metaphase, with all the chromosomes arranged on a metaphase plate (Fig. 1B); the remainder were chimeric metaphase/anaphase nuclei with most of the chromosomes on a metaphase plate except for one or two sister chromatids which had separated and begun migrating to the spindle poles (Fig. 1C,D,E). The chromosome number, gross chromosome morphology and spindle organization appear normal at this level of examination.

These data show that maternal *fzy*$^{+}$ activity is required for the preblastoderm nuclear cleavage cycles, since reduction in maternally supplied *fzy*$^{+}$ leads to mitotic arrest and a drastic reduction in the ability of the fertilized embryo to undergo nuclear division. Furthermore, maternal *fzy*$^{+}$ activity is required for the successful completion of a precise phase of the zygotic nuclear cleavage cycle, specifically the metaphase/anaphase transition.

Zygotic *fzy* is required for normal differentiation of the ventral neurogenic ectoderm

The embryonic ectoderm of *Drosophila* embryos can be subdivided into two distinct regions, the dorsal epidermal region (dER) and the ventral neurogenic region (vNR). The dER differentiates to produce the dorsal epidermis and the associated structures of the dorsal peripheral nervous system (PNS); the vNR from which are derived the neuroblasts (NBs), which produce the neurons of the central nervous system (CNS), and the ventral dermoblasts (DBs),

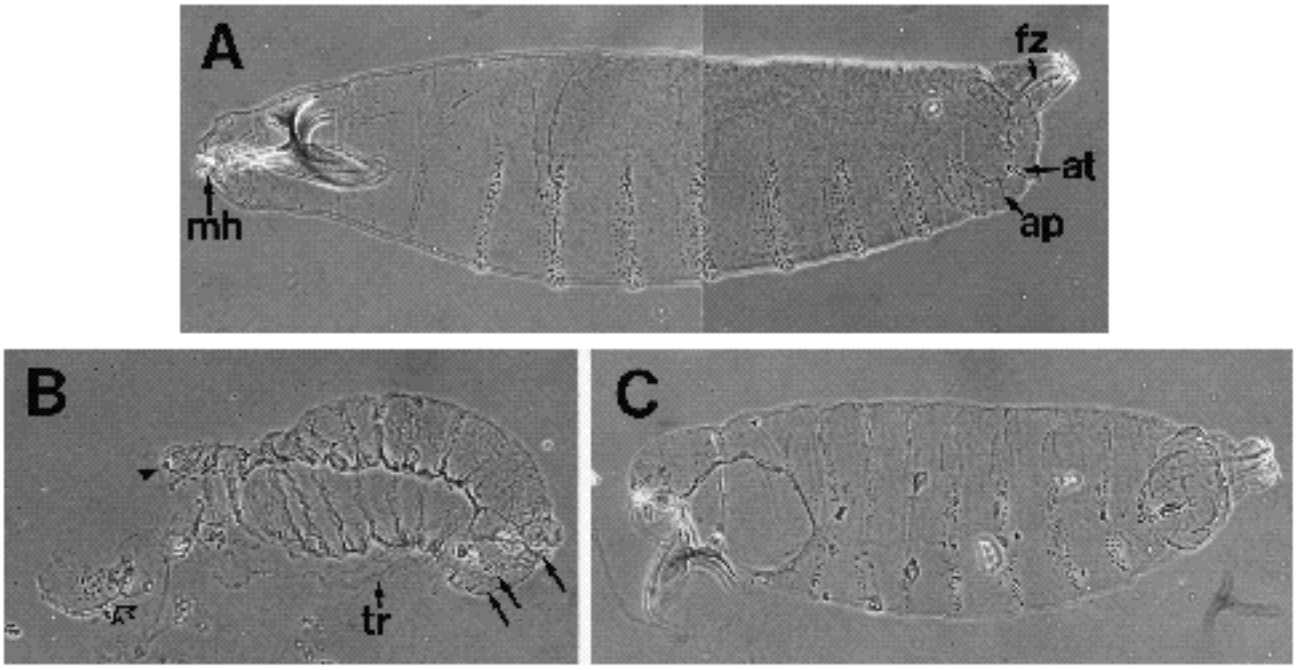

Fig. 2. Cuticle phenotypes of *fzy* embryos. Cleared cuticle preparations of pharate first instar larvae of the genotypes (A) wild type, (B) fzy^3 homozygote derived from a fzy^3/+ mother and (C) fzy^3 homozygote derived from a fzy^3/+ ; Dp(2;3)osp^3, fzy^+/+ mother. The cuticles are seen from a slightly ventrolateral perspective with anterior to the left and dorsal uppermost in all panels. The Filtzkorper (fz), anal tuft (at), anal pads (ap) and mouth hooks (mh) are indicated on the wild-type embryo (A). The differentiated cuticle of the fzy^3 embryo (B) consists only of a plate of dorsal cuticle with Filtzkorper, anal pads and anal tuft visible posteriorly (arrows), while at the anterior there are mouth hooks attached to side pieces of cephalic epidermis (arrowhead) and a piece of detached epidermis which is probably derived from the clypeolabrum (open arrow), in addition, well-developed trachea (tr) are present. The embryo in C is of the same zygotic genotype as the embryo in B and shows the extent to which the ventral epidermis and cephalic cuticle structures are rescued by doubling the dose of maternal fzy^+. In these embryos, the cuticle defects tend to be confined to the ventral thorax, which is still largely missing, and to holes and loss of denticle differentiation in the abdominal segments, particularly on or adjacent to the ventral midline.

which produce the ventral epidermis and its associated PNS structures. The majority of the cephalic epidermis is considered to be part of the vNR because of its ability to produce NBs even though it is partly derived from dorsal portions of the blastoderm. In the thoracic and abdominal regions of the embryo, the initiation of tracheal pits at the boundary between the dER and vNR, and the subsequent development of the tracheal tree, serves as a convenient morphological marker delimiting the extent of the two ectodermal regions from stage 10 of embryonic development onwards (Campos-Ortega and Hartenstein, 1985).

Cleared cuticle preparations of embryos either homozygous or hemizygous for amorphic alleles of *fzy* show that these animals die as pharate first instar larvae lacking most ventral and cephalic epidermis. The differentiated cuticle they do produce consists primarily of a plate of dorsally derived epidermis with Filtzkorper, anal pads and an anal tuft at the posterior end, while at the anterior end there are mouth-hooks and cirri attached to the dorsal plate by thin epidermal 'bridges' and a piece of detached, presumably clypeolabrally derived epidermis. A well-developed tracheal tree is also produced (Fig. 2B). Whilst the phenotype described above and shown in Fig. 2B is the typical *fzy* amorphic phenotype, it should be noted that there is noticeable variation in the cuticle phenotypes displayed even by amorphic *fzy* embryos: some embryos are less severely affected and develop occasional bridges of ventral cuticle, others are more severely affected and, in addition to lacking ventral cuticle, also have numerous small holes in the dorsally derived epidermis (data not shown).

This examination of *fzy* cuticles indicates that the epidermis usually missing in *fzy* embryos is that derived from the vNR whilst the retained portion is that derived from the dER. The extent of epidermal loss seen in these *fzy* embryos is essentially identical to that described for embryos homozygous for any of the intermediate strength zygotic neurogenic mutations (Lehmann et al., 1983). In neurogenic mutant embryos, the majority of vNR cells adopt the NB fate leading to hypertrophy of the CNS with the concomitant absence of all vNR-derived epidermal tissue (Poulson, 1937; Lehmann et al., 1983). However, despite the similarity of the cuticle phenotypes, neural hypertrophy is not the cause of the loss of ventral epidermis in *fzy* embryos, as can be seen by comparing CNSs of wild-type, *fzy* and neurogenic embryos that have been stained with anti-HRP to visualize the CNS structure (Jan and Jan, 1982; Fig. 3). The *fzy* and neurogenic embryos shown both have similar cuticle phenotypes; however, the the CNS of the *fzy* embryo shows no hypertrophy comparable to that of the neurogenic mutant. The *fzy* CNS is abnormal, both disorganized and wider compared to that of a wild-type embryo of the same stage; however, this increase in width does not reflect an

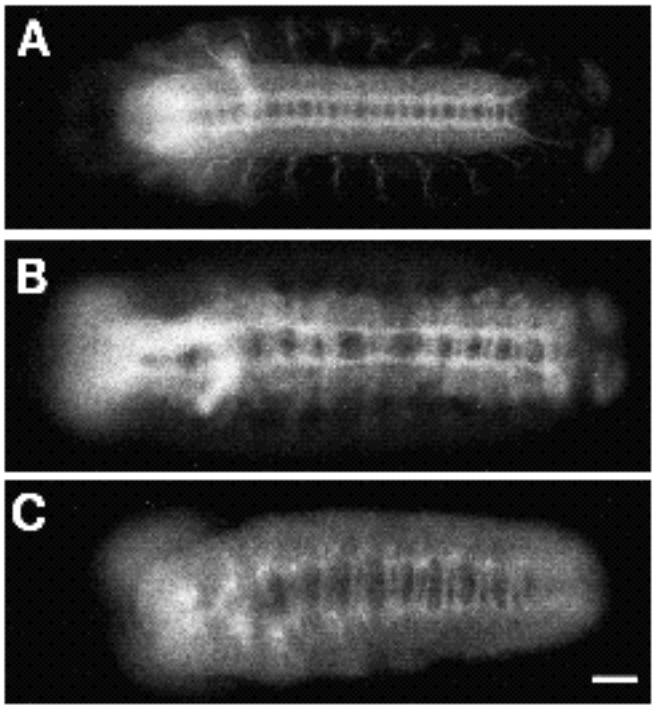

Fig. 3. The CNS of *fzy* embryos is not hypertrophied. Anti-HRP-stained CNSs of (A) wild-type, (B) fzy^{1} and (C) bib^{IIV46} stage 15/16 embryos. All are ventral views with anterior to the left. The wild-type CNS (A) has begun to contract and well-defined horizontal commissures and longitudinal connectives are visible. The CNS of the fzy^{1} embryo (B) has failed to contract properly and the commissures and connectives are poorly formed; many of the neurons are beginning to degenerate at this stage. However, compared to the bib^{IIV46} embryo (C), which has a similar cuticle phenotype to that of fzy^{1} embryos, the CNS of the fzy^{1} embryo is clearly not hypertrophied. Scale bar is approximately 20 μm.

increased number of neurons. At no point in development do we see any indication of neural hypertrophy in *fzy* embryos, as we describe in more detail below. Thus *fzy* is clearly not a neurogenic mutation.

Maternally supplied fzy^{+} activity can compensate for loss of zygotic *fzy* during development of ventral epidermis

As we have shown that maternally supplied fzy^{+} activity is present in the early embryo where it is required for the pre-blastoderm nuclear cleavage divisions, we wished to test if the maternally supplied fzy^{+} could also influence the development of the ventral DBs.

We assayed this by using a fzy^{+} duplication to vary the maternal contribution to embryos of the same zygotic *fzy* genotype (see Materials and Methods). We compared cleared cuticle preparations of parallel sets of embryos with the same zygotic *fzy* genotype, but derived from sibling mothers carrying either one or two copies of fzy^{+} (Fig. 2B,C). We found that doubling the maternal dose of fzy^{+} rescues the majority of ventral and cephalic epidermis in the mutant embryos. In addition, these experiments demonstrate that various regions of the ventral epidermis are differentially sensitive to the amount of fzy^{+} activity. The cephalic cuticle and most of the ventral cuticle of the abdominal segments is almost completely restored by the addition of an extra maternal copy of fzy^{+}. In contrast, the thoracic cuticle, including its more lateral regions, is still absent and the small holes and poorly differentiated regions of denticles that are found in the abdominal cuticle are generally restricted to the area close to and along the length of the ventral midline (Fig. 2C).

These data show that maternally supplied fzy^{+} both perdures long enough and is functionally capable of contributing to the normal development of the ventral DBs. Therefore, although we have examined and describe below the development of the ventral neurogenic ectoderm in embryos homozygous for amorphic *fzy* alleles and derived from mothers carrying only one copy of fzy^{+}, it is likely that the ventral cells of these embryos still contain some functional, maternally inherited fzy^{+} activity and we are therefore almost certainly not seeing a *fzy* null phenotype. We suspect the variability of the zygotic *fzy* cuticular phenotype described above, and of other phenotypes described below, is probably due to varying dose, perdurance or activity of maternally supplied fzy^{+}.

Lack of zygotic *fzy* causes mitotic defects and degeneration of the ventral epidermis

The most obvious phenotype seen in strong zygotic *fzy* embryos is the degeneration of tissues derived from the ventral neurogenic ectoderm. This occurs fairly late in development, beginning during stage 13 and becoming progressively worse through stages 14 and 15 until by stage 16 the ventral epidermis is completely removed (Fig. 4). Degeneration begins first in the cephalic region and then starts at anteroventral and posteroventral centers in the trunk segments, from which it spreads to the mid-trunk region and more lateral epidermis. The ventral epidermal cells undergo picnocytosis during this time (data not shown) and appear to be removed primarily by the phagocytosing activity of macrophage cells, which swarm over the exterior of the degenerating area during this process (Fig. 4B,C). The progressive nature of the ventral degeneration and the macrophage activity inferred from fixed specimens was confirmed by observing degeneration as it occurred in living *fzy* embryos (data not shown).

While it is clear that the absence of ventral cuticle seen in *fzy* embryos results from the degeneration of this tissue described above, ultimately, we wished to know what causes this degeneration to occur. To address this issue, we asked at what stage *fzy* was required for the differentiation of the ventral epidermis and at what stage we could first detect abnormalities in the ventral epidermal cells.

We conducted a series of temperature-shift experiments to determine at what stage of development zygotic fzy^{+} activity is required to promote normal development of ventral cuticle. These experiments utilized the temperature sensitivity of the cuticle phenotype of fzy^{4} homozygous embryos to assay the temporal requirements for zygotic *fzy* activity in the ventral epidermis. Embryos homozygous for fzy^{4} display a severe, almost amorphic cuticle phenotype when raised at 25°C but a much weaker phenotype when raised at 18°C (Fig. 5A,B).

The data from these temperature-shift experiments indi-

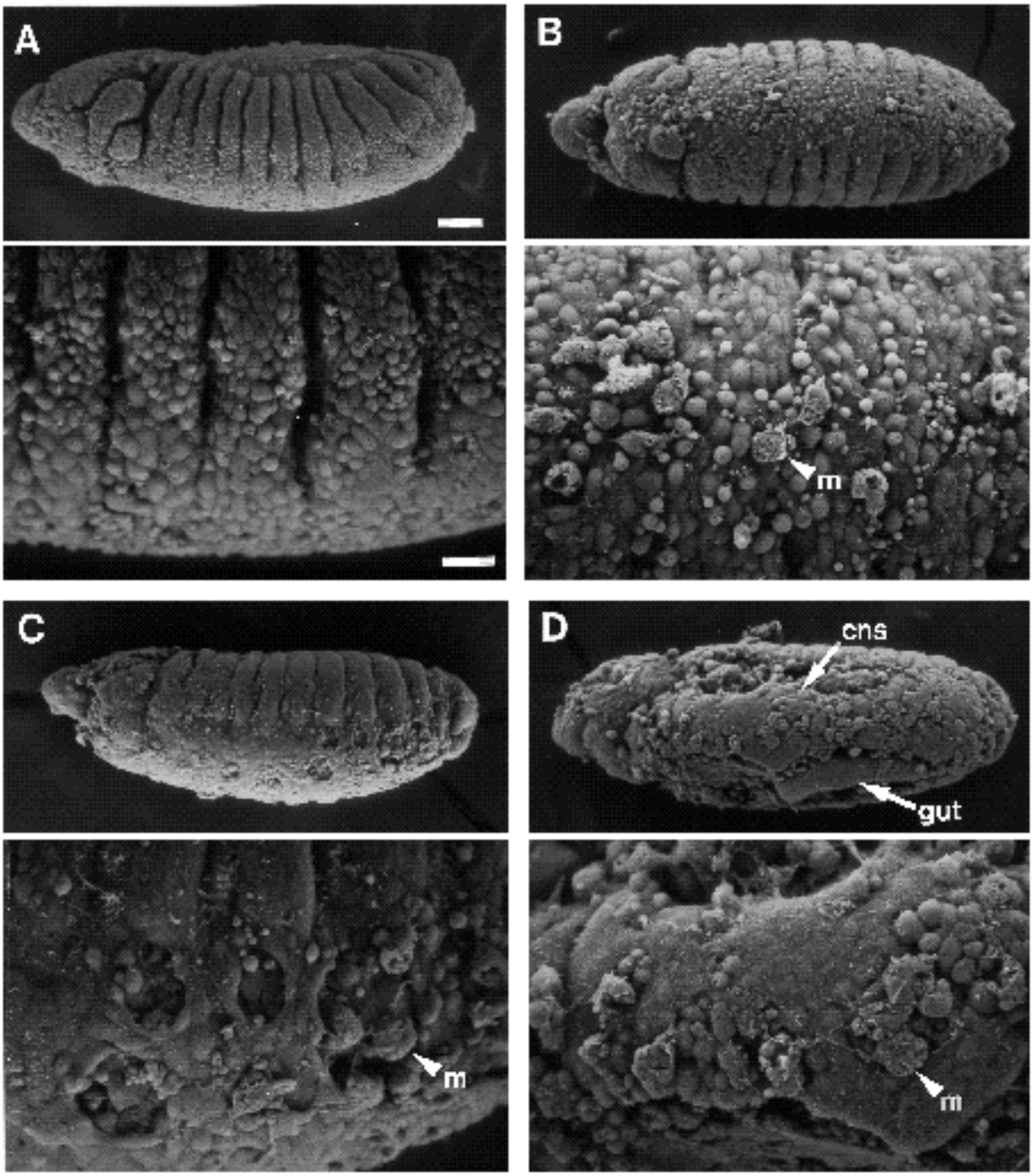

Fig. 4. Degeneration of the ventral epidermis in *fzy* embryos. SEMs of progressively older fzy^3 embryos. (A,C) Lateral views, (B,D) ventral views with anterior to the left in all panels; the lower half of each panel is a higher magnification view of part of the upper panel. (A) A stage 13 fzy^3 embryo in which degeneration of the ventral epidermis is just beginning. The only abnormalities visible at this stage are the loss of obvious segmentation in the ventralmost region and the larger, more rounded and less well organized appearance of the ventral epidermal cells compared to those of the dorsal epidermis. (B) An early stage 14 fzy^3 embryo in which ventral degeneration is more advanced. By this point, macrophages (m, arrowhead) have appeared on the ventral surface. (C) An early stage 15 fzy^3 embryo in which ventral degeneration is well advanced. Large holes are now visible throughout the ventral epidermis and there is intense macrophage activity in this region. Note the clear division between the degenerating ventral epidermis and the normally differentiating dorsal region. (D) By stage 16 the whole ventral epidermis has been removed such that the CNS and gut are clearly visible on the ventral surface of the fzy^3 embryo. Note that there are holes in the sheath surrounding the CNS through which neural cell bodies are visible and the intense activity from the macrophages which now swarm over the degenerating CNS.

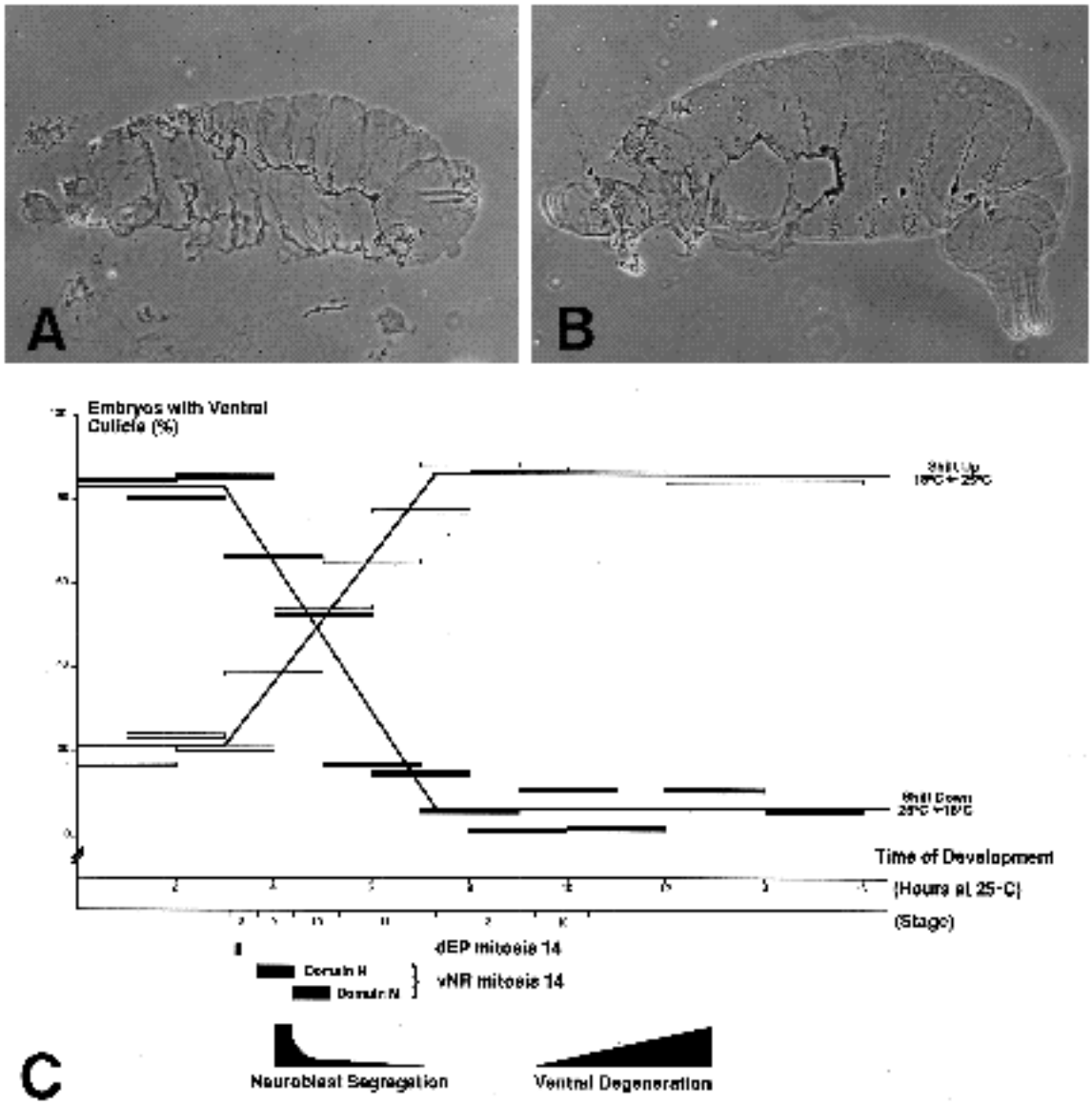

Fig. 5. Temporal requirements for zygotic *fzy* activity during differentiation of the ventral epidermis as defined by temperature-shift experiments using the fzy^4 allele. (A,B) Cleared cuticle preparations of typical fzy^4 embryos raised at 25°C (A) and 18°C (B); both are ventral views with anterior to the left and illustrate the temperature sensitivity of the phenotype of embryos homozygous for the fzy^4 allele. (C) The results of temperature-shift experiments with fzy^4 embryos presented graphically. Time points are represented by bars to indicate the spread of the ages of the embryos included at each time point (open bars for the upshift, solid bars for the downshift). The percentage of embryos from any given time point that possessed differentiated ventral cuticle is indicated on the vertical axis. Embryos were considered to have ventral cuticle if they had at least a bridge of cuticle connected to both edges of the lateral epidermis and spanning the ventral midline. Embryos were scored as either having or not having ventral cuticle, we made no attempt to rank those embryos having ventral cuticle with regard to the extent of the cuticle they possessed. The age of the embryos at each time point is indicated on the horizontal axis in both hours of development at 25°C and developmental stage (Campos-Ortega and Hartenstein, 1985). Below the horizontal axis we have indicated the times of certain relevant developmental events. Cells in the dorsal epidermal region (dER) undergo their first postblastoderm mitosis as a metasynchronous domain early in stage 8. The majority of cells in the ventral neurogenic region (vNR) enter their first postblastoderm mitosis asynchronously over an extended period of time; during stage 9 for the more lateral domain N and later during stage 10 for the ventral domain M (Foe, 1989). Neuroblast segregation begins and the majority of neuroblasts segregate during stage 9, though further neuroblasts continue to segregate for the next 3 hours (Campos-Ortega and Hartenstein, 1985). Visible degeneration of the ventral epidermis of *fzy* embryos begins during stage 13 and becomes progressively worse over the next 3 to 4 hours until all the ventral epidermis is removed.

cate that the phenocritical period for *fzy* function during differentiation of ventral epidermis is between 3.5 and 7 hours of development at 25°C (Fig. 5C). Thus the phenocritical period is substantially earlier than the onset of obvious epidermal degeneration. Furthermore, the *fzy* phenocritical period overlaps with both the timing of neuroblast segregation and the initiation of postblastoderm mitosis by the ventral DBs (Campos-Ortega and Hartenstein, 1985;

Foe, 1989): *fzy* is required by the ventral DBs as or shortly after NB segregation has occurred and during the entry of the ventral DBs, particularly the more ventral ones, into their schedule of postblastoderm mitoses (Fig. 5C).

Given the early requirement for maternal *fzy* for the preblastoderm mitoses and this correlation between the phenocritical period for *fzy* and the entry of the ventral DBs into further rounds of mitosis, we examined the mitotic activity of the ventral DBs in *fzy* embryos. Before describing this data, we will briefly summarize the cell division pattern during the relevant stages of wild-type development from the data of Foe (1989). Prior to blastoderm formation all cells of the embryo, except the pole cells, which constitute the future germline of the animal, have gone through 13 synchronous syncitial nuclear divisions. After blastoderm formation and the cellularisation of these nuclei, most cells enter their 14th mitosis as mitotic domains; that is as groups of usually adjacent cells that undergo mitosis metasynchronously and in a defined spatiotemporal pattern during subsequent developmental stages. The cells of the ventral neurogenic ectoderm, however, are an exception to this rule. In this region, cells by and large enter division individually and over an extended period of time, rather than as domains undergoing division at a strictly defined point of development. Cells in the ventrolateral part of the vNR, domain N, divide earlier, during stage 9, than those in the ventromedial area, domain M, which start to divide during stage 10 (Foe, 1989).

We examined cell division patterns in *fzy* embryos homozygous for both of the two amorphic mutations fzy^1 and fzy^3. During stage 9 and most of stage 10, we observed no obvious difference between the pattern of germ band cell division in wild-type and most *fzy* embryos. However, during stage 11 the proportion of mitotic cells in the ventral epidermis of *fzy* embryos increases, until by the end of this stage 11 almost all are in mitosis (Fig. 6). As in the fzy^{mat} embryos, the majority of these cells are in metaphase though a significant proportion of prophase cells are also present. This is in marked contrast to wild-type embryos where cell division in the ventral region has virtually ceased

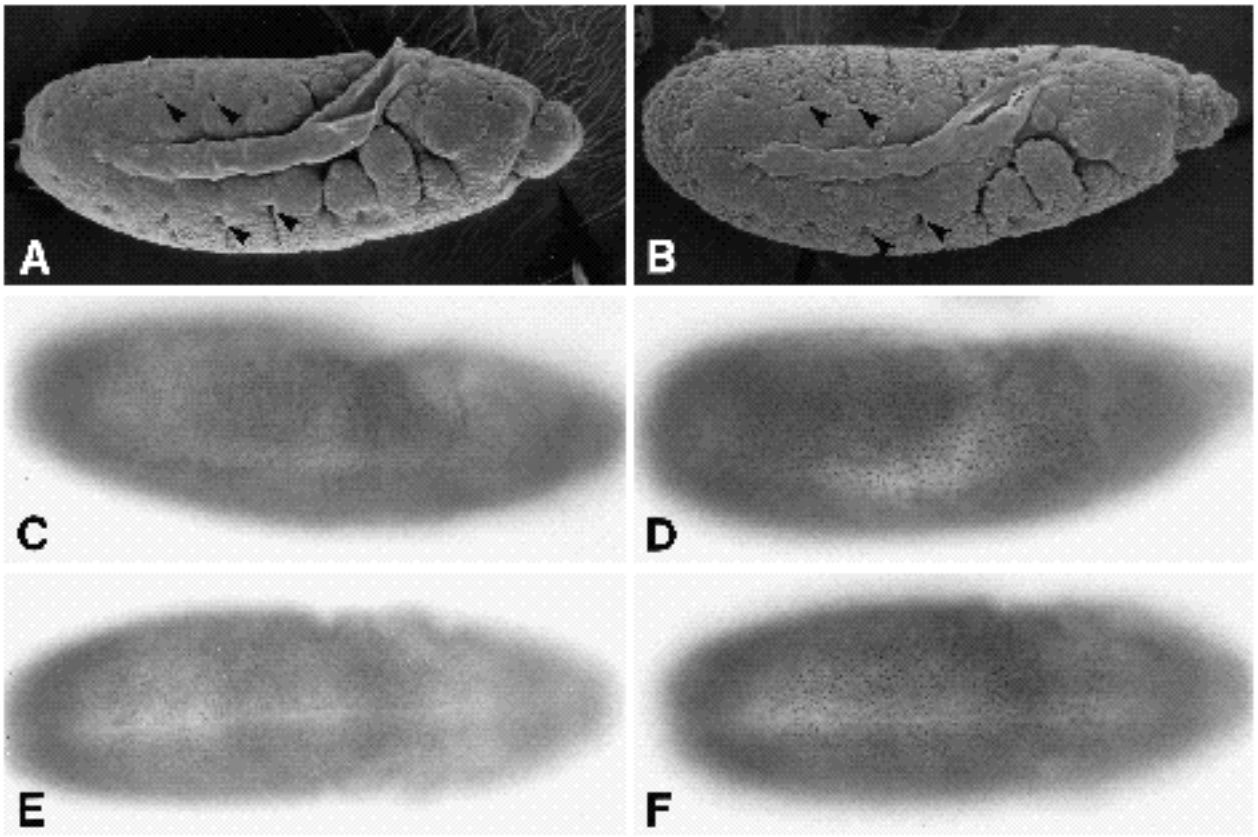

Fig. 6. Mitotic arrest in the ventral epidermis of *fzy* embryos. SEMs (A and B) and fuschin-stained whole-mount preparations (C to F) of wild-type (A,C,E) and fzy^3 (B,D,F) stage 11 embryos; anterior is to the right in all panels, which show (A,B) lateral, (C,D) ventrolateral and (E,F) ventral views respectively. (A) In the wild-type embryo at this stage, a row of tracheal pits (arrowheads) are clearly visible which serve to demarcate the boundary between the ventral neurogenic region and the dorsal epidermis. Most cells in both regions at this time are not dividing and the embryo has a fairly uniform smooth surface. However, a few cells scattered throughout the germ band can be seen to have rounded up and protrude from the surface, this is characteristic of cells undergoing mitosis. In contrast, in the *fzy* embryo (B), all the cells in the ventral neurogenic region, i.e. ventral to the tracheal pits (arrowheads), are rounded up and protruding, suggesting that all these cells are in mitosis at this time. Fuschin staining of wild-type embryos at this stage (C and E) shows that most cells in the ventral Neurogenic region are in interphase as few mitotic figures are visible. However, in equivalently staged and oriented *fzy* embryos (D and F), fuschin staining reveals that most cells of the ventral neurogenic region contain mitotic figures and that the appearance of the condensed chromatin in these cells indicates they are mostly in metaphase.

by the end of stage 11 (Fig. 6). From this time until their eventual degeneration, these cells in *fzy* embryos appear to remain arrested in metaphase. Similarly, between stages 9 and 11, most superficial cells in the cephalic region go from being in their normal interphase state to almost all being in metaphase (Fig. 6). However, we have not studied the timing of the appearance of these aberrant cephalic divisions in detail. These abnormal metaphase cells are present throughout those regions derived from the ventral neurogenic ectoderm. This can be seen most clearly in SEMs of stage 11 embryos (Fig. 6A,B).

We believe that this prevalence of metaphase cells is the consequence of a mitotic arrest at the metaphase/anaphase transition; that cells enter division normally but are then unable to exit mitosis. This would account for the almost complete lack of interphase cells seen in the ventral epidermis from stage 11 to its degeneration. Similarly, this explanation would be consistent with the affects of *fzy* mutations on other tissues, such as the female-sterile phenotype described above. The alternative explanation, that these cells are undergoing ectopic divisions, is unlikely as excess cell division is the exact opposite of the effects of *fzy* mutations seen in other tissues and, if this were the case, we should still see cells at other stages of their cycle in addition those undergoing mitosis.

This extensive mitotic arrest appears to be the primary defect that occurs throughout the epidermis of the ventral and cephalic regions and could potentially directly result in the later degeneration of these cells. While we do not know how long such a mitotically arrested epidermal cell could survive in this state in vivo, it is unlikely that such cells could participate normally in the dynamic events of embryogenesis, such as the cell movements of dorsal closure or the initiation of cell-type-specific gene expression which accompanies terminal differentiation. Since it is at the onset of these events that cells degenerate in *fzy* embryos, it could well be that this degeneration is a direct consequence of their being in mitotic arrest. Alternatively, it is possible that the late degeneration of the ventral epidermis in *fzy* embryos could be due to some other failure in the differentiation of these cells.

We addressed this issue by examining the cuticles produced by *fzy*; *string*(*stg*) double mutant embryos. Embryos homozygous for amorphic mutations in *stg* arrest cell division in the G_2 phase of cycle 13; they do not to undergo any further, postblastoderm, cell division (Edgar and O'Farrell, 1989a,b). Nonetheless, all the epidermal cells of *stg* mutants are capable of differentiating and producing cuticle. We reasoned that, if the late degeneration of the ventral epidermis is a direct result of this mitotic arrest and if the only requirement for *fzy* in the ventral epidermal cells is for their successful exit from postblastoderm mitoses, then, in a *fzy*; *stg* double mutant, the fzy^+ gene product should be dispensable, because the *stg* mutation will prevent any cells from entering postblastoderm mitoses. In this case, *stg* will be epistatic to *fzy* and these animals should now form ventral cuticle like that produced by the *stg* mutation on its own. Alternatively, if *fzy* has any other functions in addition to its mitotic one in the ventral epidermis and it is the loss of these that is directly responsible for the later degeneration rather than the mitotic arrest per se, then *fzy* would be epistatic to *stg* and the double mutant would still lack ventral epidermis. The results of this experiment shows that *stg* is epistatic to *fzy*: the *fzy*; *stg* double mutant embryos form ventral cuticle that is indistinguishable from that of *stg* mutants alone (Fig. 7). Thus it appears that the only function of *fzy* during differentiation of the ventral epidermis is to allow these cells to exit mitosis.

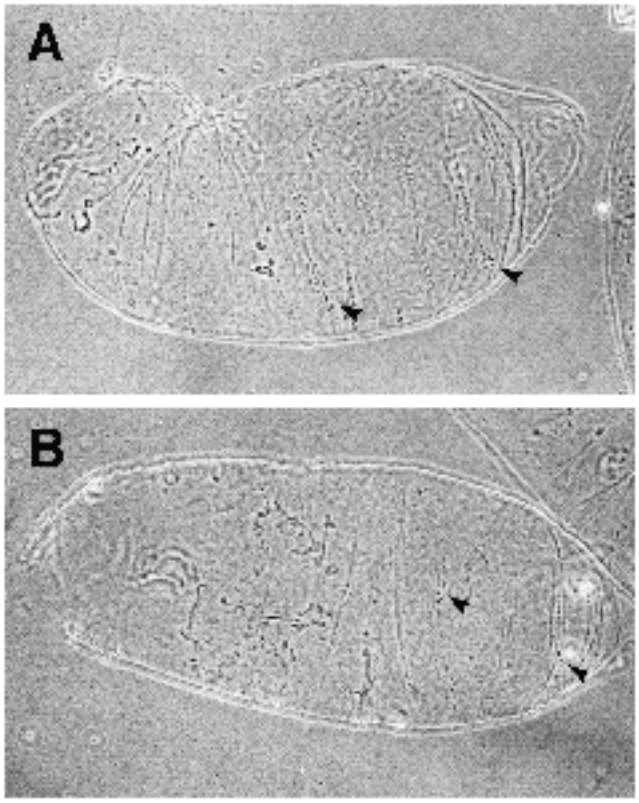

Fig. 7. *stg* is epistatic to *fzy* in the ventral epidermis. Cleared cuticle preparations of pharate first instars of the genotypes (A) stg^2 and (B) fzy^3; stg^2. Both are ventral views with anterior to the left. Both embryos have complete and fully differentiated ventral epidermis without any holes and possessing a few poorly differentiated denticle belts (arrowheads), showing that the *stg* mutation is fully epistatic to *fzy* in rescuing ventral epidermal differentiation.

Effects of *fzy* mutations on the development of the embryonic central nervous system

In addition to degeneration of the ventral epidermis, embryos homozygous for strong embryonic lethal *fzy* alleles also show CNS degeneration (Nüsslein-Volhard et al., 1984; Fig. 3). We have attempted to analyze the underlying cause of this CNS degeneration. Data obtained by staining embryos with α−HRP indicate that the early development of the CNS in *fzy* embryos appears normal, up to about stage 12. However, during subsequent development, abnormalities become apparent in the the CNS of *fzy* embryos. During stage 13, the CNS appears wider and less condensed than in wild types (Fig. 8A,B). Subsequently, the CNS of *fzy* embryos undergoes considerable cell death (data not shown) and degenerates drastically in later stages (Fig. 8C,D).

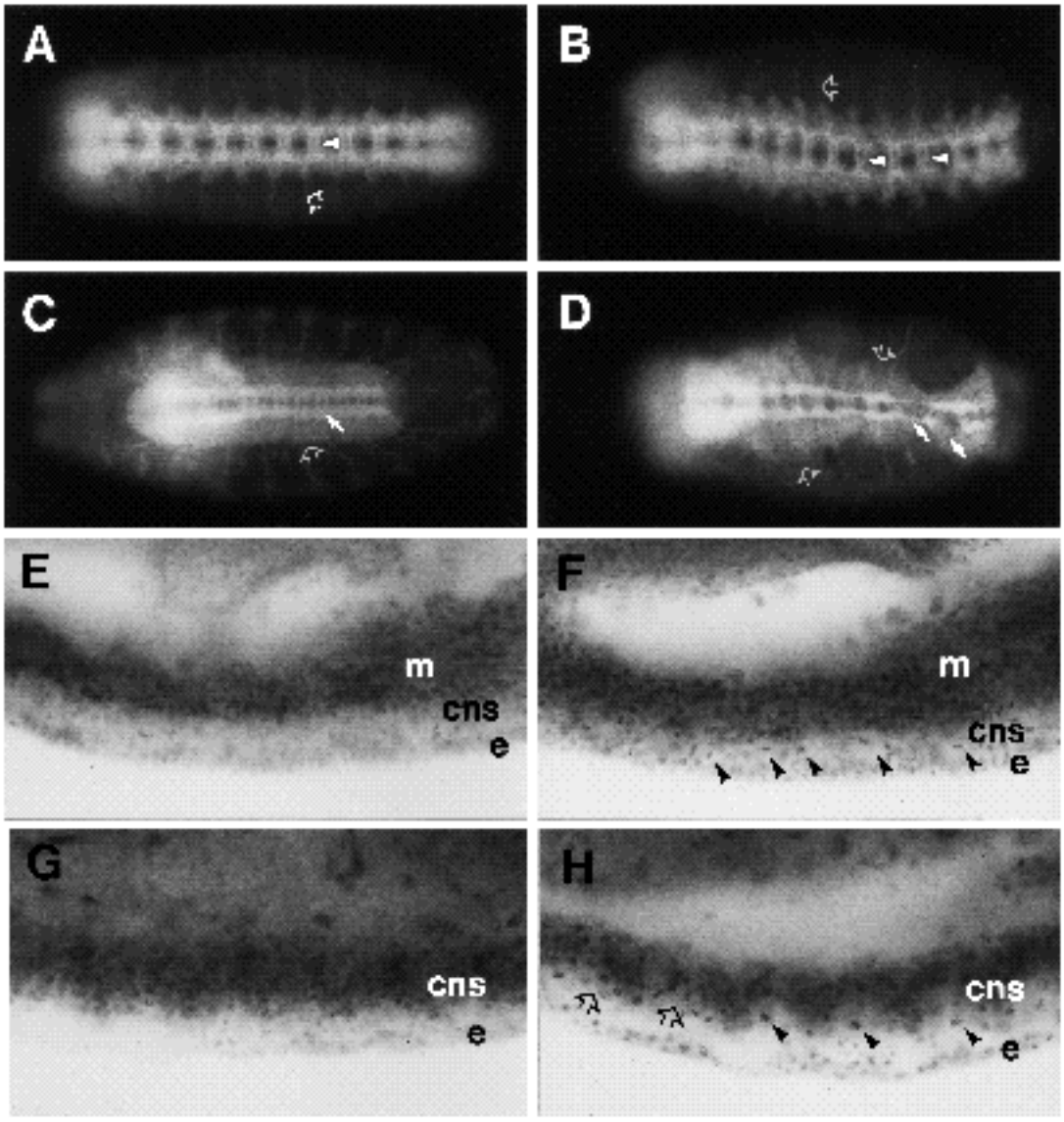

Fig. 8. CNS phenotypes of *fzy* embryos. Wild-type (A,C,E,G), *fzy*^{3} (B,D,F,H) embryos stained with anti-HRP (A-D) or fuschin (E-H); (A-D) are ventral views with anterior to the left, (E-H) are optical parasagittal sections through whole-mount embryos with anterior to the right. (A) Wild-type stage 13 embryo: the horizontal commissures (arrowhead) and axon bundles to the PNS (open arrow) are forming. (B) Stage 13 *fzy*^{3} embryo: the first clear morphological indications of CNS defects in *fzy* embryos become apparent at this stage when the CNS appears noticeably broader and the axon bundles of the commissures (arrowheads) appear thinner and less well developed when compared to the wild type, axon bundles to the PNS are present (open arrow). (C) Wild-type stage 17 embryo: by this stage embryonic CNS differentiation is essentially complete, the longitudinal connectives (arrow) have formed and the CNS has contracted. The neuronal cell bodies are tightly packed together, presumably within a perineural sheath of glial cells, and there is a distinct lateral edge to the CNS (open arrow). (D) Stage 17 *fzy*^{3} embryo: by this stage the CNS has degenerated severely, connectives have formed but frequently appear stretched or broken (arrows), commissures are poorly developed, neuronal cell bodies are still spread out laterally, the lateral border of the CNS is poorly defined (open arrows) and the whole CNS appears loose and broken. This degeneration of the CNS of *fzy* embryos is preceded by the accumulation of mitotically arrested cells within the developing CNS. In wild-type embryos during stage 11 (E) and stage 12 (G), although NBs and their progeny, the ganglion mother cells (GMCs), are still dividing at these times, only a minority of these cells are in mitosis at any given moment and few mitotic figures are visible in the developing CNS. In contrast, in a stage 11 *fzy*^{3} embryo (F), numerous metaphase figures can be seen in large cells immediately below the epidermis (arrowheads). The position, size and plane of division of these cells suggest they are NBs that have been arrested in mitosis. By stage 12 in *fzy*^{3} embryos (H), in addition to presumptive arrested NBs (arrowheads), clusters of metaphase figures can be seen at deeper layers within the developing nerve cord (open arrows) which are probably arrested GMCs. m, mesoderm; cns, central nervous system; e, epidermis.

Since examination of the preblastoderm maternal requirement for *fzy* and the degeneration of the ventral epidermis indicate that *fzy* is required for normal mitosis, we wished to ascertain if the CNS degeneration was also caused by cell division defects. Examination of the mitotic activity of cells within the developing CNS of *fzy* embryos by fuschin

staining showed no abnormalities prior to stage 11. However, from late stage 11 onwards, significantly more mitotic, primarily metaphase, cells are apparent within the CNS of *fzy* embryos compared to wild-type controls (Fig. 8 E to H). The position, size and orientation of division plane of these excess mitotic cells suggests they are either NBs or ganglion mother cells (gmc), their mitotically active daughters (Poulson, 1950; Hartenstein and Campos-Ortega, 1984). Thus, in this tissue too, lack of zygotic *fzy* activity results in abnormal cell division patterns.

We believe that the increased number of metaphase cells observed in the CNS of *fzy* embryos is due to mitotic arrest occurring in dividing cells of this tissue, which would be consistent with our previous results with the ventral epidermis. By analogy to the ventral epidermis, we assume that these arrested cells eventually die and this probably accounts for most of the cell death occurring in the CNS latter in development. We believe that the differentiated neurons that do form in the fzy^- CNS are probably those derived from early NB and gmc divisions, occurring before the maternal supply of fzy^+ runs out. However, we have not attempted to test these assumptions further.

Effects of *fzy* mutations on the development of the embryonic peripheral nervous system

While examining the embryonic phenotypes of *fzy* embryos, we found that one other tissue, the peripheral nervous system (PNS), was severely affected by *fzy* mutations. The PNS is derived from ectodermal cells known as sensilla mother cells (SMs) which, like the neuroblasts, delaminate from the surrounding epidermal cells to adopt a neural fate, beginning during stage 10 (Ghysen and O'Kane, 1989). During stages 10 through 12, the SM cells undergo a series of divisions, which generate the PNS neurons and their accompanying support cells (Bodmer et al., 1989). Given the evidence that *fzy* is required for cell division, we examined the development of the PNS in *fzy* embryos to determine if the absence of PNS neurons in these animals is also due to cell division defects. Because there are many other defects occurring in the development of the ventral ectodermally derived tissues in *fzy* embryos, we confined our analysis of PNS development to the dorsal PNS underlying the apparently normal dorsal epidermis to avoid any complications or indirect effects caused by these other defects.

When *fzy* embryos were stained with mAb 22.C.10, very few mAb 22.C.10-positive neurons were detected (Fig. 9B). Similarly, when *fzy* embryos carrying a *prospero*:β-gal enhancer trap line, which expresses β-gal in the neurons and certain of their support cells (Vaessin et al., 1991), were stained with an anti-β-gal antibody, little if any *pros*:β-gal expression could be detected (data not shown). It is clear then that almost all of the PNS neurons and at least some of the terminally differentiated PNS support cells, are absent in *fzy* embryos. However, when *fzy* embryos carrying the 37A enhancer trap line, in which β-gal is expressed in the SM cells as well as all their progeny (Ghysen and O'Kane, 1989), were stained with an anti-β-gal antibody, β-gal expression could be detected. The early 37A: β-gal expression pattern, when the SM cells are being born, appears normal in *fzy* embryos (data not shown) but by latter stages, when the SM cells have undergone division, the pattern in *fzy* embryos is altered. There are fewer β-gal-positive cells and the β-gal expression is more diffuse (Fig. 9C,D). Thus, in *fzy* embryos, it appears that the SM cells are born normally but produce fewer progeny and those appear to be unable to terminally differentiate as judged by their failure to express neuron-specific markers such as 22.C.10. These data are consistent with the hypothesis that in *fzy* embryos the SM cells and/or their early progeny, are unable to complete their normal division pattern to produce the neurons and support cells of the PNS, presumably due to mitotic arrest.

Two other pieces of data support this hypothesis. When *fzy* embryos are stained with the α-Ubx antibody, from stage 11 onwards groups of large, heavily staining, Ubx-positive cells can be seen subepidermally in each of the abdominal segments; these are absent from the wild-type controls (Fig. 9E). We believe these are SM cells or their progeny for the following reasons. Firstly, Ubx is known to be expressed in the PNS and its precursors in the abdominal segments (White and Wilcox, 1984). Secondly, the subepidermal location of these cells, between the epidermis and the dorsal musculature, and their constant and reiterating pattern in each abdominal segment is consistent with the known pattern of SM cell distribution and PNS development: furthermore, this pattern of Ubx-positive cells is similar to the pattern of 37A expressing cells. We conclude, therefore, that these are indeed the SM cells or their progeny. There are two points to note from this pattern of Ubx expression. Firstly, these Ubx-positive cells are noticeably larger and rounded up compared to the neighboring dorsal epidermal cells; this distinct morphology suggests that they are in fact in mitosis since mitotic cells round up in this manner. In addition, the Ubx antigen, which is normally located in the nucleus (White and Wilcox, 1984; Beachy et al., 1985), is spread throughout the bodies of these cells; this displacement of a nuclear antigen is also consistent with these cells being in mitosis. Secondly, these cells continue to strongly express Ubx even after Ubx expression has decayed completely in the surrounding dorsal epidermis and in the epidermis and PNS of control siblings. A similar perdurance of Ubx expression is seen in the mitotically arrested cells of the ventral epidermis before they degenerate (data not shown). This suggests that in *fzy* embryos the development of the PNS precursor cells has been arrested at a stage of embryogenesis prior to the completion of their last division and the subsequent decay of Ubx expression in the terminally differentiating PNS cells.

Lastly, we have examined the dorsal epidermal regions of *fzy* embryos by fuschin staining to look at their mitotic activity. As expected, we find prominent subepidermal clusters of metaphase cells in consistent and reiterating patterns in each of the trunk segments, which are not found in wild-type embryos (Fig. 9F,G). Again the subepidermal locations and segmental distribution of these metaphase cells is consistent with the known patterns of PNS precursors. We believe, therefore, that at least some of these metaphase cells represent mitotically arrested PNS precursor cells.

Taken together, these data strongly support the hypothesis that the absence of most the PNS in *fzy* embryos can also be attributed directly to a failure of cell division by the PNS precursors. Furthermore, it appears that, in this

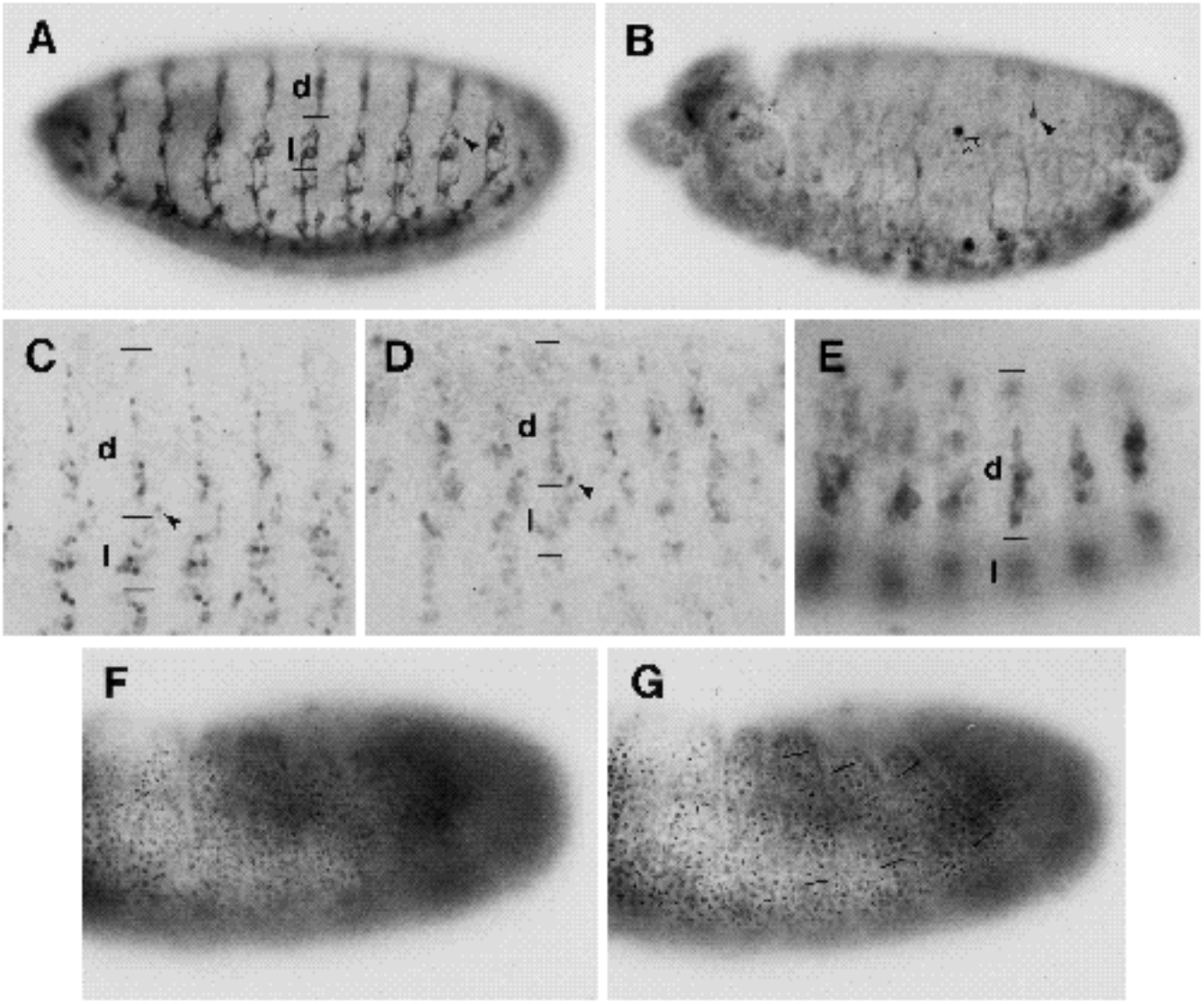

Fig. 9. Development of the PNS in *fzy* embryos. Antibody-stained (A-E) and fuschin-stained (F,G) whole mounts of wild-type (A,C) and *fzy*^{3} (B,D-G) embryos. Anterior is to the left and dorsal uppermost in all panels. (A) Lateral view of a wild-type stage 16 embryo stained with antibody mAb 22.C.10, which recognizes an antigen expressed on the membranes of all PNS neurons. Abdominal segments A1 to A7 have an identical pattern of PNS structures; the extent of the dorsal (d) and lateral (l) neuronal clusters are indicated (bars) in one abdominal segment. These neurons and their support cells are derived from the dorsal epidermal region. One other specific neuron, the v´ch1 neuron (Dambly-Chaudiere and Ghysen, 1986), which is derived from the ventral neurogenic region but migrates dorsally to underlie the dorsal epidermis, is indicated in another segment (arrowhead). In a *fzy*^{3} embryo of the same stage stained with mAb 22.C.10 (B), almost the entire complement of PNS neurons are seen to be absent. Only one well-differentiated neuron is present in the abdominal region (arrowhead), which from its position and distinct morphology is most likely a v´ch1 neuron - this particular neuron regularly escapes in *fzy* embryos and forms normally in about 10% of the abdominal hemi-segments. Large, round subepidermal cells, which are strongly mAb 22.C.10-positive, are also frequently seen in *fzy* embryos (open arrow). We believe these are most likely progenitors of PNS neurons that have arrested in division but nonetheless express the 22.C.10 antigen. (C) A higher magnification view of a wild-type embryo expressing the 37A P-element enhancer trap, which is expressed in all cells, both neuronal and support, of the PNS, and is visualized with an anti-β-gal antibody. The dorsal and lateral clusters are indicated (bars) as is the most strongly staining cell of the v´ch1 organ (arrowhead). Note that the 37A staining in the wild-type embryo is nuclear. (D) A *fzy*^{3} embryo at a comparable stage to C stained for 37A, again dorsal and lateral clusters are indicated as is a strongly staining cell of a v´ch1 organ (bars and arrowhead). There are fewer 37A-positive cells present in each cluster and the staining is more diffuse. (E) A *fzy*^{3} embryo comparable to C and D stained anti-Ubx antibody, which reveals stereotyped clusters of large, round subepidermal cells in each abdominal segment. These appear to be arrested precursors of the PNS neurons and support cells. Again note that the Ubx protein, which is normally nuclearly localized, in these cells is spread throughout the cytoplasm. (F) Surface and (G) subsurface views of the same fuschin-stained *fzy*^{3} embryo. Clusters of mitotic cells (bars) are found in regular patterns just below the epidermis in each abdominal segment. From their position and pattern, it appears likely that at least some of these are mitotically arrested PNS precursors.

tissue too, loss of *fzy* activity results in a metaphase arrest of the affected cells.

DISCUSSION

In this paper, we have described the effects of mutations at the *fizzy* locus on development in *Drosophila melanogaster*. We have shown that *fzy* is required maternally for the preblastoderm nuclear cleavage divisions of the embryo and required zygotically for the development of the embryonic ventral epidermis, CNS and PNS. We have analyzed the embryonic phenotypes in detail and shown that they can be attributed to defects in cell division by the precursor cells of the affected tissues. We have shown that loss of *fzy* activity leads to metaphase arrest and consequent failure to exit mitosis in the affected cells. We conclude therefore, that the *fzy* locus is a novel *Drosophila* cell cycle gene, the product of which is required for normal cell division and specifically for the progress from metaphase to anaphase during mitosis.

The clearest example of *fzy*'s involvement in mitosis is seen in the maternal contribution to preblastoderm development of the fertilized embryo. These early syncitial nuclear cleavage divisions are driven by maternal products and occur remarkably quickly, each cycle of DNA replication and mitosis taking approximately 10 minutes (Zalokar and Erk, 1976; Foe and Alberts, 1983; Glover, 1989). The blockage of these divisions in the absence of sufficient maternally supplied *fzy* strongly suggests that *fzy* is required for successful progress through mitosis. Furthermore, the observation that, while most of the nuclei in fzy^{mat} embryos are at metaphase, a few nuclei are chimeric metaphase/anaphase hybrids (i.e. with most of their chromosomes at a metaphase plate but a few having migrated to the spindle poles) indicates that *fzy* is required specifically for the metaphase-to-anaphase transition during mitosis.

Analysis of the zygotic embryonic phenotypes are consistent with this hypothesis. In all the affected tissues that we examined in detail (the ventral epidermis, CNS and PNS), we see perturbation of the usual pattern of cell division and an accumulation of mitotic, particularly metaphase cells.

The degeneration of the ventral epidermis can be attributed directly to this cell division defect. Throughout the ventral epidermis the epidermal cells begin to accumulate in mitosis from stage 11 onwards and appear to remain that way up to the point when they begin to picnocytose, degenerate and die. Our data indicate that it is likely that the degeneration of this tissue is a direct result of these cells being caught inappropriately in mitosis, being unable to differentiate normally and being removed by death or phagocytosis. The phenotype of *fzy*; *stg* double mutant embryos strongly supports this view. The simplest explanation of the observation that an amorphic *stg* mutation rescues the differentiation of the ventral epidermis in a *fzy* background is that *fzy* is required solely for mitosis and, by preventing cells from undergoing further cell division by introducing the *stg* mutation, *fzy* function now becomes dispensable.

The absence of most of the PNS in *fzy* embryos can also be attributed directly to a cell division defect. Our data indicate that the sensilla mother cells are present in *fzy* embryos and that they and/or their progeny arrest in mitosis. This failure of the PNS precursor cells in *fzy* embryos to undergo normal division can account directly for the observed absence of most PNS structures.

We have also observed cell division defects in the developing CNS of *fzy* embryos which could account for the later abnormalities and degeneration of this tissue. However, we think that in this tissue secondary factors might also contribute to the degeneration that we observe. For example, during normal development the cells of the CNS and ventral epidermis are closely apposed up to stage 12, after which these two tissues then physically separate (Campos-Ortega and Hartenstein, 1985). This separation does not occur in fzy^{-} embryos; instead the degenerating ventral epidermis remains firmly attached to the CNS whilst at the same time being pulled laterally by the dorsal epidermis as it undergoes dorsal closure (I. Dawson, unpublished data). This could well account for the lateral spreading of the CNS observed in fzy^{-} embryos at this and later stages and contribute, by physical disruption, to CNS degeneration. Nonetheless, while such secondary effects may contribute to the final CNS phenotype, the initial defect seen in this tissue and which we attribute directly to loss of *fzy* function, is again failure of normal cell division.

The function of *fzy* during development

The *fizzy* phenotype is, to the best of our knowledge, unique. No other loci in *Drosophila* are known to be mutable to give this combination of phenotypes. One other locus, *poly-homoeotic* (*ph*), is mutable to give a ventral degeneration phenotype superficially similar to that of *fzy* embryos (Dura et al., 1987; Smouse et al., 1988). However, *fzy* and *ph* mutations do not interact genetically in the ventral epidermis or elsewhere (I. A. Dawson, unpublished data), their other phenotypes are completely different (Dura et al., 1985; I. A. Dawson unpublished data) and no cell cycle defects have been reported for *ph* mutations. Therefore we think it unlikely that they are defective in the same process. Furthermore, the *fzy* phenotype is very different from the phenotypes of other identified mutations that affect cell division and mitosis in *Drosophila*. Firstly, most mitotic mutations identified in *Drosophila* to date result in larval/pupal rather than embryonic lethality (Gatti and Baker, 1989). This is thought to be because all the embryonic cell divisions, not just the preblastoderm ones, can be completed from maternally supplied products of these genes and therefore the zygotic absence of these products only becomes apparent in postembryonic development. Moreover, of the cell cycle mutations identified so far that do result in embryonic lethality, such as *string*, *pebble* and *cyclin A*, all act globally within the embryo; all cells are affected (Edgar and O'Farrell, 1989a and 1989b; Lehner and O'Farrell, 1989; Hime and Saint, 1992); in contrast, *fzy* clearly affects only a subset of the embryonic cells.

This observation raises the question of why do *fzy* mutations show this cell and tissue specificity? The most likely explanation for this probably lies in the perdurance of the maternal contribution of fzy^{+} coupled with the relative timing of postblastoderm cell divisions. Our data from vary-

ing the maternal dosage shows that maternally supplied *fzy* does perdure and can function during the postblastoderm divisions. This maternal activity might perdure long enough to drive the early postblastoderm divisions but run out before the later ones. Data from fuschin staining support this hypothesis. Mitotic arrest, as seen by the build up of metaphase cells only becomes pronounced relatively late during embryogenesis, from stage 11 onwards, and only in tissues which are still actively dividing, such as the ventral epidermis, the CNS and the PNS. By this time the precursors of other embryonic tissues, such as the dorsal epidermis, the midgut and hindgut, have completed most of their divisions (Campos-Ortega and Hartenstein, 1985), which would explain why these tissues are able to escape and differentiate normally in *fzy* embryos. However, an alternative explanation, which we cannot rule out at present, is the possibility that there could be redundant functions that are expressed and able to compensate for loss of *fzy* function in the normally developing tissues.

In addition, it is also possible that other factors, besides the timing of cell division, may play a role in determining which cells are affected. Some cell types could, for whatever reason, require more *fzy* activity than others or the maternal supply could undergo attrition at different rates in different tissues. While the accumulation of arrested metaphase cells in the embryo generally seems to support a model based on timing of division, we cannot rule out the additional involvement of other influences. For instance, both within the vNR and amongst the progeny of the SM cells, cell-cell interactions are important for determining cell fates and the subsequent developmental pathway a cell will follow (reviewed by Artavanis-Tsakonas and Simpson, 1991; Campos-Ortega and Jan, 1991). This in turn determines the timing and pattern of mitoses undergone by these cells and it is likely that cell cycle regulation in these cells is linked to the cell fate choices that these cells make. It is intriguing that these two tissues are particularly sensitive to loss of *fzy* function but at present we cannot determine if this is in any way due to such additional influences or solely a matter of timing.

It is difficult to address the issue of whether timing is the only factor involved in the tissue specificity of the *fzy* phenotypes at present. In part this is because the relative timing and patterns of the later cell divisions are currently less well documented than the earlier ones; but more so because the perdurance of maternal fzy^+ and the variability of the *fzy* phenotype, particularly in the accumulation of arrested metaphase cells between individual embryos, hinder precise analysis. We do not know to what extent the maternal supply of fzy^+ activity actually contributes to the progress of the postblastoderm cell divisions and therefore do not know what the postblastoderm cell division phenotype would be in a truly null *fzy* embryo - whether all cell divisions would now be affected equally or whether tissue specificity would still exist. Unfortunately, the usual means of removing maternally contributed products in such situations, by creating germ line clones of amorphic alleles, would not work in this case. Since maternally supplied fzy^+ activity is required for preblastoderm development, embryos derived from such clones would arrest prior to the postblastoderm stages we wished to examine. Furthermore, data from clonal analysis with amorphic *fzy* alleles in the adult epidermis indicates that such clones are cell lethal, as might be expected for a gene whose product is required for cell division (I. Dawson, unpublished results). Since the female germline cells must divide to produce the oocyte, clones incapable of cell division would not be recoverable anyway.

On balance though, we conclude that the data that we report here supports the notion that the perdurance of the maternal product coupled with the timing of cell division seems likely to be the major, if not the only, factor responsible for the tissue specificity of the *fzy* embryonic phenotype.

The function of *fzy* during mitosis

We have shown that *fizzy* is required for the exit of cells from metaphase during mitosis, which raises the question of exactly how and where *fzy* functions during the cell cycle. We can make some predictions about this based on the phenotypic data. Since cells enter mitosis on schedule in *fzy* animals, it is clear that *fzy* is not required for timing or patterning cell division. Furthermore, since cells enter division normally, this suggests that the earlier stages of the cell cycle, in this case the S and G_2 phases (Edgar and O'Farrell, 1989b) proceed normally and do not require *fzy* function. It should be noted however, that as a cell progresses from one phase of the cell cycle to the next it passes through various checkpoints which ensure that earlier events are successfully completed before proceeding onto the next phase. One of these checkpoints occurs at metaphase (Nishimoto et al., 1992). It is therefore, formally possible that *fzy* could be functioning earlier in the cell cycle than the metaphase/anaphase transition but that the effects of its absence might not become apparent until this point.

However, it seems simplest at present to assume that *fzy* is most likely acting during exit from mitosis at the metaphase/anaphase transition. This still leaves a number of alternative possibilities. For one, it is possible that *fzy* plays a regulatory role. The state of activity of the MPF (cdc2 kinase/cyclin) complex appears to be the master regulator of mitotic progression. This complex is known to undergo alterations as mitosis progresses. Specifically, the destruction of cyclin B at the metaphase/anaphase transition (Whitfield et al., 1990), presumably following its ubiquitinilation (Glotzer et al., 1991), inactivates MPF kinase activity, which is essential for the cell to exit from mitosis (Murray et al., 1989). This process presumably requires the activities of ubiquitinilating enzymes and the ubiquitin degradation system (reviewed by Jentsch, 1992). In addition, since MPF inactivation appears to be a checkpoint for exit from mitosis, this process may also require the normal activities of 'checkpoint' genes, candidates for which have recently been identified in *S. cerevisiae* (Hoyt et al., 1991; Li and Murray, 1991). Furthermore, protein phosphatases, such as *dis*2 in *S. pombe* and *bim*G in *A. nidulans* are also required for anaphase events to occur normally (Ohkura et al., 1989; Kinoshita et al., 1990; Doonan and Morris, 1989). These reports have shown that considerable modifications to the cell cycle regulatory apparatus occur during mitosis at the metaphase/anaphase transition;

fizzy could be required for any one of these processes since perturbation of any of these systems could lead to a metaphase arrest phenotype.

However, it is also equally possible that *fzy*, rather than having a regulatory function, could encode a structural component of the mitotic apparatus. Likely possibilities in this case would be as a component of either the spindle fibers or the kinetochores, as the integrity of both these structures is required for normal anaphase and perturbation of either one can result in metaphase arrest (Sluder, 1979; Yen et al., 1991). It should be noted that, at least at the level of analysis that we have conducted so far, we see no gross defects in either spindle fiber or chromosome morphology and arrangement (this work and I. Dawson, unpublished observations). This suggests that, if *fzy* has a structural role, it is one which is specific and necessary for the anaphase separation or movement of chromosomes rather than the general organization of the mitotic apparatus.

Whilst we cannot at present distinguish between a regulatory or structural role for *fzy* during mitosis, we have one piece of data that may be relevant to how *fzy* functions. That is our observation of chimeric metaphase/anaphase nuclei in fzy^{mat} embryos, where we see that most chromosomes are at the metaphase plate but one or two sister chromatids have frequently separated and begun migrating towards the spindle poles. This indicates that *fzy* does not act globally but rather can affect each chromosome individually and separately from its neighbors. Such a localized function suggests that the *fzy* gene product may directly associate with or modify chromosomes, kinetochores or the spindle fibers. While such an association would be expected if *fzy* was a structural component of the mitotic apparatus, it should be noted that mitotic regulatory proteins such as the cyclin-cdc2 complex and type 1 protein phosphatase also show specific localized associations with elements of the mitotic apparatus (Alfa et al., 1990; Pines and Hunter, 1991; Maldonado-Codina and Glover, 1992; Fernandez et al., 1992).

We hope that further resolution of *fzy*'s function during mitosis will prove tractable to molecular analysis which is currently in progress.

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REFERENCES

Alfa, C. E., Beach, D., Ducommun, B. and Hyams, J. S. (1990). Distinct nuclear and spindle pole populations of cyclin-cdc2 in fission yeast. *Nature*, **347**, 680-682.

Artavanis-Tsakonas, S. and Simpson, P. (1991). Choosing a cell fate: a view from the Notch locus. *Trends in Genetics* **7**, 403-408.

Ashburner, M., Detwiler, C., Tsubota, S. and Woodruff, R. C. (1983). The genetics of a small autosomal region of *Drosophila melanogaster* containing the structural gene for alcohol dehydrogenase. VI. Induced revertants of *Scutoid. Genetics* **104**, 405-431.

Ashburner, M., Thomson, P., Roote, J., Lasko, P. F., Grau, Y., El Messal, M., Roth, S. and Simpson, P. (1990). The genetics of a small autosomal region of *Drosophila melanogaster* containing the structural gene for Alcohol dehydrogenase. VII. Characterization of the region around the *snail* and *cactus* Loci. *Genetics* **126**, 679-694.

Beachy, P. A., Helfand, S. L. and Hogness, D. S. (1985). Segmental distribution of bithorax complex proteins during *Drosophila* development. *Nature* **313**, 545-551.

Bodmer, R., Carretto, R. and Jan, Y. N. (1989). Neurogenesis of the peripheral nervous system in *Drosophila* embryos: DNA replication patterns and cell lineages. *Neuron* **3**, 21-32.

Booher, R., Alfa, C. E., Hyams, J. S. and Beach, D. (1989). The fission yeast cdc2/cdc13/suc1 protein kinase: regulation of catalytic activity and nuclear localization. *Cell* **58**, 485-497.

Brinkley, B. R., Ouspenski, I. and Zinkowski, R. P. (1992). Structural and molecular organization of the centromere-kinetochore complex. *Trends in Cell Biol.* **2**, 15-21.

Campos-Ortega, J. A. and Hartenstein, V. (1985). *The Embryonic development of* Drosophila melanogaster. Berlin, Heidelberg: Springer-Verlag.

Campos-Ortega, J. A. and Jan, Y. N. (1991). Genetic and molecular basis of neurogenesis in *Drosophila melanogaster*. *Ann. Rev. Neurosci.* **14**, 399-430.

Dambly-Chaudiere, C. and Ghysen, A. (1986). The sense organs of in the *Drosophila* larva and their relation to the embryonic pattern of sensory neurons. *Roux's Arch. Dev. Biol.* **195**, 222-228.

Doonan, J. H. and Morris, N. R. (1989). The *bim*G gene of *Aspergillus nidulans*, required for completion of anaphase, encodes a homolog of mammalian phosphoprotein phosphatase 1. *Cell* **57**, 987-996.

Draetta, G., Piwinca-Worms, H., Morrison, D., Druker, B., Roberts, T. and Beach, D. (1988). Human cdc2 protein kinase is a major cell-cycle regulated tyrosine kinase substrate. *Nature* **336**, 738-744.

Draetta, G., Luca, F., Westendorf, J., Brizuela, L., Ruderman, J. and Beach, D. (1989). cdc2 protein kinase is complexed with both cyclin A and cyclin B: evidence for proteolytic inactivation of MPF. *Cell* **56**, 829-838.

Dunphey, W. G., Brizuela, L., Beach, D. and Newport, J. W. (1988). The *Xenopuscdc2* protein is a component of MPF, a cytoplasmic regulator of mitosis. *Cell* **54**, 423-431.

Dunphy, W. G. and Newport, J. W. (1988). Unraveling of mitotic control mechanisms. *Cell* **55**, 925-928.

Dunphy, W. G. and Newport, J. W. (1989). Fission yeast p13 blocks mitotic activation and tyrosine dephosphorylation of the *Xenopus* cdc2 protein kinase. *Cell* **58**, 181-191.

Dura, J.-M., Brock, H. W. and Santamaria, P. (1985). *Polyhomeotic*: a gene of *Drosophila melanogaster* required for correct expression of segmental identity. *Mol. Gen. Genet.* **198**, 213-220.

Dura, J.-M., Randsholt, N. B., Deatrick, J., Erk, I., Santamaria, P., Freeman, J. D., Freeman, S. J., Weddell, D. and Brock, H. W. (1987). A complex genetic locus, *polyhomeotic*, is required for segmental specification and epidermal development in *D. melanogaster*. *Cell* **51**, 829-839.

Edgar, B. A. and O'Farrell, P. H. (1989a). Genetic control of cell division patterns in the *Drosophila* embryo. *Cell* **57**, 177-187.

Edgar, B. A. and O'Farrell, P. H. (1989b). The three postblastoderm cell cycles of *Drosophila* embryogenesis are regulated in G2 by *string*. *Cell* **62**, 469-480.

Endow, S. A., Henikoff, S. and Soler-Neidziela, L. (1990). Mediation of meiotic and early mitotic chromosome segregation in *Drosophila* by a protein related to kinesin. *Nature* **345**, 81-83.

Fernandez, A., Brautigan, D. L. and Lamb, N. J. C. (1992). Protein phosphatase type 1 in mammalian cell mitosis: chromosomal localization and involvement in mitotic exit. *J. Cell Biol.* **116**, 1421-1430.

Foe, V. E. (1989). mitotic domains reveal early commitment of cells in *Drosophila* embryos. *Development* **107**, 1-25.

Foe, V. E. and Alberts, B. M. (1983). Studies of nuclear and cytoplasmic behavior during the five mitotic cycles that precede gastrulation in *Drosophila* embryogenesis. *J. Cell Sci.* **61**, 31-70.

Gatti, M. and Baker, B. S. (1989). Genes controlling essential cell cycle functions in *Drosophila melanogaster*. *Genes Dev.* **1**, 924-930.

Gautier, J., Norbury, C., Lohka, M., Nurse, P. and Maller, J. (1988). Purified maturation-promoting factor contains the product of a *Xenopus* homolog of the fission yeast cell cycle control gene *cdc2*$^+$. *Cell* **54**, 433-439.

Gautier, J., Matsukawa, T., Nurse, P. and Maller, J. (1989). Dephosphorylation and activation of *Xenopus* p34 protein kinase during the cell cycle. *Nature* **339**, 626-629.

Ghiara, J. B., Richardson, H. E., Sugimoto, K., Henze, M., Lew, D. J., Wittenberg, C. and Reed, S. I. (1991). A cyclin B homolog in S. cerevisiae: Chronic activation of the cdc28 protein kinase by cyclin prevents exit from mitosis. *Cell* **65**, 163-174.

Ghysen, A. and O'Kane, C. (1989). Neural enhancer-like elements as specific cell markers in *Drosophila. Development* **105**, 35-52.

Glotzer, M., Murray, A. W. and Kirschner, M. W. (1991). Cyclin is degraded by the ubiquitin pathway. *Nature* **349**, 132-138.

Glover, D. M. (1989). Mitosis in *Drosophila. J. Cell Sci.* **92**, 137-146.

Gould, K. and Nurse, P. (1989). Tyrosine phosphorylation of the fission yeast cdc2$^+$ protein kinase regulates entry into mitosis. *Nature* **342**, 39-45.

Hartenstein, V. and Campos-Ortega, J. A. (1984). Early neurogenesis in wild-type *Drosophila melanogaster. Roux's Arch. Dev. Biol.* **193**, 308-325.

Hime, G. and Saint, R. (1992). Zygotic expression of the *pebble* locus is required for cytokinesis during the postblastoderm mitoses of *Drosophila. Development* **114**, 165-171.

Hoyt, M. A., Totis, L. and Roberts, B. T. (1991). *S. cerevisiae* genes required for cell cycle arrest in response to loss of microtubule function. *Cell* **66**, 507-517.

Hunt, T. (1991). Destruction's our delight..... *Nature* **349**, 100-101.

Hyman, A. A. and Mitchison, T. J. (1991). Two different microtubule-based motor activities with opposite polarities in kinetochores. *Nature* **351**, 206-211.

Jan, L. Y. and Jan, Y. N. (1982). Antibodies to horseradish peroxidase as specific neuronal markers in *Drosophila* and in grasshopper embryos. *Proc. Nat. Acad. Sci. USA* **79**, 2700-2704.

Jentsch, S. (1992). Ubiquitin-dependent protein degradation: a cellular perspective. *Trends in Cell Biol.* **2**, 98-103.

Jimenez, J., Alphey, L., Nurse, P. and Glover, D. M. (1990). Complementation of fission yeast *cdc2*^{ts} and *cdc25*^{ts} mutants identifies two cell cycle genes from *Drosophila*: a *cdc2* homolog and *string*. *EMBO J.* **9**, 3565-3571.

Kinoshita, N., Ohkura, H. and Yanagida, M. (1990). Distinct, essential roles for types 1 and 2A protein phosphatases in the control of the fission yeast cell division cycle. *Cell* **63**, 405-415.

Labbé, J. C., Capony, J. -P., Caput, D., Cavadore, J. C., Derancourt, J.-M., Kaghad, M., Lelias, M., Picard., A. and Dorée, M. (1989). MPF from starfish oocytes at the first meiotic metaphase is a heterodimer containing one molecule of cdc2 and one molecule of cyclin B. *EMBO J.* **8**, 3053-3058.

Lee, M. G. and Nurse, P. (1987). Complementation used to clone a human homolog of the fission yeast cell cycle control gene *cdc2. Nature* **327**, 31-35.

Lee, M. G. and Nurse, P. (1988). Cell cycle control genes in fission yeast and mammalian cells. *Trends in Genetics* **4**, 287-290.

Lehmann, R., Jiminez, F., Dietrich, U. and Campos-Ortega, J. A. (1983). On the phenotype and development of mutants of early neurogenesis in *Drosophila melanogaster. Roux's Arch. Dev. Biol.* **192**, 62-74.

Lehner, C. F. and O'Farrell, P. H. (1989). Expression and function of *Drosophila* cyclin A during embryonic cell cycle progression. *Cell* **56**, 957-968.

Lehner, C. F. and O'Farrell, P. H. (1990). *Drosophila cdc2* homologues: a functional homolog is coexpressed with a cognate variant. *EMBO J.* **9**, 3573-3581.

Li, R. and Murray, A. W. (1991). Feedback control in mitosis of budding yeast. *Cell* **66**, 519-531.

Lindsley, D. L. and Zimm, G. (1992). *The Genome of* Drosophila melanogaster. San Diego, California, USA: Academic Press, Inc.

McDonald, H. B., Stewart, R. J. and Goldstein, L. S. B. (1990). The kinesin-like *ncd* protein of Drosophila is a minus end-directed microtubule motor. *Cell* **63**, 1159-1165.

Maldonado-Codina, G. and Glover, D. M. (1992). Cyclins A and B associate with chromatin and the polar regions of spindles, respectively, and do not undergo complete degradation at anaphase in syncitial *Drosophila* embryos. *J. Cell Biol.* **116**, 967-976.

Mitchison, T. J. and Sedat, J. (1983). Localization of antigenic determinants in whole *Drosophila* embryos. *Dev. Biol.* **99**, 261-264.

Morla, A. O., Draetta, G., Beach, D. and Wang, J. Y. J. (1989). Reversible tyrosine phosphorylation of cdc2: dephosphorylation accompanies entry into mitosis. *Cell* **58**, 193-203.

Murray, A. M., Solomon, M. J. and Kirschner, M. W. (1989). The role of cyclin synthesis and degradation in the control of maturation promoting factor activity. *Nature* **339**, 280-286.

Nishimoto, T., Uzawa, S. and Schegel, R. (1992). Mitotic checkpoints. *Current Opinion in Cell Biology* **4**, 174-179.

Nurse, P. (1990). Universal control mechanism regulating onset of M-phase. *Nature* **344**, 503-508.

Nüsslein-Volhard, J., Wieschaus, E. and Kluding, H. (1984). Mutations affecting pattern of the larval cuticle in *Drosophila melanogaster*. I. Zygotic loci on the second chromosome. *Roux's Arch. Dev. Biol.* **193**, 267-282.

Ohkura, H., Kinoshita, N., Miyaiani, S., Toda, T. and Yanagida, M. (1989). The fission yeast *dis*2$^+$ gene required for chromosome disjoining encodes one of two putative type 1 protein phosphatases. *Cell* **57**, 997-1007.

Paschal, B. M. and Vallee, R. B. (1987). Retrograde transport by the microtubule-associated protein MAP 1C. *Nature* **330**, 181-183.

Pfarr, C. M., Coue, M., Grissom, P. M., Hays, T. S., Porter, M. E. and McIntoch, J. R. (1990). Cytoplasmic dynein is localized to kinetochores during mitosis. *Nature* **345**, 263-265.

Pines, J. and Hunter, T. (1991). Human cyclins A and B1 are differentially localized in the cell and undergo cell cycle dependent nuclear transport. *J. Cell Biol.* **115**, 1-17.

Poulson, D. F. (1937). Chromosomal deficiencies and embryonic development of *Drosophila melanogaster*. *Proc. Nat. Acad. Sci. USA* **23**, 133-137.

Poulson, D. F. (1950). Histogenesis, organogenesis and differentiation in the embryo of *Drosophila melanogaster*, Meigen. In *Biology of Drosophila* (ed. M. Demarec). pp. 168-274. New York: Wiley.

Sawin, K. E. and Scholey, J. M. (1991). Motor proteins in cell division. *Trends in Cell Biol.* **1**, 122-129.

Simanis, V. and Nurse. (1986). The cell cycle control gene *cdc2*$^+$ of fission yeast encodes a protein kinase potentially regulated by phosphorylation. *Cell* **45**, 261-268.

Sluder, G. (1979). Role of spindle microtubules in the control of cell cycle timing. *J. Cell Biol.* **80**, 674-691.

Smouse, D., Goodman, C., Mahowald, A. and Perrimon, N. (1988). *polyhomeotic*: a gene required for the embryonic development of axon pathways in the central nervous system of *Drosophila. Genes Dev.* **2**, 830-842.

Steuer, E. R., Wordeman, L., Schroer, T. A. and Sheetz, M. P. (1990). Localization of cytoplasmic dynein to mitotic spindles and kinetochores. *Nature* **345**, 266-268.

Van der Meer, J. M. (1977). Optically clean and permanent whole mount preparation for phase-contrast microscopy of cuticular structures of insect larvae. *Drosophila Inform. Serv.* **52**, 160.

Vaessin, H., Grell, E., Wolff, E., Bier, E., Jan, L. Y. and Jan, Y. N. (1991). *prospero* is expressed in neuronal precursors and encodes a nuclear protein that is involved in the control of axon outgrowth in *Drosophila. Cell* **67**, 941-953.

Walker, R. A., Salmon, E. D. and Endow, S. A. (1990). The *Drosophila claret* segregation protein is a minus-end directed motor molecule. *Nature* **347**, 780-782.

White, R. A. H. and Wilcox, M. (1984). Protein products of the bithorax complex in *Drosophila*. *Cell* **39**, 163-171.

Wieschaus, E. and Nüsslein-Volhard, C. (1986). Looking at Embryos. In Drosophila, *A Practical Approach* (ed. D. B. Roberts). Oxford, England: IRL Press.

Whitfield, W. G. F., Gonzalez, C., Maldonado-Codina, G. and Glover, D. M. (1990). The A- and B-type cyclins of Drosophila are accumulated and destroyed in temporally distinct events that define separate phases of the G2-M transition. *EMBO J.* **9**, 2563-2572.

Yen, T. J., Compton, D. A., Wise, D., Zinkowski, R. P., Brinkley, B. R., Earnshaw, W. C. and Cleveland, D. W. (1991). CENP-E, a novel human centromere-associated protein required for progression from metaphase to anaphase. *EMBO J.* **10**, 1245-1254.

Zalokar, M. and Erk, I. (1976). Division and migration of nuclei during early embryogenesis of *Drosophila melanogaster*. *J. Microbiol. Cell* **25**, 97-106.

Zipursky, S. L., Venkatesh, T. R., Teplow, D. B. and Benzer, S. (1984). Neuronal development in the *Drosophila* retina: monoclonal antibodies as molecular probes. *Cell* **36**, 15-26.

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