1991

Abnormal Chromatin (Abc), a Maternal-Effect Locus in Drosophila Melanogaster

Kristin B. Vessey, *Bowling Green State University - Main Campus*
Rachael L. Ludwiczak, *Bowling Green State University - Main Campus*
Amy S. Briot
Eileen M. Underwood, *Bowling Green State University*

abnormal chromatin (abc), a maternal-effect locus in Drosophila melanogaster

KRISTIN B. VESSEY, RACHAEL L. LUDWICZAK, AMY S. BRIOT and EILEEN M. UNDERWOOD

Department of Biological Sciences, Bowling Green State University, Bowling Green, Ohio 43403, USA

Summary

Mutations in the maternal-effect gene *abnormal chromatin* (*abc*) in *Drosophila melanogaster* result in a variety of defects involving nuclear replication/division. Three recessive alleles of this gene, which maps near 51F on chromosome 2, all result in female sterility. They cause slower embryonic development that is usually abnormal from the earliest nuclear divisions and arrested by the sixth one. Nuclei tend to be large and erratically distributed, some intensely staining. Mitotic asynchrony is common. Few embryos reach the gastrula stage and none hatch. With the weakest allele, *fsPL*, bridges between nuclei are common; abnormal chromatin clumps that resemble yolk nuclei occur before the other nuclei reach the surface; and spindle anomalies and DNA wads with numerous centrosomes are seen. Females with the stronger alleles, *fsA5* and *fs27*, lay fewer eggs and a smaller proportion of embryos reach blastoderm; developmental arrest occurs earlier, usually with several large nuclei distributed along the length of the embryo. Chorion defects occur in all three mutants. Mitotic asynchrony, nuclear bridging, endoreduplication and nuclear behavior aberrant from the first division suggest that the *abc* gene product operates in DNA replication/nuclear division. Larval (homozygous F_1) neuroblast chromosome structure and mitotic indices are normal, indicating that any mitotic function is strictly maternal, i.e. *abc* is not a general mitotic gene. Thus *abc* is one of a few known genes with a maternal effect that appears to function in the embryonic cell cycle.

Key words: maternal-effect mutant, *Drosophila*, cell cycle.

Introduction

Early embryonic development in many metazoans requires rapid DNA synthesis and nuclear divisions and is accompanied by cytoskeletal changes. It therefore provides an avenue for examining such basic cell-biology processes. Analysis of mutants defective in some aspect of a process can illuminate steps involved in it. Isolation of many cell-division-cycle mutants in budding yeast (e.g. see Hartwell *et al.* 1974; Hartwell and Weinert, 1989) has enabled intensive work to be done on mitosis and the cell cycle: at least five parallel pathways of dependent interactions and over 50 genes appear to be involved. Less is known about the cell cycle in more complex eukaryotes. Molluscs, echinoderms, amphibians and arthropods have been subjects of cell-cycle studies, many of which focussed on embryos (for reviews, see McIntosh and Koonce, 1989; Murray and Kirschner, 1989). In *Drosophila*, the search throughout the genome for female-sterile (fs) mutations that halt development has been extensive (e.g. X chromosome: Gans *et al.* 1975; Komitopoulou *et al.* 1983; Mohler, 1977; Orr *et al.* 1989; chromosome 2: Schüpbach and Wieschaus, 1989; Szabad *et al.* 1989; chromosome 3: Erdelyi and Szabad, 1989). It has produced a rapidly growing understanding of genes and pathways involved in oogenesis and the storehouse of maternal molecules that direct such aspects of embryogenesis as cleavage, polarity and nuclear migration. Many of the female-sterile mutations, however, are known only as single alleles and

have not yet been examined in detail. Here we report on three allelic, recessive, maternal-effect lethal mutations in *Drosophila melanogaster* that affect early nuclear behavior and halt development, usually before the cellular blastoderm stage.

In normal *Drosophila* development (see Campos-Ortega and Hartenstein, 1985, for general review), fertilization is followed by nine nuclear divisions in the center of the embryo at approximately 9-min intervals. During the last two divisions the nuclei start migrating to the surface, where the future germline nuclei are set aside in the posterior end and the rest make a single layer and divide four more times (Foe and Alberts, 1983; Zalokar and Erk, 1976). There is no further division in the next hour, which is when cell membranes form; by 3.5 h at 25°C the embryo has reached the cellular blastoderm stage and gastrulation begins.

A review by Glover (1989) includes more than 70 loci implicated by mutations to be involved in the cell cycle in *Drosophila*, but again most of these have only one allele. In a recent study Gatti and Baker (1989) examined a group of mutations that were lethal at the larval–pupal transition (late lethals), expecting some to be involved in mitosis because this is a period when imaginal cells are dividing to produce the adult insect: 30/59 proved to be cell-cycle mutants. Mutations in a few loci that affect nuclear division in the embryo have been studied in detail in the hope of understanding mitotic events in general as well as the extremely fast cleavages unique to the early

embryo. These include *polo* (Sunkel and Glover, 1988), which has spindle abnormalities that are suspected to be caused by centrosome defects, and *asp* (*abnormal spindle*: Gonzalez *et al.* 1990; Ripoll *et al.* 1985), in which a structural element in the spindle is changed.

The *abc* locus is a previously unstudied gene on the second chromosome in region 51F-52A. Recent work on this unexplored area has revealed several maternal-effect genes (Underwood *et al.* 1990; Underwood and Lengyel, 1988). The embryos laid by females hemizygous for the *abc* mutations (ABC embryos) exhibit development that is slower and is arrested long before hatching, often between the third and sixth nuclear divisions. We have studied them with a variety of cytological tools: DNA stains to observe the nuclei; antibodies to actin and tubulin (cellular components of the cytoskeleton also involved in division) and to centrosomes (nucleation sites for mitotic spindles); neuroblast squashes for chromosome structure and mitotic data; and ultrastructural analysis of the chorion. Results from these studies lead us to suspect that the gene may be involved in DNA replication and/or nuclear division in the early embryo.

Materials and methods

Fly stocks

All visible mutants are as described by Lindsley and Grell (1968) or Lindsley and Zimm (1985, 1987). The three alleles of *abc* (*fsPL*, *fs27*, *fsA5*) will be referred to by their allelic designations. Mutants *fs27* and *fsA5* were derived from a screen aimed at saturating the region uncovered by the deficiency *Df(2R)XTE11* (51E;52A6–10), using γ-irradiation of *cn bw sp* (*fs27*) or *cn bw* (*fsA5*) males (Underwood *et al.* 1990). *mat(2)synPL63* (abbreviated here as *fsPL*) was induced with EMS on a *cn bw* chromosome (Schüpbach and Wieschaus, 1989). The original *fsPL* chromosome also contained an allele of the female-sterile mutation *fruh* (T. Schüpbach, personal communication). We have eliminated this second mutation from the chromosome through recombination and find the ABC phenotype unaffected. All were maintained at 25°C by standard lab culture over *CyO* (=*In(2LR)O,Cy dp^{lvl} pr cn²*) or *GlaCyO* (=*In(2LR)Gla, In(2LR)O, Cy dp^{lvl}*) balancers, as was the deficiency, *Df(2R)XTE11*. 'Wild types' used for phenotypic comparisons were Oregon R and the parental chromosome, either *cn bw* or *cn bw sp*.

Embryos

(1) Collections: virgin *mutant/CyO* females were crossed to *XTE11/CyO* males, *mutant/XTE11* F$_1$ females were crossed to Oregon R males, and F$_2$ embryos were collected at 25°C on molasses–agar plates plus yeast paste at timed intervals. All subsequent operations were at room temperature, ~23°C. (2) Dechorionation: embryos were put into water, commercial bleach was added to a concentration of about 50%, and after 2 min they were rinsed several times with water. (3) Vitelline membrane removal and fixation: the method of Mitchison and Sedat (1983) as modified by Karr and Alberts (1986) was scaled-down (1/10) for use in Eppendorf tubes. (a) For antibody studies, the embryos were pipetted into a tube containing 0.5 ml PEM (see Mitchison and Sedat, 1983) + 0.5 ml *n*-heptane + 10–100 µl 37% formaldehyde (with anti-tubulin, 1 µl 0.5 mM taxol in dimethyl-sulfoxide was added before the formaldehyde, the tube shaken for <10 s, and formaldehyde added), shaken for 10 min, the aqueous portion was removed by Pasteur pipette, 0.75 ml methanol added and shaken for 10 min; two rinses followed, first in methanol, then in 100% ethanol, and the embryos were stored at 4°C in 70% ethanol. (b) For staining with Hoechst 33258 alone, embryos were fixed in 0.9 ml *n*-heptane+90 µl Hoechst buffer B+10 µl 37% formaldehyde (Mitchison and Sedat, 1983) and the same procedure was used.

Immunofluorescent staining

The technique of Karr and Alberts (1986) was used except that some primary antibody incubations occurred overnight at 4°C and staining with Hoechst was for 10 min. The primary antibodies were diluted 1/500, the secondaries 1/1000.

Antibodies

The anti-centrosome Rb188 was a kind gift from D. Glover and W. Whitfield, as was taxol from M. Suffness (NCI). The primary monoclonal antibodies, anti-β-tubulin and anti-actin, were purchased from Amersham. Secondary antibodies goat anti-rabbit IgG and goat anti-mouse IgG conjugated with rhodamine came from Organon Teknika and goat anti-mouse IgG conjugated with fluorescein from US Biochemical Corp.

Microscopy

Embryos were viewed with epifluorescence using a Zeiss Axiophot photomicroscope. Film used was Kodak Tech Pan 2415 (ASA 100).

Cuticle

Preparations were by the methods of Matthews and Kaufman (1987), mounting in polyvinyl lactophenol (Bio/medical Specialties, Inc.).

Chorion

Fixation for transmission electron microscopy of chorion fine structure was a modification (lengthening embedding times) of the Kalt and Tandler (1971) method. Embryos were embedded in Spurr's (Electron Microscopy Sciences), sectioned on an LKB Microtome III and viewed on a Zeiss EM10.

Neuroblasts

Third-instar larvae were obtained from cultures transferred daily and supplemented with liquid yeast. Squashes were as described by Karess and Glover (1989). Fields were defined by a 63× Zeiss Plan-apochromat phase-contrast objective and 10× eyepieces on a Zeiss Axiophot microscope.

Results

We examined the nuclei, mitotic apparatus, elements of the cytoskeleton, and chorion of eggs and embryos produced by mutant females, as well as larval neuroblast chromosomes, to help define the role of this gene in early development. Complementation analysis indicated the three mutations are alleles (Underwood *et al.* 1990). They provide a range in severity of defects: fsA5 is the strongest (has the most severe effect) and *fsPL* has the weakest effect, permitting embryos to develop furthest.

Employing a chromosome with a deficiency in the area, *Df(2R)XTE11*, flies were made hemizygous for one of these mutations. Hemizygosity was used because both *fs27* and *fsA5* are homozygous semi-lethal: only two-thirds (*fs27*) or one-fifth (*fsA5*) of the expected number reach the adult stage; the rest die as pupae. This semi-lethality may be due to unrelated lethal(s) (outside the deficiency) induced on the chromosomes during mutagenesis (Underwood *et al.* 1990). Adult hemizygous females showed reduced viability: about 15% died within 4 days of eclosion, compared to no deaths in the hemizygous *cn bw* (*sp*) control. In addition the egg-laying rate was reduced compared to the control (hemizygous parental) rate: *fsPL* was 2/3 and *fs27* and *fsA5* 1/10 the control rate. Embryos from homozygous *fsPL* mothers rarely progressed beyond 64 nuclei. Here we define FSPL, FS27 and FSA5 embryos as those produced by hemizygous females; none of these mutant embryos survived its first 24 h or hatched. The

mutant phenotype was almost always evident in such embryos from the earliest nuclear divisions, regardless of their genotype, i.e. there was no paternal rescue to adulthood of these maternal-effect mutants.

Mutations in abc lead to aberrant nuclear division and the formation of large nuclei

All three alleles have in common: large nuclei, some of which stain intensely and are located along the anterior–posterior (A–P) axis; erratic distribution of nuclei; and mitotic asynchrony. In many embryos there are some bright and some faint nuclei of similar size.

Fig. 1 compares embryonic DNA stained with Hoechst 33258 during the first three nuclear divisions in controls and FSPL. Even at the 2-nuclei stage, the presence of bridges distinguishes FSPL (Fig. 1B) from parental *cn bw* (Fig. 1A). Similarly, the mutant nuclei in the 4-nuclei stage (Fig. 1D) may be joined by bridges, whereas the parental (Fig. 1C) nuclei are not. At the 8-nuclei stage the control (Fig. 1E) nuclei are arranged in a sphere and mitotically synchronized (the fuzzier nuclei are in a different focal plane), while the mutant (Fig. 1F) nuclei already show irregular distribution and mitotic asynchrony.

Many embryos from all three mutants show distribution of nuclei that differs from wild-type by the third cleavage. In wild-type the nuclei are arranged as if on the surface of a sphere that spreads into an ellipse with further cleavage. In the mutants these early nuclei are often not in a sphere and may not form an ellipse, but are rather aligned row-like along the A–P axis (Fig. 2E). In wild-type the nuclei become smaller with successive divisions, so that by pole cell formation the nuclei are small and of uniform size within each embryo. The nuclei in the mutants frequently appear to remain large and there appear sometimes (on the basis of size and/or staining intensity) to be subgroups of nuclei (Fig. 2E); in addition irregularly shaped nuclei that resemble yolk nuclei may be present (Fig. 2C).

Yolk nuclei abberant. Yolk nuclei in wild-type embryos (Fig. 2B) become evident in the interior during migration of nuclei toward the embryo's surface, starting with cleavage 7. After three mitoses (stages 8–10) the yolk nuclei stop dividing but continue replicating DNA and become polyploid, as seen by increased staining intensity (Foe and Alberts, 1983). They are initially spread throughout the embryo (with relatively few in the pole cell region) and appear gradually to withdraw into the central portion along the A–P axis with the remaining yolk. They maintain their integrity as individual nuclei, but sometimes clump together in small bunches.

ABC embryos (Fig. 2D), on the other hand, commonly have less uniform distribution of yolk nuclei, some of which may be near the pole cells (seen also in *engrailed* embryos (Karr et al. 1985)). Abnormal chromatin clumps that look like yolk nuclei may show up early (Fig. 2C), i.e. before the other nuclei have surfaced. There is a strong tendency to clump, so that an embryo may appear at low magnification to have a small number (e.g. 7) of huge, brightly staining yolk nuclei that are often strongly channelled along the A–P axis (Fig. 2D).

An opaque Hoechst-staining ring occurs in the posterior (Fig. 2F) of some embryos of all three mutants, least often in FSPL (5 %) and in about 20 % of the other two. The wild-type control shows such a ring in <1 % of its embryos. The ring is generally in the presumptive pole cell position.

Blastoderm abnormal. In wild-type embryos, nuclei that reach the surface are uniformly distributed and mi-

totically synchronized prior to gastrulation (Foe and Alberts, 1983; Karr et al. 1985) (Fig. 2A,B). The phenotype in the small number of mutant ABC embryos that develop that far (Fig. 2C) is variable but characteristically includes abnormal chromatin clumps, unusual distribution of nuclei, and some chromatin extending between nuclei that are otherwise well-separated (i.e. nuclear bridges). Pole cells often form in those embryos with nuclei in the posterior part. In the 30 % of FSPL embryos where nuclei reach the surface (Fig. 2D), abnormal blastoderm forms in most (4/5), while the rest (1/5) have a normal surface pattern; about a third of those with surface nuclei attempt to gastrulate, and a fifth of those gastrulating survive long enough to make some aberrant cuticle (2 % of total embryos laid).

In embryos that reach blastoderm, ABC surface nuclei vary in location, extent and mitotic stage (Fig. 2D). Often there is a single row of nuclei in a different mitotic stage bordering regions with and without surface nuclei (Fig. 5B,D,F). Distribution of nuclei within one area may be normal, but in over half these embryos there are regions of different mitotic stages that are distinct from the ends-to-middle mitotic waves seen in wild type (parasynchrony: Zalokar and Erk, 1976); a similar phenotype has been described by Karr and his coworkers (1985) in *engrailed* embryos. In addition, the region containing surface nuclei may be compressed relative to the rest of the embryo, as if girdled.

Mitosis normal in larval neuroblasts. To see if the early embryonic phenotype reflects weak expression of a general mitotic defect, we assessed the frequency of cell division and examined chromosomal structure during mitosis in neuroblasts (Gatti et al. 1974) from F_1 third-instar larvae. Homozygotes are *cn bw* and thus distinguishable by the color of their Malpighian tubules (white) from their heterozygous (yellow) sibs. For each genotype, mitotic figures in at least 10 fields containing actively dividing cells were counted from each of 5–11 individuals and averaged to give the mitotic index (mitotic figures/field). The proportion of mitotic figures in anaphase was similar across genotypes. No differences in mitotic index more extreme than 0.5–1.5 times the control values were observed (Table 1), a range that Gatti and Baker (1989) defined as equivalent to the control. Neither were chromosomal aberrations (in ploidy, condensation or integrity) seen, with or without colchicine and hypotonic shock.
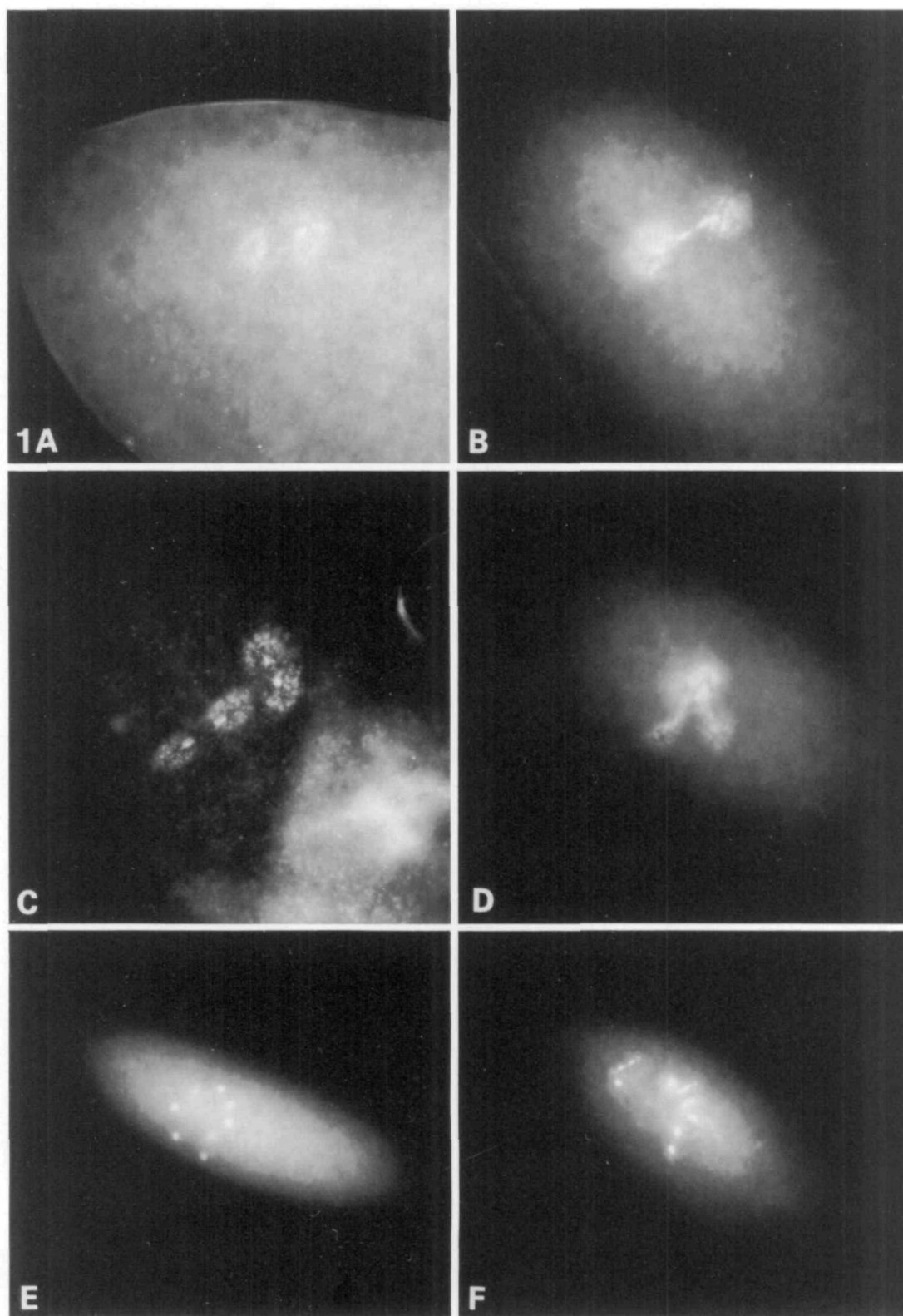
Mutations in abc affect egg structure and development

Chorion structure abnormal. The normal chorion comprises two layers of approximately equal depth: the

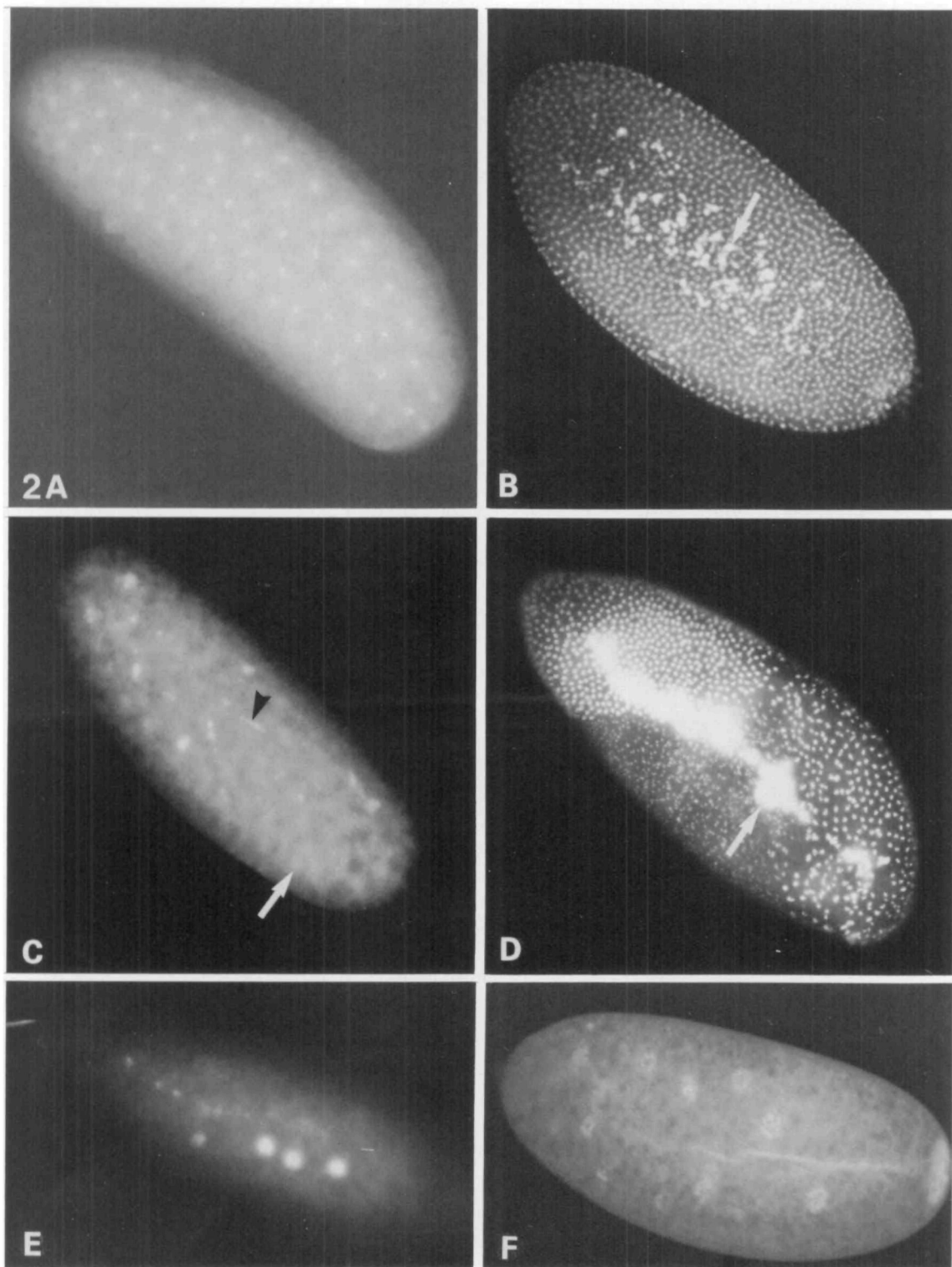**Table 1.** *Analysis of mitotic figures in neuroblasts*

Genotype*	Fields (individuals)	Mitotic figures	Anaphases (%)	Mitotic index†
A. *OR*	88 (8)	1149	255 (22)	13.1
cn bw	58 (6)	968	242 (25)	16.7
B. *fsA5/fsA5*	143 (12)	2311	484 (21)	16.2
fsA5/GlaCyO	111 (9)	1910	474 (25)	17 2
C. *fs27/fs27*	124 (11)	1935	458 (24)	15.6
fs27/CyO	133 (11)	1673	428 (26)	12.6
D. *fsPL/fsPL*	51 (5)	746	225 (30)	14.6
fsPL/GlaCyO	77 (7)	1063	397 (37)	13.8

*A Wild type; B–D, homozygous mutants and their heterozygous sib controls.
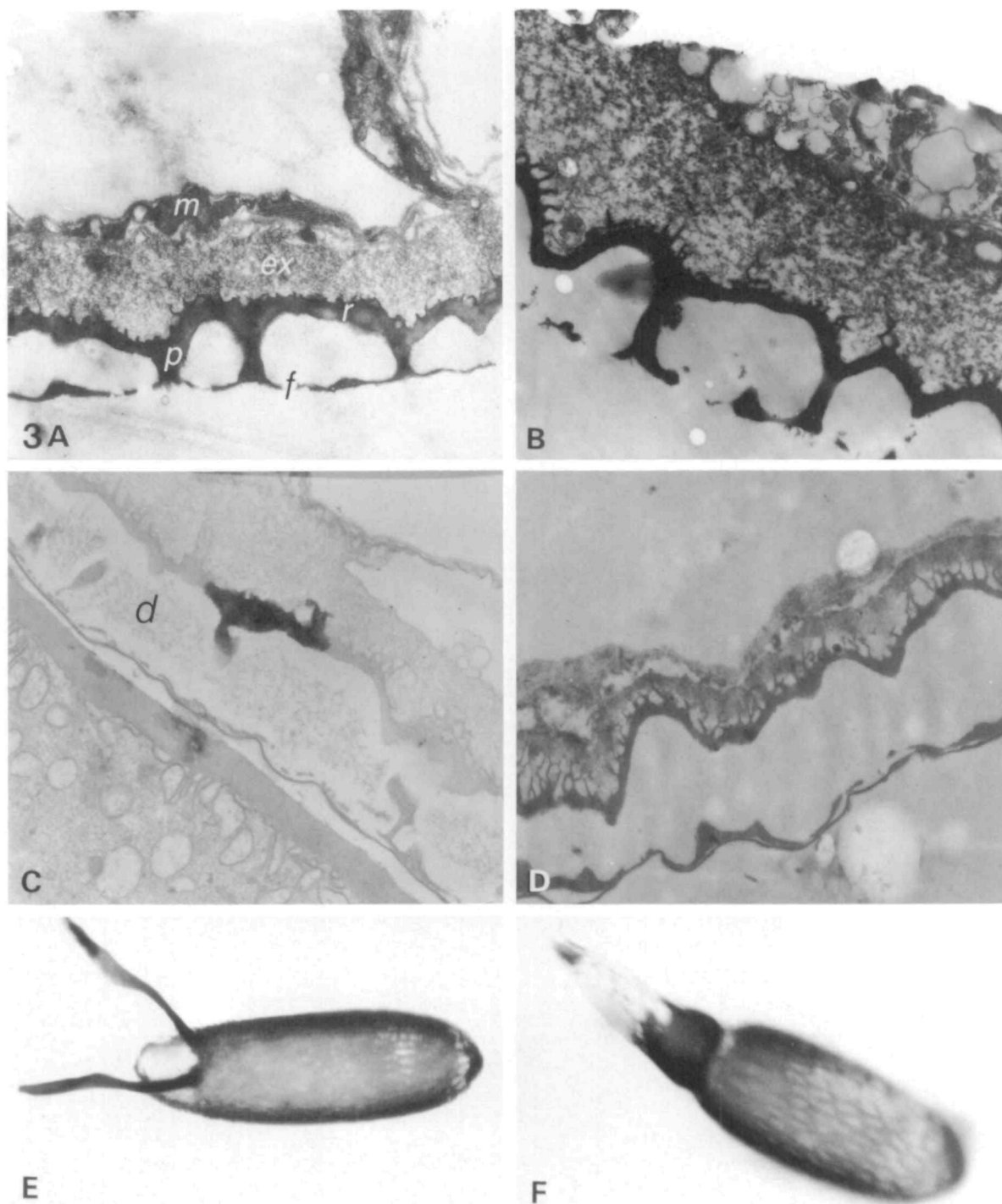†Mitotic index, no. of mitotic figures/no. of fields.

Fig. 1. Nuclear stages 2–4. (A, C, E) Wild type: (A) 2 nuclei, no bridge. (C) 4 nuclei in anaphase. (E) 8 nuclei showing spherical arrangement and mitotic synchrony. (B, D, F) FSPL: (B) 2 nuclei bridged; (D) 4 nuclei bridged; (F) 8 nuclei with neither spherical distribution nor mitotic synchrony. Note: the starbursts of small dots surrounding the nuclei in A–D may indicate the presence of a bacterial endosymbiont (D. Glover, T. Karr, personal communication). A–D, ×400; E–F, ×200.

Fig. 2. Typical phenotypes of the three alleles compared with wild type after Hoechst staining of the DNA. (A) Wild type at ~1 h has nuclei of uniform size and distribution. (B) Wild type at syncytial blastoderm: nuclei cover the surface uniformly and are mitotically synchronized; yolk nuclei (arrow) are small and slightly clumped. (C) FSPL at ~1 h has irregularly shaped and distributed brightly stained nuclei with some bridges (arrowhead) as well as more uniformly sized, fainter nuclei approaching the surface (arrow). (D) FSPL at syncytial blastoderm: surface nuclei are abnormally distributed and in different mitotic stages; yolk nuclei (arrow) are extremely clustered and channelled. (E) FS27 at ~1 h has 9 nuclei strung out A–P, with one group of 4 much larger and brighter. (F) FSA5 at ~1 h has 12 nuclei extending along two-thirds of the embryo instead of in a sphere; an opaque ring (arrowhead) in the posterior is in the presumptive pole cell region; the white line is an artifactual fold in the vitelline membrane. The anterior end of the embryo is at the upper left. ×200.

Fig. 3. Chorions produced by hemizygous females: ultrastructure of stage 14 oocytes (A–D) and surface morphology of laid eggs (E–F). (A) Wild type: uniform exochorion (ex) and endochorion, which is subdivided into continuous roof (r), solid pillars (p) in a uniform pattern, and floor (f); muscle (m). There is no debris in the space between pillars. (B) FSPL: less compact exochorion, normal roof, 'weak' pattern of pillars, and floor that has more breaks than normal. (C) FS27: defective exochorion, thinner roof than normal, no pillars, and debris (d) between roof and floor. (D) FSA5: underdeveloped exochorion, thin roof, no pillars, and nonuniform, 'clumpy' floor. ×12500. (E) Control, cn bw/XTE11. ×116. (F) Mutant, A5/XTE11: note dorsal chorionic appendages, which are shorter, fatter and fused into a ring. ×120.

exochorion is fairly homogeneous, while the endochorion is divisible into roof and floor connected by pillars (Fig. 3A). Abutting the floor are the waxy layer and vitelline membrane. All three of the abc mutants contained chorion defects in mature stage-14 oocytes and

laid eggs (Fig. 3), most severe in FSA5 and least in FSPL. The structure of the exochorion was much thinner in FSA5 (Fig. 3D). In the endochorion, both FSA5 and FS27 had few pillars, the roof was thin and debris was often seen in the spaces between the pillars (Fig. 3C,D); FSPL had fewer

than normal pillars (Fig. 3B). The fact that 10% of FSA5 and FS27 embryos did not survive dechorionation suggests problems as well with the innermost eggshell layer, the vitelline membrane.

Chorion defects were visible in whole mounts in 30% of the eggs from mothers hemizygous for one of the stronger alleles and 3% of FSPL eggs, compared with 0.3% of eggs from the hemizygous control. We describe two overlapping classes of defects, one involving the appendages and one the overall thickness of the chorion. The dorsal chorionic appendages are respiratory elaborations on the anterior, dorsal side of the embryo that extend outward in antenna-like fashion (Fig. 3E). Changes in the dorsal appendages occurred in both FSA5 and FS27, more frequently in the former. There was a range of egg morphs: in some eggs shorter and fatter appendages extended laterally around the embryo and sometimes fused at the base in the ventral midline, forming a ring around the embryo (Fig. 3F). Variable expressivity was apparent, in that a single female laid different morphs in a 24-h period. The appendages adhered to each other in many of these embryos and sometimes occluded the micropyle (point of sperm entry), which may have been a factor in the increased number of apparently unfertilized eggs observed in these mutants. In some cases the egg shape itself differed, with a collar-like region at the anterior end (Fig. 3F); and in a few, this end was missing, so that the appendages came off the top of a shorter egg. In the other class of defect, the chorion was translucent instead of opaque as in wild type; since the appendages were present, this probably means thin or missing exochorion.

Unfertilized eggs 'normal'. At least one mutation affecting DNA replication (*gnu*) shows a mutant phenotype in the absence of fertilization (Freeman and Glover, 1987), so we looked at unfertilized eggs and found that the ABC mutant phenotype was not produced. No changes in the cortical actin or tubulin patterns were seen in unfertilized eggs laid by virgin females (mutants or control) from 0–24-h collections, nor was there evidence of mitotic apparatus except in polar bodies. Degenerative changes in the cytoplasm were visible in many of the eggs; these appeared earlier and were more severe in unfertilized ABC eggs than in unfertilized control eggs, suggesting that *abc* females hold their eggs. The large asters associated with degeneration in fertilized FSPL eggs were never seen in unfertilized eggs from either group (data not shown).

Embryogenesis delayed. A developmental profile (Fig. 4) was obtained from four successive 1-h collections of embryos by determining the proportions of embryos at different nuclear division stages: in the first group ages (counted from the time of egg deposition) in the population ranged from 0.5–1.5 h and in the last, 3.5–4.5 h. Development was slower in FSPL: there was a preponderance of earlier stages and progression to the 32-nuclei stage took longer than in wild type. By 2.5 h, wild-type embryos had most of their nuclei at the surface, but most FSPL embryos still had 16 or fewer nuclei. By 4 h most wild types were gastrulating, in contrast to the mutant embryos, of which about a third had nuclei at the surface, over half still had less than 16 nuclei and a very few were attempting gastrulation.

About 2% (17/850) of these FSPL embryos lived long enough to make some cuticle (at 13–16 h), but in each one it was aberrant. Some of the denticle belts were missing and parts of others were fused, twisted, and/or partial; there was no obvious pattern to the defects when different
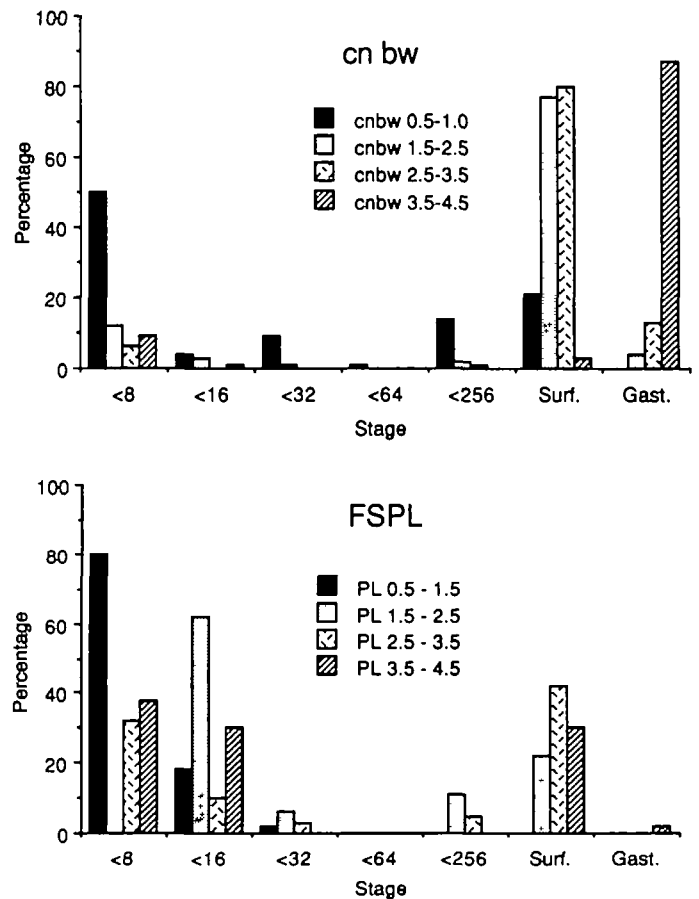

Fig. 4. Developmental timing. Embryos from 4 different age groups (in hours) were classed according to nuclear stage: <8, stages 1–4; <16, stage 5; <32, stage 6; <64, stage 7; <256, stages 8–9; Surf., stages 10–14, blastoderm; Gast., beyond cellular blastoderm.

embryos were compared (data not shown). Because FS27 and FSA5 embryos cease nuclear division very early developmental profiles were not performed.

The three alleles can be ranked in terms of increasing severity of defect: *fsPL<fs27<fsA5*. In a 0–4-h collection of embryos, most FSPL have 16 or fewer nuclei, FS27 have 8 or fewer, and FSA5, 4 or fewer. There is a subgroup of embryos with nuclei that reach the surface, ~30% in FSPL and ~2% in the other two mutants. In FS27 embryos there are rarely >32 nuclei and in FSA5 rarely >16; thus developmental arrest usually has occurred by the 5th or 6th nuclear division. This same ranking is found in the chorion defects.

Cytoskeletal abnormalities

Actin and tubulin. Because actin is involved in cytoplasmic movement and nuclear migration, as tubulin is in the mitotic apparatus, we used antibodies to them in seeking clues to their behavior in the mutants. There is a transition in wild-type embryos from a surface lawn of actin to actin caps and rings around the nuclei during different mitotic stages once the nuclei have surfaced. Tubulin-containing structures in the cortex are also reorganized during migration (Karr and Alberts, 1986). In the rare cases when nuclei in the ABC embryos reach the surface, both the actin and tubulin surface patterns change as they do in wild type; the pattern is less regular,

however, and depending on the local condition of nuclei may be at different stages in different parts of an embryo. Otherwise there is no change in the surface pattern for either actin or tubulin (data not shown).

Because antibodies to tubulin visualize the mitotic apparatus of nuclei, mitotic stages and various anomalies could be discerned. Normal spindles and behavior were common in the mutants. Those embryos showing mitotic asynchrony (Fig. 2D) in regions with surface nuclei had contiguous patches (not individual nuclei) in different stages. Multiple spindles from a common pole or half-spindles occurred, and the latter might have one or no asters (Fig. 5F). Spindles were frequently in disarray (differing in size, stage, integrity) in the part of an embryo lacking surface nuclei.

Centrosomes. Because centrosome abnormalities occur in some other maternal-effect mutants, we examined the ABC mutants using the anti-centrosome antibody Rb188 (Whitfield *et al.* 1988), which reacts with both centrosomes and nuclear membranes (Fig. 5A). Centrosome behavior in these mutants seemed normal with no large-scale independent replication divorced from nuclear replication as in *gnu* (Freeman and Glover, 1987) or *asp* (Gonzalez *et al.* 1990), but centrosomal division was sometimes asynchronous (Fig. 5B). Small tracts of centrosomes and asters without visible nuclei were common in mutant embryos that reached blastoderm (Fig. 5B,D,F). In FSPL the brightly stained DNA wads (Fig. 5D) often had several spindles and numerous (8–22) centrosomes. Degeneration occurred earlier in mutant embryos, including loss of centrosomes from nuclei (or *vice versa*), so that clusters of centrosomes without nuclei or with bits of chromatin appeared. The numbers of spindle anomalies and DNA wads were higher in older FSPL embryos and may be part of the degenerative process. Very large asters developed on the surface, typically not associated with nuclei. In contrast, wild-type embryos of the same ages contained no multiple spindles, no DNA wads, no large surface asters, and no centrosomes without nuclei (Fig. 5A–C).

Discussion

The *abc* locus is one of a few maternal-effect genes that appear to affect the cell cycle in the early *Drosophila* embryo (see review by Glover, 1989). Early nuclei in the F_2 ABC embryos behave differently from wild type: cleavage usually ends prematurely and often with only a few large nuclei, nuclei do not always separate cleanly, some nuclei stain more intensely than others, distribution of nuclei may be abnormal, unusual chromatin clumps develop, mitosis is often asynchronous, development is slower, syncytial blastoderm is abnormal; in addition, the chorion is defective. Thus the mutated *abc* gene product made by hemizygous (and homozygous *fsPL*) mothers is inadequate to support development up to the larval stage, and in most of the embryos not even up to gastrulation. Therefore this gene is essential in the initial stages of development. Since nuclear behavior is often aberrant from the first divisions, we consider it likely that the embryonic nuclear cycle is being disrupted and that the gene is involved in some aspect of DNA replication or nuclear division.

Mitotic divisions in homozygous larval neural ganglia appear normal and no chromosomal defects are apparent, evidence that if the gene acts in cell division its main role is maternal rather than zygotic. Many F_2 embryos with either of the stronger alleles appear not to develop and

most of the rest arrest earlier than FSPL. The large nuclei characteristic of early arrest appear to be in interphase, suggesting that progress through this stage is not completed. Thus this gene might act in DNA synthesis or in preparing for or triggering mitosis (see reviews by Glover, 1989; Murray and Kirschner, 1989).
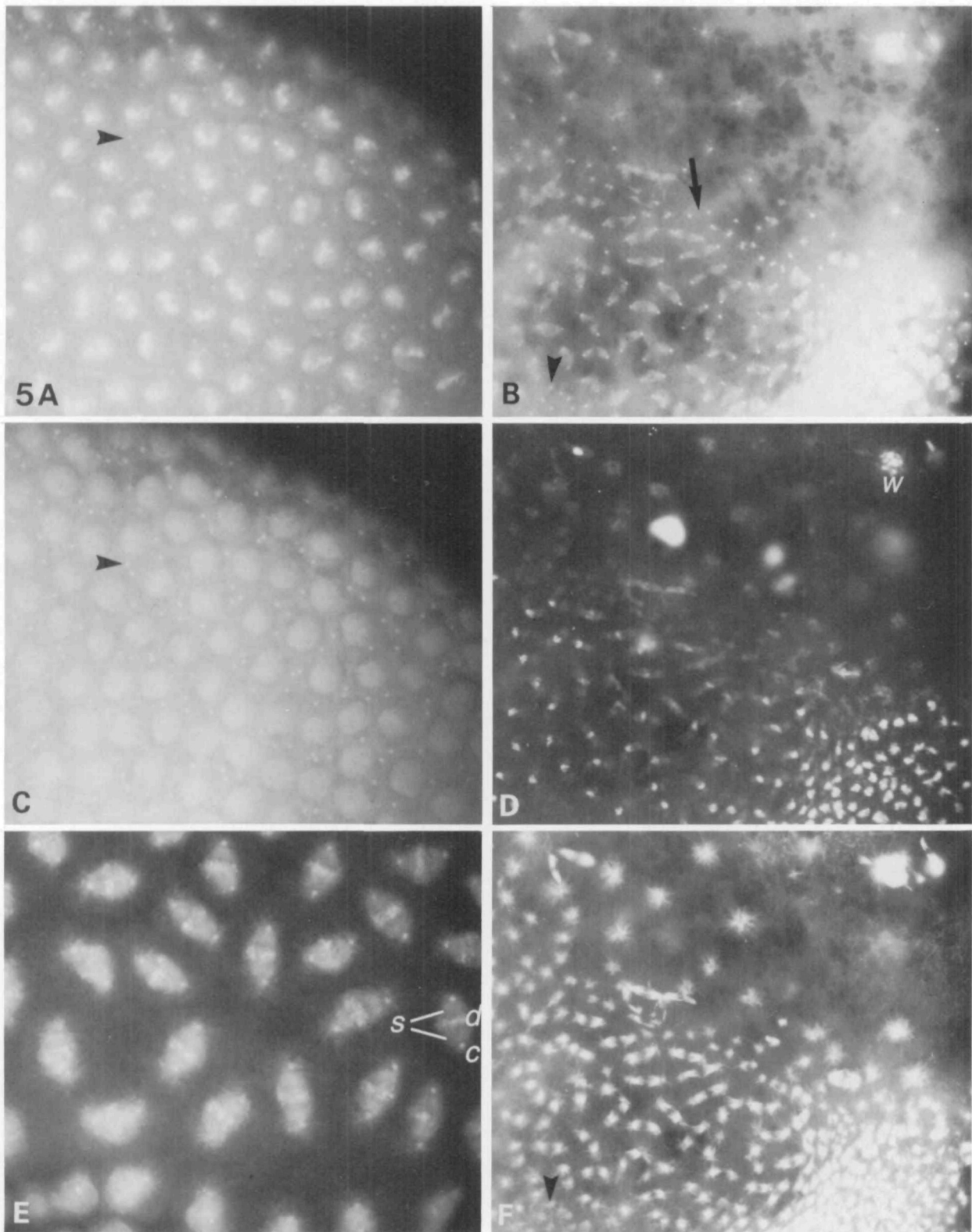
A defect in DNA synthesis could explain the seemingly disparate phenotypic characteristics of chorion defects and early developmental arrest if there is under-replication of DNA in the follicle cells and/or nurse cells, which are polyploid and interact in producing the mature oocyte. Under-replication could cause insufficient amounts of the eggshell and internal oocyte components to be produced. The chorion genes themselves are not underamplified when compared with an internal control, rDNA levels (Underwood *et al.* 1990), but this does not rule out lower ploidy in follicle cells.

The connections between nuclei seen frequently in the F_2 embryos may be telophase bridges, reflecting difficulties involving the mitotic apparatus, sister chromatid separation, or incomplete DNA replication. An apparent phenocopy of this bridging characteristic has been produced by aphidicolin, a DNA synthesis inhibitor that interferes with DNA polymerase α (Ikegami *et al.* 1979), in sea urchin embryos (Brachet and DePetrocellis, 1981) and *Drosophila* embryos (Raff and Glover, 1988). The telophase bridges between nuclei have been attributed to the spindles' difficulty in separating incompletely replicated DNA (Raff and Glover, 1988). Zahner and Cheney (1990) interpret nuclear clustering in their *quartet* embryos as indicative of chromosome separation problems. Failure of chromosome separation with continued DNA replication is a cause of endoreduplication. The increased staining seen in a subset of nuclei in some ABC embryos would suggest endoreduplication.

The slower development we see in FSPL might be related to the cell-cycle delay observed in sea-urchin (Ikegami *et al.* 1979), starfish (Nagano *et al.* 1981) and *Drosophila* (Raff and Glover, 1988) embryos treated with aphidicolin. While the polymerase–aphidicolin interaction affects DNA replication, it also causes an unexplained delay in mitosis (Raff and Glover, 1988). Abnormal tubulin can also retard mitosis (Sluder, 1979; Sluder and Begg, 1983). The frequent failure of nuclei in the ABC mutant embryos to become arranged in a sphere and then an ellipse during the first 5 cleavages (as wild type does: see Zalokar and Erk, 1976) suggests some type of distribution/ migration problem. Alternatively, it could be explained by a developmental delay in nuclear division: the embryos are 'older' and try to allocate the nuclei appropriately, but due to delay in nuclear division and/or failure of separation (both of which may be related to incomplete DNA replication) there are too few nuclei to show the normal pattern.

In FSPL embryos, the abnormal clumps may be yolk nuclei that appear relatively premature only because the other nuclei are delayed in reaching the surface. Since wild-type yolk nuclei continue to divide in synchrony with the rest of the nuclei in cleavages 8–10 (Foe and Alberts, 1983), their polyploid state must develop after that. A second possibility is that the brightly staining, abnormal chromatin represents early polyploidy in yolk nuclei due to separation failure.

The ABC phenotype can be seen only after fertilization, in contrast to *gnu* (Freeman and Glover, 1987). Centrosomes in *gnu* embryos replicate independently of nuclei, migrate to the surface where they continue to replicate

Fig. 5. Centrosomes. (A, C, E) Wild type: Centrosomes (C) associated with surface nuclei (Rb188 anti-centrosome antibody also stains nuclear membrane), here in prometaphase, are seen also in a double exposure showing DNA (A) (arrowheads are reference points). (E) Metaphase mitotic figures in a triple exposure of a different embryo showing centrosomes (c) spindles (s), and DNA (d). (B, D, F) FSPL: views of one triply stained embryo using different filters (note that the anti-tubulin–FITC conjugate leaks through in B and D): centrosomes in B, DNA in D, tubulin in F. Note: the DNA wad (w), the cluster of centrosomes without obvious DNA (arrowhead), the string of centrosomes (arrow) bordering the sparsely populated upper region. A, C, E, ×1000; B, D, F ×630.

without nuclei, and show a uniform distribution (Raff and Glover, 1988). FSPL embryos have centrosome replication occurring in conjunction with nuclear replication and distribution of centrosomes is often nonuniform, probably reflecting aberrant distribution of nuclei and nuclear breakdown. The occasional small tracts of centrosomes associated with asters but not visibly with DNA may also represent nuclear breakdown. Additionally, free centrosomes have been seen in other mitotic mutants that display syncytial embryonic phenotypes (e.g. *asp*, see Gonzalez *et al.* 1990; *aurora*, Leibowitz and Glover, personal communication).

The aberrant patterns of surface nuclei in FSPL seem to reflect loss of different groups of nuclei from the surface (on the basis of observations of living embryos). The apparent randomness of this variable distribution is reminiscent of gynander patterns, where a stochastic event can have a variable result depending on the point in development and the random orientation of the spindle during and following the event (Garcia-Bellido, 1979). Such an event could also explain why in early embryos some nuclei look normal and some abnormal from the beginning. The variable phenotype may be a generic response to such a stochastic event: a similar phenotype has been observed in several lines with female-sterile mutations at other loci on the second chromosome (K. Harding and E. Wieschaus, personal communication). Alternatively, such genes may act in the same pathway. Other explanations for variable phenotypes in maternal-effect mutants are environmental factors such as temperature, food and female age (Schüpbach and Wieschaus, 1989) and leaky mutations (Gatti and Baker, 1989).

Because the earliest nuclear cycles are rapid and internal, they are not easy to dissect. Phenotypic analysis of mutants defective in the cell cycle is currently the major means by which one can approach this complex process. Here we have presented such an analysis of one of a few known *Drosophila* maternal-effect genes in which mutations disrupt the cell cycle during early cleavage. Many loci must be active in the pathways related to early nuclear proliferation, and only through a detailed analysis of mutations in such genes can we hope to understand the processes involved in this aspect of early development. Further pursuit of the *abc* gene is in progress in our laboratory.

References

BRACHET, J. AND DEPETROCELLIS, B. (1981). The effects of aphidicolin, an inhibitor of DNA replication, on sea urchin development. *Expl Cell Res.* 135, 179–189.

CAMPOS-ORTEGA, J. AND HARTENSTEIN, V. (1985). *The Embryonic Development of* Drosophila melanogaster. New York: Springer-Verlag.

ERDELYI, M. AND SZABAD, J. (1989). Isolation and characterization of dominant female sterile mutations of *Drosophila melanogaster.* I. Mutations on the third chromosome. *Genetics* 122, 111–127.

FOE, V. AND ALBERTS, B (1983). Studies of nuclear and cytoplasmic behaviour during the five mitotic cycles that precede gastrulation in *Drosophila* embryogenesis. *J. Cell Sci.* 61, 31–70.

FREEMAN, M. AND GLOVER, D. (1987). The *gnu* mutation of *Drosophila* causes inappropriate DNA synthesis in unfertilized and fertilized eggs. *Genes Dev.* 1, 924–930.

GANS, M., AUDIT, C. AND MASSON, M. (1975). The isolation and characterisation of sex-linked female sterile mutants in *Drosophila melanogaster. Genetics* 81, 683–704.

GARCIA-BELLIDO, A. (1979). Genetic analysis of the achaete-scute system of *Drosophila melanogaster. Genetics* 91, 491–520.

GATTI, M. AND BAKER, B. S. (1989). Genes controlling essential cell-cycle functions in *Drosophila melanogaster. Genes Dev.* 3, 438–453.

GATTI, M., TANZARELLA, C. AND OLIVIERI, G. (1974). Analysis of chromosome aberrations induced by X-rays in somatic cells of *Drosophila melanogaster. Genetics* 77, 701–719.

GLOVER, D. (1989). Mitosis in *Drosophila. J. Cell Sci.* 92, 137–146.

GONZALEZ, C., SAUNDERS, R. D. C., CASAL, J., MOLINA, I., CARMENA, M., RIPOLL, P. AND GLOVER, D. M. (1990). Mutations at the *asp* locus of *Drosophila* lead to multiple free centrosomes in syncytial embryos, but restrict centrosome duplication in larval neuroblasts. *J. Cell Sci.* 96, 605–616.

HARTWELL, L., CULOTTI, J., PRINGLE, J. AND REID, B. (1974). Genetic control of the cell division cycle in yeast. *Science* 183, 46–51.

HARTWELL, L. AND WEINERT, T. (1989). Checkpoints: controls that ensure the order of cell cycle events. *Science* 246, 629–634.

IKEGAMI, S., AMEMIYA, S., OGURO, M., NAGANO, H. AND MANO, Y. (1979). Inhibition by aphidicolin of cell cycle progression and DNA replication in sea urchin embryos. *J. cell. Physiol.* 100, 439–444.

KALT, M. R. AND TANDLER, B. (1971). A study of fixation of early amphibian embryos for electron microscopy. *J. Ultrastruct. Res.* 36, 633–640.

KARESS, R. E. AND GLOVER, D. M. (1989). *rough deal:* A gene required for proper mitotic segregation in *Drosophila. J. Cell Biol.* 109, 2951–2961.

KARR, T. AND ALBERTS, B. (1986). Organization of the cytoskeleton in early *Drosophila* embryos. *J. Cell Biol.* 102, 1494–1509.

KARR, T. L., ZEHRA, A., DREES, B. AND KORNBERG, T. (1985). The *engrailed* locus of *D. melanogaster* provides an essential zygotic function in precellular embryos. *Cell* 43, 591–601.

KOMITOPOULOU, K., GANS, M., MARGARITIS, L., KAFATOS, C. AND MASSON, M. (1983). Isolation and characterization of sex-linked female-sterile mutants in *Drosophila melanogaster. Genetics* 105, 897–920.

LINDSLEY, D. AND GRELL, E. (1968). *Genetic Variations of* Drosophila melanogaster. Carnegie Inst. Washington, DC.

LINDSLEY, D. AND ZIMM, G. (1985). The genome of *Drosophila melanogaster.* Part 1: Genes A-K. *Drosoph. Inform. Serv.* 62, 1–227.

LINDSLEY, D. AND ZIMM, G. (1987). The genome of *Drosophila melanogaster.* Part 3: Rearrangements *Drosoph. Inform. Serv.* 65, 1–203.

MATTHEWS, K. A. AND KAUFMAN, T. C. (1987). Developmental consequences of mutations in the 84B α-tubulin gene of *Drosophila melanogaster. Devl Biol.* 119, 100–114.

McINTOSH, J. AND KOONCE, M. (1989). Mitosis. *Science* 246, 622–628.

MITCHISON, T. AND SEDAT, J. (1983). Localisation of antigenic determinants in whole *Drosophila* embryos. *Devl Biol.* 99, 261–264.

MOHLER, J. (1977). Developmental genetics of the *Drosophila* egg. I. Identification of 50 sex-linked cistrons with maternal effects on embryonic development. *Genetics* 85, 259–272.

MURRAY, A. AND KIRSCHNER, M. (1989). Dominoes and clocks: the union of two views of the cell cycle. *Science* 246, 614–621.

NAGANO, H., SETSURO, H., OKANO, K. AND IKEGAMI, S. (1981). Achromosomal cleavage in fertilized starfish eggs in the presence of aphidicolin. *Devl Biol.* 85, 409–415

ORR, W, GALANOPOULOS, V., ROMANO, C. AND KAFATOS, F (1989). A female sterile screen of the *Drosophila melanogaster* X chromosome using hybrid dysgenesis. Identification and characterization of egg morphology mutants. *Genetics* 122, 847–858.

RAFF, J. AND GLOVER, D. (1988). Nuclear and cytoplasmic mitotic cycles continue in *Drosophila* embryos in which DNA synthesis is inhibited with aphidicolin. *J. Cell Biol.* 107, 2009–2019.

RIPOLL, P., PIMPINELLI, S., VALDIVIA, M. AND AVILA, J. (1985). A cell division mutant of *Drosophila* with a functionally abnormal spindle. *Cell* 41, 907–912.

SCHÜPBACH, T. AND WIESCHAUS, E. (1989). Female-sterile mutations on the second chromosome of *Drosophila melanogaster.* I. Maternal effect mutations. *Genetics* 121, 101–117.

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

SLUDER, G. AND BEGG, D. (1983). Control mechanisms of the cell cycle: role of the spatial arrangement of spindle components in the timing of mitotic events. *J. Cell Biol.* 97, 877–886.

SUNKEL, C. AND GLOVER, D. (1988). *polo,* a mitotic mutant of *Drosophila* displaying abnormal spindle poles. *J. Cell Sci.* 89, 25–38.

SZABAD, J., ERDELYI, M., HOFFMANN, G., SZIDONYA, J. AND WRIGHT, T. (1989). Isolation and characterization of dominant female sterile

mutations of *Drosophila melanogaster*. II. Mutations on the second chromosome. *Genetics* 122, 823–835

UNDERWOOD, E. M., BRIOT, A. S., DOLL, K. Z., LUDWICZAK, R. L., OTTESON, D. C., TOWER, J., VESSEY, K. B. AND YU, K. (1990). Genetics of 51D-52A, a region containing several maternal-effect genes and two maternal-specific transcripts in *Drosophila Genetics* 126, 639–650.

UNDERWOOD, E. M. AND LENGYEL, J. A. (1988). *Drosophila* genes encoding maternal-specific and maternal-differential RNAs. *Devl Genet.* 9, 23–35.

WHITFIELD, W., MILLAR, S., SAUMWEBER, H., FRASCH, M. AND GLOVER, D (1988) Cloning of a gene encoding an antigen associated with the centrosome in *Drosophila. J. Cell Sci.* 89, 467–480.

ZAHNER, J E. AND CHENEY, C M. (1990). *quartet*: a *Drosophila* developmental mutation affecting chromosome separation in mitosis. *Devl Genet.* 11, 27–40.

ZALOKAR, M. AND ERK, I. (1976). Division and migration of nuclei during early embryogenesis of *Drosophila melanogaster. J. Microsc. Biol. Cell* 25, 97–106