Development 104, 483–493 (1988)
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Requirements for X-linked zygotic gene activity during cellularization of early *Drosophila* embryos

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Summary

To examine the requirements for X-chromosomal transcription during precellular stages of *Drosophila* embryogenesis, attached X-chromosomes and XY translocations were used to generate embryos deficient for large cytologically defined regions of that chromosome. Embryos that lack all X-chromosome material ('nullo-X embryos') develop normally to the cycle-14 syncytial blastoderm stage, but fail to partition their nuclei to single cells during cellularization. The cellularization defects can first be detected in the abnormal distribution of cortical actin and nuclei during early cycle 14. The same defects are produced by deletions of only a single region on the X-chromosome, between 6F and 7A. Nullo-X embryos carrying a duplication of this region cellularize and develop normally to the onset of gastrulation.

Key words: *Drosophila*, gene activity, X-linked, cellularization, transcription, X-chromosome.

Introduction

Although early embryonic development depends heavily on gene products provided during oogenesis, substantial zygotic RNA is synthesized prior to the cellular blastoderm stage (Zalokar, 1976; Lamb & Laird, 1976; McKnight & Miller, 1976; Anderson & Lengyel, 1979, 1981; Sina & Pellegrini, 1982; Karr *et al.* 1985; Edgar & Schubiger, 1986). If the products of this zygotic gene activity are required for the nuclear replication cycles or cellularization, their elimination should cause defined, reproducible alterations in those early events. We have carried out large scale EMS-mutagenesis screens to detect lethals affecting embryonic development, but identified no zygotically active loci with morphological effects prior to gastrulation (Nüsslein-Volhard *et al.* 1984; Jürgens *et al.* 1984; Wieschaus *et al.* 1984). From the phenotypes of the point mutations obtained in those screens, it seemed that the cleavage and cellularization that precede ventral furrow formation might depend solely on maternal gene products. On the other hand, since our mutagenesis screens would only have detected mutant embryos that differentiate an abnormal cuticle, it is possible that genes that block development very early may not have been detected.

That some zygotic gene activity is required prior to gastrulation is suggested by the abnormal cellularization observed in embryos treated with various inhibitors of RNA synthesis (Zalokar & Erk, 1976; Gutzeit, 1980; Arking & Parente, 1980). Moreover, in one of the earliest studies of the embryo's requirement for zygotic gene activity, Poulson (1940) found that embryos with no X-chromosome ('nullo-X embryos') become abnormal prior to cellularization. Poulson's results were interpreted to indicate the existence of at least one gene on the X-chromosome required for normal morphology prior to gastrulation. Subsequent studies seem to confirm Poulson's basic conclusion, although some of the details of the exact phenotypes have been questioned (Scriba, 1964).

In the experiments described in the following paper, we have re-examined the phenotype of embryos totally lacking an X-chromosome. These experiments verify the requirement for zygotic gene activity prior to gastrulation, but revise the description of the abnormalities that result when the X-chromosome is removed. In addition, XY translocations were used to generate embryos deficient for smaller overlapping regions of the X-chromosome. This procedure has localized the cellularization requirement

to a single two-band region of the chromosome. Based on the phenotype of embryos that lack this region, we propose a model for the normal initiation of cellularization in *Drosophila* cycle-14 embryos.

Materials and methods

Genotypes and stocks used

The attached-X chromosome used in almost all experiments was *C(1)DX, y f*, although the basic phenomena were confirmed using the other attached-X chromosomes commonly used in our laboratory (i.e. C(1)RM, w cv; C(1)RM, y su^{wa} w^a B). All the attached-X stocks used in these experiments carry the maternal effect mutant *klarsicht* (Wieschaus & Nüsslein-Volhard, 1986) which increases the clarity of the egg cytoplasm and allows development to be followed more easily in living embryos.

The XY translocations used to localize zygotically required regions were obtained from the Bowling Green and Pasadena Stock Centers. Before being used, each translocation was tested to determine the frequency with which the two X-chromosomal fragments segregate from each other during meiosis. Translocation-bearing males were crossed to *svb runt/FM7* females (Gergen & Wieschaus, 1985) and the fraction of the phenotypically *runt* progeny that were svb^+ was used as a measure of the fraction of the gametes containing the distal, but not the proximal, part of the X-chromosome.

Observation of living embryos

In the initial stages of each analysis, 40–100 embryos from a given cross were examined in Voltalef 3S oil on Petri dishes using a stereomicroscope at ×50. Once the gross morphology of the progeny from a given cross had been characterized in this manner, photomicrographs and time-lapse video films of single embryos were made with a compound microscope, using bright-field optics at higher powers of magnification (generally ×400).

Staining of actin and DNA

Embryos of the appropriate age were transferred to a fine-mesh wire sieve, dechorionated in commercial bleach (5·25 % sodium hypochlorite) for 2 min, rinsed in water, then transferred from the sieve to a 20 ml snap-cap vial containing a 1:1 mixture of heptane and 8 % formaldehyde and shaken for 5 min. The fixed embryos were pipetted (from the heptane/formaldehyde interface with a minimum of heptane) onto double-stick tape in the bottom of a small Petri dish and covered with 8 % formaldehyde. The vitelline membranes were removed under a dissecting microscope with a fine tungsten needle. The devitellinized embryos were then washed for 10–30 min in PBS.

Actin was stained with rhodamine-labelled phalloidin. 10 μl of a 3·3 μM-rhodamine–phalloidin/methanol solution was evaporated and redissolved in 200 μl PBS. The fixed embryos were transferred into this rhodamine–phalloidin/PBS solution for 20 min, then washed 5 min in PBS. They were then transferred to Hoechst 33258 dye at 100 μm ml^{-1} in PBS for 4 min to stain the nuclei. The stained embryos were mounted in 1:1 glycerol:PBS and examined at ×16, ×25 and ×100 using a Zeiss compound microscope with fluorescence optics.

Sectioned material

Embryos were dechorionated with commercial bleach, fixed at a heptane–25 % glutaraldehyde interface and hand-peeled in 8 % glutaraldehyde in PBS. They were then postfixed in osmium tetroxide, dehydrated in ethanol and propylene oxide and embedded in Epon 812. Sections to be examined in the light microscope were cut at 2 μm on an LKB ultramicrotome, stained with a solution of 1 % methylene blue and 1 % azure blue and mounted in DPX mountant (BDH Chemicals Ltd, Poole, England). Sections of embryos were examined and photographed under bright-field optics on a Zeiss photomicroscope. Sections for transmission electron microscopic examination were cut at 0·05–0·06 μm.

Results

Cellularization of wild-type embryos

The initial mitotic divisions in *Drosophila* occur in the egg cytoplasm without any intervening cytokinesis. The nuclei are partitioned into separate cells only after they have migrated to the surface and reached a surface density of about 6000 per embryo. Their spacing and arrangement are somewhat irregular but approximate a densely packed hexagonal array. The first signs of cellularization are visible in living embryos about 10 min after the completion of the 13th mitotic cycle, when plasma membrane invaginates down from the overlying surface between adjacent nuclei (Foe & Alberts, 1983). The initial process is extremely regular and uniform. The advancing membrane separates each nucleus from all of its neighbours and initially moves at approximately the same rate over the entire surface of the egg. The membranes between adjacent cells are closely apposed, except at the base of the furrow where they separate, forming a 'furrow canal' (Fullilove & Jacobson, 1971). After about 40 min, the rate at which the cleavage furrows advance into the egg increases dramatically. In the next 15 min, the length of the cell–cell interface doubles and the cells reach their final size.

In the very early stages of cellularization, the surface of the blastoderm is covered with folds and microprojections (Turner & Mahowald, 1976; Warn & Macgrath, 1983). These surface projections remain throughout the slow phase of cellularization and during this phase the furrow presumably elongates by incorporation of new membrane material (Lundquist & Emanuelsson, 1979). During the rapid elongation of the fast phase, the apical surface of the cells smooths out and the microprojections may serve as a source of membrane during the final stages of

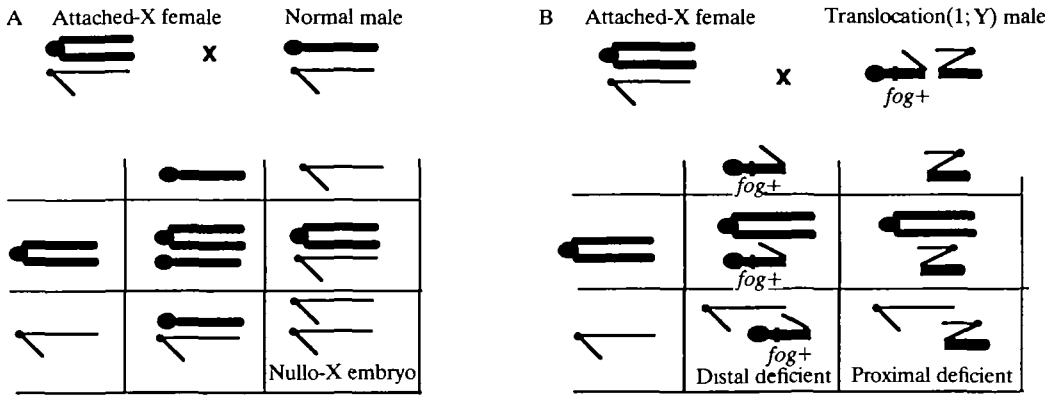

Fig. 1. (A) Production of nullo-X embryos. Embryos with no X-chromosome were obtained as the progeny of females carrying an attached-X chromosome (*drawn as thick bars*) and a normal Y-chromosome (*drawn as thin bars*). Such females make normal eggs. During meiosis, both X-chromosomes migrate to one pole and the Y-chromosome to the other. If the daughter nucleus receiving the Y-chromosome becomes the female pronucleus, the resultant egg will have no maternally derived X-chromosome. When fertilized by a Y-bearing sperm, a 'nullo-X' embryo is produced which has no X-chromosome. Such embryos should be produced among the progeny of this cross at a frequency of about 25%. (B) Production of proximal and distal duplications using XY translocations. Males bearing XY translocations produce two kinds of sperm, lacking either the regions of the X-chromosome proximal or distal to the translocation breakpoint. When such males are mated to attached-X females, one quarter of the embryos will be deficient for all distally located genes and one quarter will be deficient for all proximally located genes. Because embryos lacking the distal fragment of the X-chromosome still possess a wild-type allele of the proximally located *folded-gastrulation* (=fog^+) gene, they can be distinguished from the other deficiency class by the formation of a posterior midgut.

elongation (Turner & Mahowald, 1976). The ventral side of the embryo completes cellularization about 5–10 min earlier than the dorsal side. This may be due to differences in the rate of elongation or differences in the timing of the transition between slow and fast phases.

Gastrulation begins with a contraction on the ventral surface, resulting in the formation of a furrow running in the anterior–posterior direction in the middle two thirds of the embryo. About 10 min after the furrow begins to form, the cells at the posterior end of the embryo underlying the pole cells become very thick and their nuclei migrate basally. This results in the cup-shaped posterior midgut invagination, which eventually carries the pole cells into the interior of the embryo.

Phenotype of nullo-X embryos

Embryos with no X-chromosome can be obtained by mating attached-X/Y females to normal X/Y males (Fig. 1A). Because such embryos have two Y-chromosomes, the total chromatin per nucleus and thus the nuclear/cytoplasmic ratio is not appreciably altered. Such 'nullo-X' embryos go through mitotic cycles, form pole cells and reach the late syncytial blastoderm stages at the same times as normal wild-type embryos. The first abnormalities in living embryos are observed about 15 min after the completion of the final mitotic division. In normal embryos, the advancing front of the invaginating cell membrane can be observed as a line running parallel to the surface of the egg (Fig. 2A). In nullo-X embryos, this line is very difficult to resolve and when it can be seen over short stretches of the egg, it is intermittent or extremely irregular (Fig. 2B).

During the first 40 min of cycle 14, the clear cortical layer of the syncytial blastoderm in nullo-X embryos remains uniform in thickness over the entire surface of the egg. At about 40 min into cycle 14, when the cell membranes have reached the base of the nuclei and the embryo would normally begin the 'fast', contractile, phase of cellularization, the general appearance of the nullo X embryos undergoes a dramatic alteration. At the posterior pole, the yolk pushes to the surface trapping the pole cells in a yolky cell-free matrix (Fig. 2D). Similar, although more variable, breaks in the cortex occur in the ventral and anterior regions of the embryo. On the ventral side of the embryo, the cortical cytoplasm and associated nuclei become clumped and irregular; on the dorsal and lateral sides, cellularization continues but the cells that are formed are variable in size. Analysis of sectioned embryos reveals that many of these cells contain between two and five nuclei (Scriba, 1964; Fig. 3A,B).

The abnormal cellularization in nullo-X embryos can be correlated with an altered F-actin distribution soon after the embryos enter cycle 14. In wild-type embryos at this stage, the cortical actin is organized into a hexagonal network, which overlies the nuclei

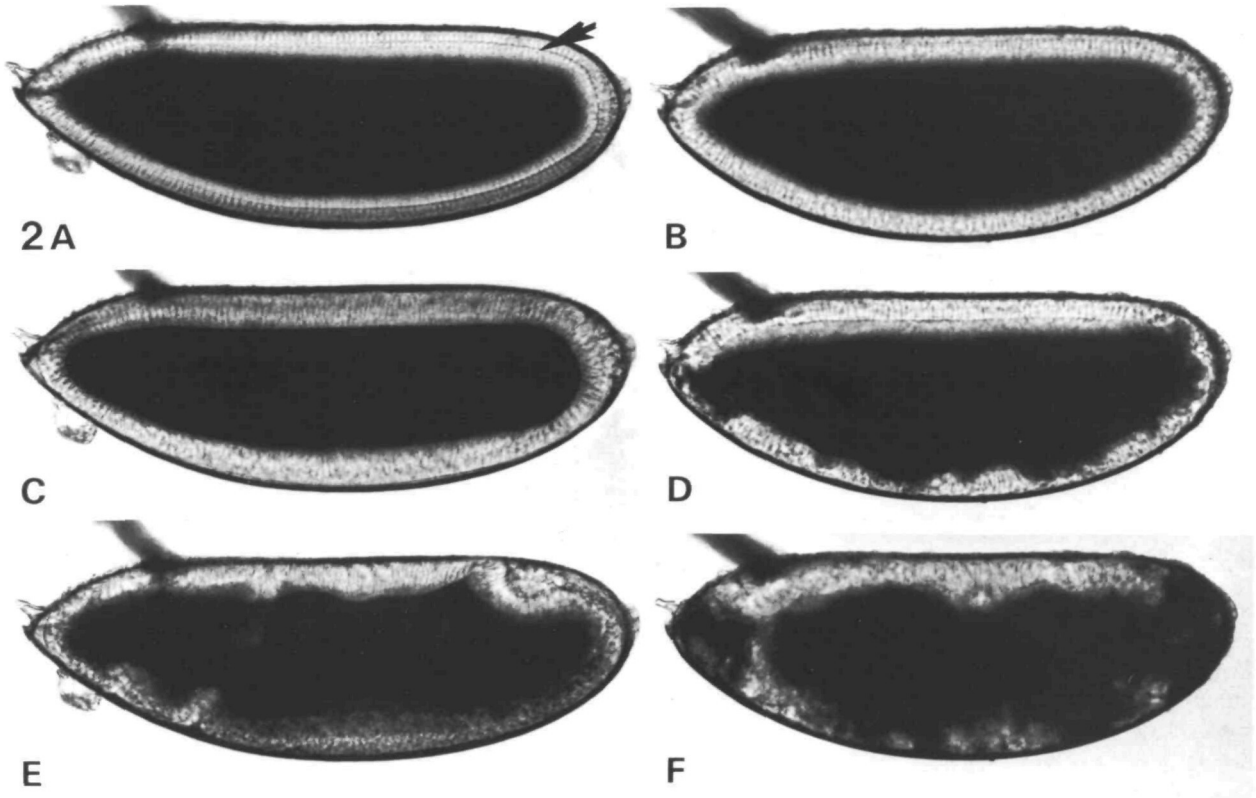

Fig. 2. Time-lapse photomicrograph of early cycle 14 in normal and nullo-X embryos. In early cycle 14, the advancing front of cell membrane can be distinguished as a dark line (→) in the normal embryo (A) but not in the nullo-X embryo (B). By the time cellularization has been completed in the normal embryo (C) and gastrulation begun (E), the ventral surface of the nullo-X embryo has become irregular (D) and cells are restricted to the dorsal surface (F).

(Fig. 3C). Contraction of this network presumably pulls the plasma membrane down between adjacent nuclei (Warn & Macgrath, 1983; see Discussion). In nullo-X embryos, the F-actin fibres separating adjacent nuclei are of varying thickness (Fig. 3D). The spacing of the nuclei is also not uniform (Fig. 3E,F) and groups of nuclei can be recognized on the surface of whole-mount embryos stained with Hoechst 33258, a DNA-specific dye. As cellularization advances, not all adjacent sets of nuclei are separated by cell membranes and the embryos assume an increasingly abnormal appearance. Despite these abnormalities, the depth of the advancing cell membranes is uniform in a given region of the egg (Fig. 3G,H). No evidence for lagging cellularization fronts is observed.

The description of nullo-X embryos outlined above differs from that of Poulson (1945) and Scriba (1963), both of whom concluded that nullo-X embryos become visibly abnormal by cycle 10 when the nuclei have migrated into the cortex. Our description, limiting the defects to cycle 14, was based initially on the appearance of living embryos viewed with a stereomicroscope. Because of the discrepancies with the earlier work, our conclusions were confirmed using time-lapse video tapes made with a compound microscope at ×400. Of the 52 videotaped embryos used in the analysis, six wild-type and four nullo-X embryos had been taped beginning in early mitotic stages prior to pole cell formation. No differences were detected between the two classes of embryos in the length of the 10–13 mitotic cycles (9·8±0·5, 10·0±2·5, 14·7±3·1 and 21·6±1·2 min per cycle in the normal embryos *versus* 8·3±2·1, 10·5±1·3, 13·7±2·9 and 21·2±0·8 min per cycle in the nullo-X embryos), nor in the total time from pole cell formation to the onset of cycle 14. Neither have we observed any abnormalities in cycle 10 through 13 embryos that were stained with Hoechst dye to follow the distribution of nuclei, or with RH–phalloidin to follow the morphology of the actin caps overlying each nuclei (Warn *et al.* 1984; Karr & Alberts, 1985). The earlier analyses of Poulson (1940) and Scriba (1964) were based on sectioned material and it is possible that they mistook some of the grossly disrupted late blastoderms for abnormal earlier mitotic stages. This would be understandable given the rapidity of the transformations occurring during late cellularization in nullo-X embryos.

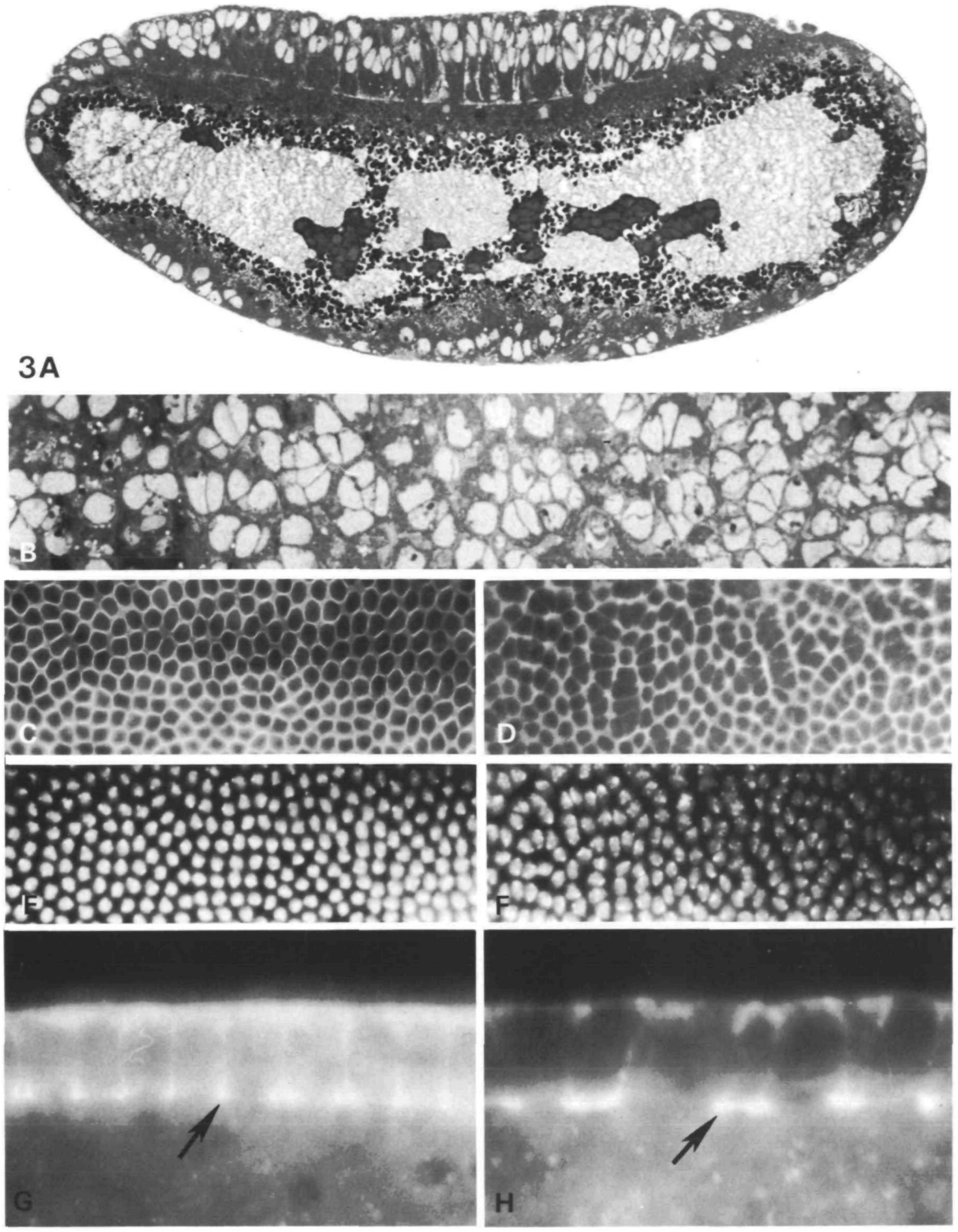

Fig. 3. Cellular defects in nullo-X embryos. By gastrulation, the cells in a nullo-X embryo are restricted to the dorsal and lateral surfaces (A) and, in tangential view, can be seen to be composed of irregular clusters of several nuclei (B). In contrast to the regular hexagonal array of actin and nuclei observed at the beginning of cycle 14 in normal embryos (C,E), the bridges of cortical actin that separate nuclei in nullo-X embryos are irregular in thickness (D) and the nuclei show early signs of clumping (F). As cellularization continues, the cleavage furrow (→) invaginates to a uniform depth in both the normal (G) and the mullo-X embryo (H), but the cells formed in the nullo-X embryo are irregular in size. Approximate enlargements: (A) ×300; (B) ×1100; (C–F) 640; (G–H) ×1600.

Table 1. *Chromosomal aberrations used to generate deficiency-embryos*

Name	Cytology	Ref.	Phenotype
Df(1)mal	19A to centromere	a,b	*fog;*
Dp(1,Y)mal	18F to centromere	a,b	*nullo fog*$^+$;
T(1,Y)125	15D, YL	d	*nullo fog*$^+$; *fog*
T(1,Y)J1	13A, YL	f	*nullo fog*$^+$; *fog*
T(1,Y)B32)	12E,YL	f	*nullo fog*$^+$; *fog*
T(1,Y) B49	11D, YL	f	*nullo fog*$^+$, *fog*
T(1,Y)147	8F,YS	d	*nullo fog*$^+$; *fog*
T(1,Y)131	6E, YS	d	*nullo fog;*
T(1,Y)149	6E, YS	d	*nullo fog;*
T(1,Y)B36	5C,YS	f	*nullo fog;*
T(1,3)0R64	3A;65A	a	normal
Dp(1,f)z	3E7–F1;19–20	a	*nullo fog*$^+$
Df(1)cr^{J4}	7A2;7C1, sxl$^+$	b,e	normal
Df(1)Sxl^{10RA}	6F5;7A, cm$^+$sxl$^-$	e	normal
Df(1)cr^{J6}	6E1;7C1; cm$^-$ sxl$^-$	b,e	*nullo fog*$^+$
Df(1)sxl^{bt}	6E;7A,cm$^-$ sxl$^-$	e	*nullo fog*$^+$
Df(1)HA32	6E4–5;7A6,cm$^-$ sxl$^+$	b,e	*nullo fog*$^+$
Df(1)6F1–2	6F1–2, cm$^+$sxl$^-$	c	*nullo fog*$^+$
Dp(1;3)sn^{13a1}	6C11;7C9, cm$^+$sxl$^+$	b	*nullo*$^+$ *fog*
Dp(1;3)sn$^{13a1\text{-}red}$	n.d., cm$^-$sxl$^-$	e	*nullo fog*
y$^+$*Yct*$^+$	6D3;7C5–6, cm$^+$sxl$^+$	e	*nullo*$^+$ *fog*$^+$

Phenotype refers to the abnormalities produced by the aberrant chromosome in male (or Y-bearing) progeny derived from attached-X mothers. Two classes of deficiency embryos are produced in equal numbers by XY translocations and where relevant both phenotypes are given. The term 'nullo' refers to the cellularization abnormalities observed in nullo-X embryos and described in the text. The term 'fog' refers to the failure to form a posterior midgut, characteristic of folded gastrulation.

References: a, Lindsley & Grell (1968); b, Craymer & Roy (1980); c, Lim (1979), personal communication; d, Nicoletti & Lindsley (1960); e, Nicklas & Cline (1983), T. Cline, personal communication; f, Stewart & Merriam (1976), J. Merriam, personal communication.

Genetic localization of the cellularization defect to 6F1–2

To determine whether the cellularization defects observed in nullo-X embryos are associated with a requirement for a single defined region of the X-chromosome, males bearing various XY translocations were mated to attached-X females. Each such cross generates two classes of deletion embryos, one lacking the proximal regions of the X-chromosome and one lacking the distal regions (Fig. 1B). It was possible to distinguish between these classes using the *folded gastrulation* gene located near the centromere in 20A3-B4. Embryos lacking *fog*$^+$ fail to form a posterior midgut invagination at the onset of gastrulation (Zusman & Wieschaus, 1986; Fig. 4A). The *fog* gene provides a particularly good marker for the presence of the proximal X-fragment because the initial invagination of the posterior midgut does not require complete cellularization. Nullo-X embryos with only a small *fog*$^+$ fragment of the proximal X-chromosome show the typical cellularization defects, but do make a clearly defined posterior midgut.

In the initial mapping, ten translocations were used with breakpoints between 3A and 20A (Table 1, Fig. 5). In each cross, a quarter of the embryos showed the cellularization defects similar to those observed in nullo X-embryos. For translocations with breakpoints at or distal to 6E, the defects were observed only in the embryos deficient for the proximal fragment (i.e. those not forming posterior midguts (Fig. 4B)). For translocations with breakpoints at or proximal to 8F, the defects were only observed in the distally deficient embryos (Fig. 4C,D). This is the expected result if the defect is produced by the absence of a single gene located between 6E and 8F.

To obtain a more precise localization, we examined the process of cellularization in embryos homozygous for various deletion chromosomes with breakpoints in the 6E–8F region. The phenotype is produced by four independently derived deletions, all of which lack polytene bands 6F1–2; i.e. *Df(1)cr*^{J6}, *Df(1)Sxl*^{bt}, *Df(1)HA32*, *Df(1)Lim 6F1–2* (Table 1, Fig. 4F,H). It was not observed using adjacent deletions which do not affect this region (*Df(1)Sxl*^{10RA}, *Df(1)cr*^{J4}). Sectioned deletion embryos show clusters of three to five nuclei in many of the cells (Fig. 6B). The most remarkable feature of these embryos, however, is that in spite of their defects, the embryos subsequently make normal posterior midguts and elongate their germ bands normally (Fig. 5J). By midgastrulation, the light-microscopic appearance of such embryos is remarkably similar to wild-type embryos at the same stage (Fig. 5L). This perhaps explains why the phenotype was not detected in

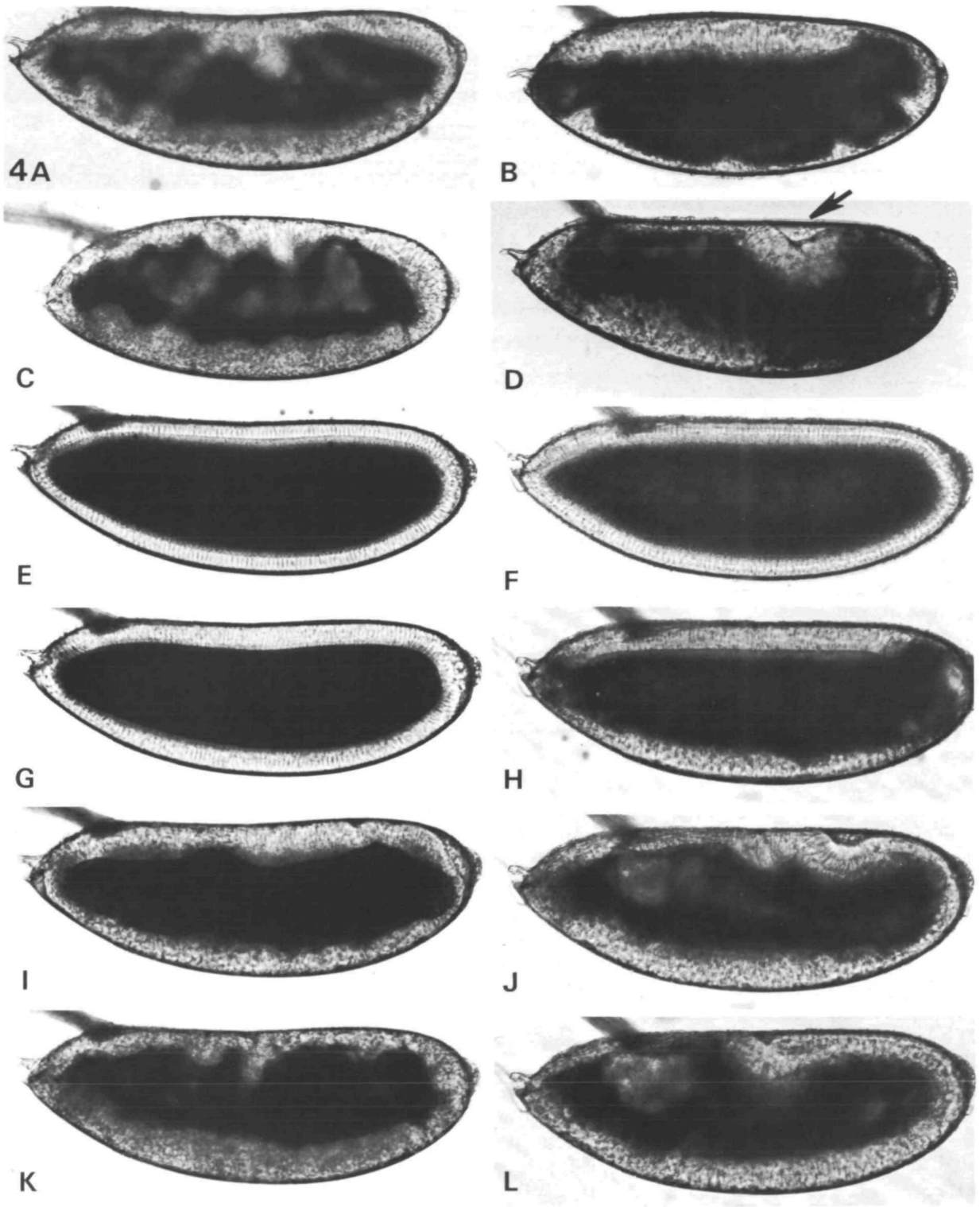

Fig. 4. Development of embryos deficient for various portions of the X-chromosome. (A) Early gastrula deficient for 18F to 20 (=*Df(1)mal*^{12}), note failure to form posterior midgut due to absence of folded gastrulation. (B) Early gastrula deficient for 6E to base (=Y/distal segregant of T(1;Y) 131). (C) Early gastrula deficient for 8F to base (=Y/distal segregant of T(1;Y) 147). (D) Early gastrula deficient for 1A to 8F (Y/proximal segregant of T(1;Y) 147), note the posterior midgut (→) and the abnormal cellularization. (E,G,I,K) Progressive stages in the development of a nullo-X embryos carrying a duplication covering 6E to 7B (=*Dp(1;3)sn13a1;*). (F,H,J,L) Progressive stages in the development of a embryo hemizygous for Df(1)B20, lacking 6E to 7B.

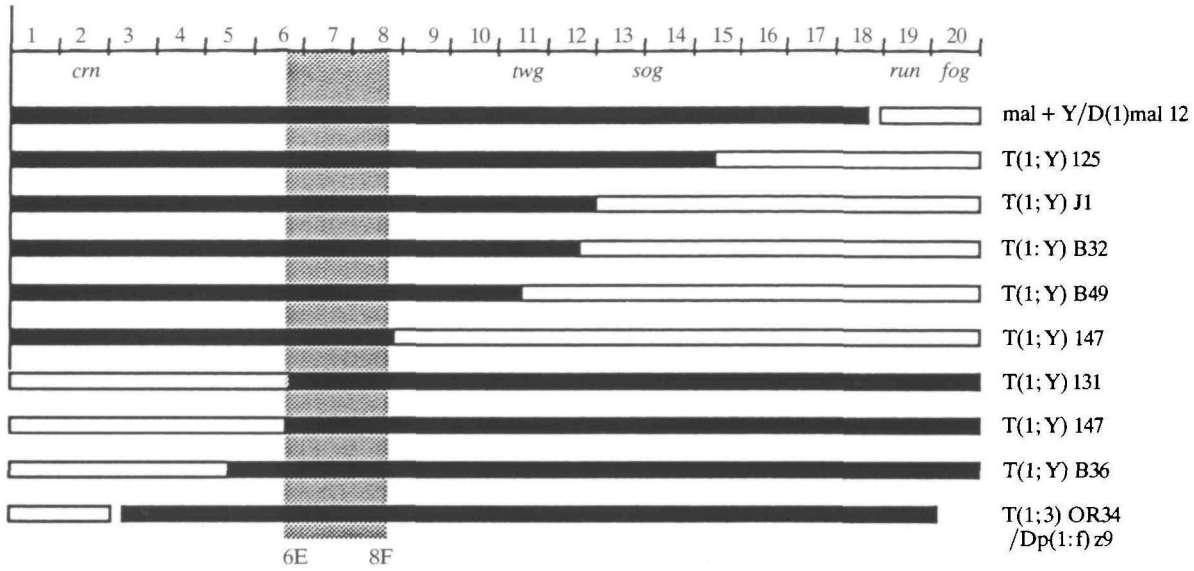

Fig. 5. XY-translocations localize the cellularization defects observed in embryos lacking the X-chromosome to the 6E–8F region of the X-chromosome. Black bars indicate the deficiency segregant from each translocation that produces the cellularization phenotype observed in nullo-X embryos. The shaded area indicates the region of the X-chromosome responsible for the cellularization phenotype.

earlier screens of embryos hemizygous for various X-chromosomal deletions (Wieschaus *et al.* 1984). Embryos deleted for 6F1–2 never complete normal development and remain very poorly differentiated, with grossly abnormal heads and internal morphology. They do, however, differentiate cuticle and, judging from the number of denticle bands, appear to have normal segmentation (Fig. 7).

Nullo-X embryos carrying *Duplication(1,3)sn*^{13a1} which covers bands 6E to 7B cellularize normally, based on examination of living embryos (Fig. 4E,G) as well as EM sections (Fig. 7B). They become distinguishable from sibling embryos in the same egg collection only at the onset of gastrulation due to their failure to make a posterior midgut (Fig. 5I,K). Their normal development up to that point argues that there are no other zygotically active genes elsewhere on the X-chromosome with effects on cellularization comparable to 6F1–2. The failure to form a posterior midgut is due to the absence of the *folded gastrulation* gene and can be corrected by using a duplication that covers both 6F1–2 and *fog* (i.e. *Dp(1;Y)ct*$^{+}$*131*, data not shown). Subsequent gastrulation in such embryos is abnormal in the cephalic furrow region, probably complicated by the elimination of other zygotically active X-linked genes (*twisted gastrulation*, *short gastrulation*, *runt* and *crooked neck*) known to have direct or indirect effects on that structure.

Discussion

One of the most striking features of early *Drosophila* development is the rapidity of the events between fertilization and the onset of gastrulation. Our previous mutagenesis experiments suggested that early-acting, zygotically required, loci are rare (Nüsslein-Volhard *et al.* 1984; Jürgens *et al.* 1984; Wieschaus *et al.* 1984). Most gene products required for early morphological transformations appear to be supplied to the egg maternally. The reliance on maternal transcription makes sense, given the longer time available for oogenesis and the high synthetic capacity of the polytene nurse cells associated with each oocyte. In this view, the rare early-acting zygotic loci might encode only those functions whose staging or localization makes it difficult for them to be supplied maternally.

The cellularization defects in nullo-X embryos are already apparent in the hexagonal network of F-actin which overlies the nuclei at the beginning of cycle 14. We do not know that this defective network is the primary alteration in mutant embryos and it is possible that detailed analyses using probes for other cytoskeletal elements might reveal even earlier abnormalities in nullo-X embryos. On the other hand, the defective actin arrays do offer a direct explanation for the subsequent defects in cellularization. If the fibres of this network in normal embryos are interconnected over the entire surface of the egg, their contraction would produce a force capable of pulling the plasma membrane down into cleavage furrows (like a net contracting over the surface of an inflated balloon). In such a model, the actin network in *Drosophila* may be analogous to the contractile ring observed in the cortex of dividing cells in many other

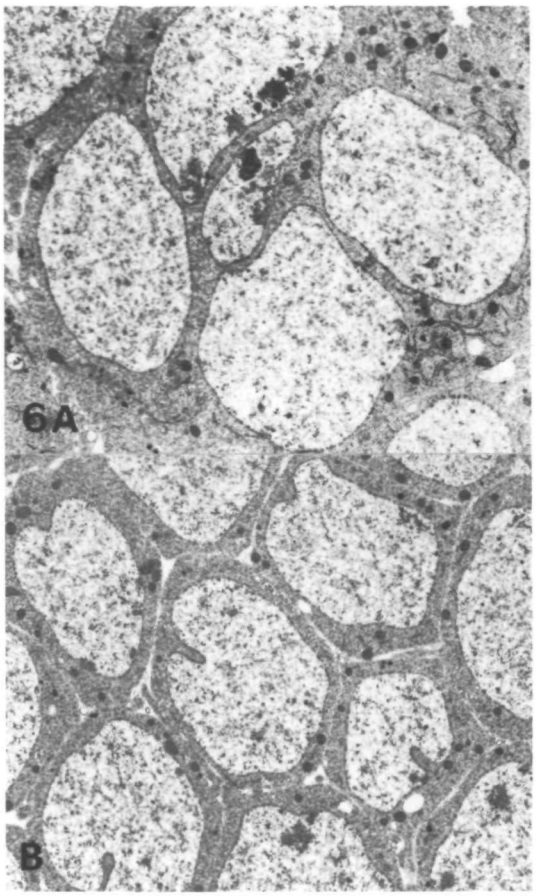

Fig. 6. Transmission electron micrographs of cellularization in early *Drosophila* embryos. (A) Abnormal multinucleate cells in embryos deficient for the same region (i.e. $Df(1)sxl^{b1}/Y$) and (B) normal cellularization in nullo-X embryos carrying a duplication for 6E to 7B Dp (i.e. $Dp(1:3)sn^{13a1}$).

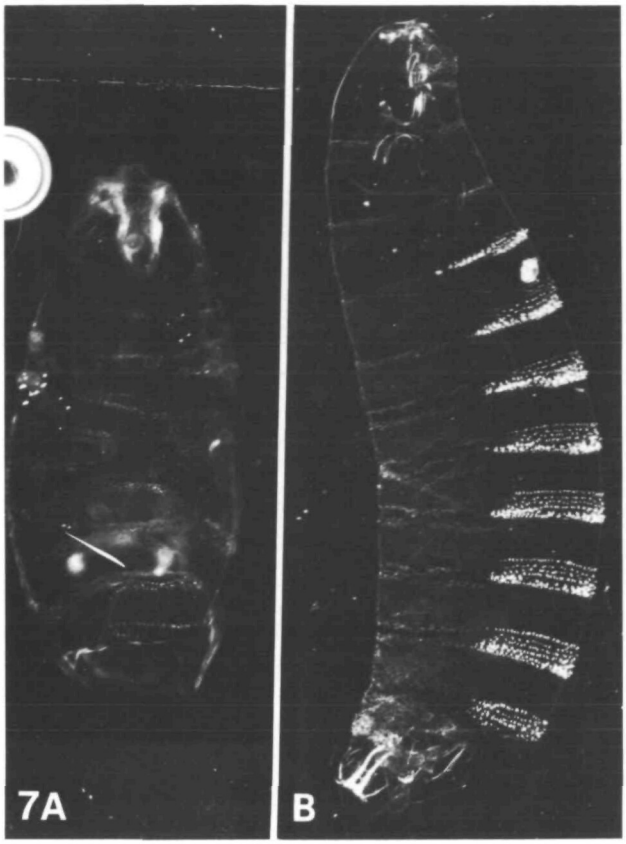

Fig. 7. Final cuticle differentiation in embryos lacking the 6E–7B region. (A) *Df(1) HA32/Y*; (B) normal.

organisms (Schroeder, 1973; Rappaport, 1974; White & Borisy, 1981). Contraction at the base of the furrow would maintain the straight (taut) appearance of the membranes as they elongate between adjacent nuclei (Warn & Macgrath, 1983), an observation difficult to explain if membrane is simply added in the cleavage furrow with nothing to stretch it out. The contractile nature of the F-actin at the base of the cleavage furrow is supported by its colocalization of myosin (Warn *et al.* 1980). Moreover treatment of *Drosophila* eggs with cytochalasin or other drugs known to disrupt actin-associated microfilaments block the progress of cellularization (Edgar *et al.* 1987).

For normal cellularization in the model outline above, the pattern of the contractile network must correspond precisely to that of the underlying nuclei. In *Drosophila*, the earliest interaction between the nuclei and the cortical actin is thought to occur at cycle 9, when nuclei first migrate into the surface cortex (Karr & Alberts, 1985). The first visible consequence of this migration is a reorganization of the initially homogeneous surface actin into 'caps' which overlie the individual nuclei (Warn *et al.* 1984; Karr & Alberts, 1985). As the nuclei continue to divide, these caps divide as well, until at cycle 14, the margins of the caps overlying adjacent nuclei become juxtaposed. It is at these juxtapositions that the actin takes on the characteristic morphology of hexagonal arrays and where cell membranes will eventually arise. When nullo-X embryos were stained with RH-phalloidin and Hoechst during cycles 10–13, the actin and nuclear patterns appeared to be normal. The first abnormalities in these embryos were observed at the beginning of cycle 14, not in the juxtaposition of the actin caps, but in the transition from that pattern to a contractile hexagonal array.

One way that actin localized at cap boundaries might be sharpened up into a cleanly defined hexagonal array is through the initiation of the contractile process itself. In this view, contraction of actin/myosin fibres at the cap juxtapositions initiates furrow formation, which in turn aligns and concentrates more and more actin fibres in the hexagonal array (White & Borisy, 1981). For the array to maintain its regular spacing relative to the nuclei, the contractile forces must be distributed uniformly at all cap juxta-

positions. Any initial irregularities will be enhanced as more actin filaments slip into those furrows that form first. Irregularities in the initiation of contractile forces would thus result in some of the subdivisions in the hexagonal arrays being lost, squeezing adjacent nuclei together and ultimately cellularizing multiple nuclei into a single cell. The similarity of this predicted phenotype to that observed in 6F1–2 embryos suggests a possible role for the 6F1–2 product in the initiation of furrow formation. On the other hand, it should be emphasized that, in the absence of data on other cytoskeletal components, the relationship between the 6F1–2 gene product and the phenomena we have observed may be more indirect.

The failure to detect the 6F1–2 requirement in earlier mutagenesis screens

Although the cellularization defects in embryos deficient for 6F1–2 are quite dramatic, the embryos do not die immediately. They continue to develop and eventually differentiate an abnormal cuticle. One can reasonably ask why mutations in the locus were missed in our earlier EMS–mutagenesis experiments in which cuticle preparations from lethal stocks were scored at the end of embryogenesis (Wieschaus *et al.* 1984). Given its proximity to the *Sxl* gene, the region between 6E and 7B has also been analysed in mutagenesis screens not based on morphological criteria (Nicklas & Cline, 1983). None of the 122 EMS-induced lethals obtained mapped to the 6F1–2 region between *car* and *Sxl* and none cause the cellularization phenotypes characteristic of nullo-X embryos. Other more recent screens of X-chromosomal embryonic lethals have also failed to identify point mutations in this region (Eberl & Hilliker, 1988).

All these observations suggest that the locus causing the cellularization defects in nullo-X embryos may be particularly insensitive to point mutagens like EMS. Early-acting loci may have to produce large amounts of product in a short period of time, and to allow such high synthesis rates, the genes may be tandemly repeated. In such a model, embryonic phenotypes might only be produced when a substantial fraction of the copies are eliminated. Mutations in a single repeat may not even result in lethality.

This view is attractive in that it suggests an explanation for another curious feature of the 6F1–2 deletion phenotype, namely that it does not toally block cellularization but instead only decreases the probability that any given actin bridge will be maintained. In that sense, the phenotype is variable and incompletely penetrant, more like what one would expect of a hypomorphic mutation rather than of a deletion that totally eliminates all function. If the gene is repeated, it is not necessary that all repeats be clustered in the 6F1–2 region, only a fraction sufficient to produce a phenotype when eliminated. If the remaining copies are located on the autosomes, even embryos that lacked all X-chromosomal material would not show a truly amorphic phenotype or be totally blocked in cellularization. It might be possible to detect the other loci involved in the process by generating deficiencies for defined portions of the autosomes, following procedures similar to those used in this study. In the accompanying paper (Merrill *et al.* 1988), we have used this approach and have identified two such loci on the tip of the third chromosome.

We thank John Merriam, Tom Cline and the stock centers at Pasadena and Bowling Green for providing some stocks used in these experiments. We also thank my colleagues at Princeton for their help and advice during the course of the experiments and during the preparation of the manuscript. The experiments presented in this paper were supported by NIH Grant HD 15587.

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(Accepted 25 July 1988)