Mitotic domains reveal early commitment of cells in *Drosophila* embryos

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Summary

In embryos of *Drosophila melanogaster* all the nuclei in the syncytial egg divide with global synchrony during the first 13 mitotic cycles. But with cellularization in the 14th cycle, global mitotic synchrony ceases. Starting about one hour into the 14th interphase, at least 25 'mitotic domains', which are clusters of cells united by locally synchronous mitosis, partition the embryo blastoderm surface into a complex fine-scale pattern. These mitotic domains, which are constant from one embryo to the next, fire in the same temporal sequence in every embryo. Some domains consist of a single cell cluster straddling the ventral or dorsal midline. Most consist of two separate cell clusters that occupy mirror-image positions on the bilaterally symmetric embryo. Others comprise a series of members present not only as bilateral pairs but also as metameric repeats. Thus a domain can consist of either one, two, or many (if metamERICALLY reiterated) clusters of contiguous cells. Within each cluster, mitosis starts in a single cell or in a small number of interior cells then spreads wave-like, in all directions, until it stops at the domain boundary. Each domain occupies a specific position along the anteroposterior axis – as determined by the expression pattern of the *engrailed* protein, and along the dorsoventral axis – as determined by cell count from the ventral midline.

The primordia of certain larval structures appear to consist solely of the cells of one specific mitotic domain. Moreover, cells in at least some mitotic domains share specific morphogenetic traits, distinct from those of cells in adjacent domains. These traits include cell shape, spindle orientation, and participation by all the cells of a domain in an invagination. The specialized behaviors of the various mitotic domains transform the monolayer cell sheet of the blastoderm into the multilayered gastrula. I conclude that the fine-scale partitioning of the newly cellularized embryo into mitotic domains is an early manifestation of the commitment of cells to specific developmental fates.

Key words: mitotic domains, cell determination, gastrulation, morphogenesis, fate map, *Drosophila* embryology, cell cycle control.

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gastrulation require the specialized function of various mitotic domains.

The midline cleft produced by permanent invagination of the mesoderm, and the transient cephalic fold, create useful visual landmarks during germ band elongation.

As the embryo elongates more than two-fold; the amnioserosa folds up ahead of the extending germ band.

Formation of the posterior midgut and proctodeum carry the pole cells inside the embryo.

Formation of the anterior gut tube occurs piecewise.

Oriented division puts the brain primordium inside the developing head; other brain neuroblasts may arrive by ingestion.

Ventral cord neuroblasts ingress.

1 Introduction

During embryogenesis in Drosophila melanogaster, the first 10 cycles of nuclear division occur synchronously throughout the entire syncytial egg (Rabinowitz, 1941; Sonnenblick, 1950; Turner and Mahowald, 1976; Zalokar and Erk, 1976). Initially, all of the dividing nuclei occupy the interior of the egg, but late in cycle 7 about three quarters of these nuclei begin migrating outwards toward the surface, leaving behind a small population of 'yolk' nuclei (Foe and Alberts, 1983). Following their 10th mitosis, these yolk nuclei cease dividing and become polyploid. During cycle 9, the first of the migrating nuclei reach the surface of the egg, at the posterior pole; late in cycle 10 these cellularize and thereafter lose mitotic synchrony with the remainder of the nuclei. These early-forming posterior cells are the germ cell progenitors, called 'pole cells'. The remainder of the migrating nuclei reach the egg surface early in cycle 10 and there distribute themselves in a monolayer. This monolayer of blastoderm nuclei, still syncytial, undergoes the next four mitotic division cycles (cycles 10–13) metachronously; that is, mitosis is nearly synchronous but starts first in nuclei near the embryonic poles then spreads wavelike towards the equator (Foe and Alberts, 1983). Thus, the blastoderm nuclei near the anterior and posterior poles are the first to begin and the first to conclude division. For any given cycle, all nuclei have about the same cycle length even though cycles 10 through 13 show a progressive increase in length – the durations of cycles 10, 11, 12, and 13 are about 7-6, 9-7, 12-6, and 16 min respectively at 25°C – (this is a slight revision of the cycle lengths reported in Foe and Alberts, 1983 – see Methods).

*The cycles of synchronous division are numbered consecutively, and each cycle is defined as starting with the beginning of interphase and concluding with the end of mitosis (Zalokar and Erk, 1976; Foe and Alberts, 1983).

4 Discussion

4.1 Mitotic domains occupy fixed positions on the embryo's surface.

4.2 Mitotic domains mark embryonic primordia.

4.3 Mitotic domains may turn out to be phylogenetically general; the techniques for visualizing them certainly are.

4.4 Patterned mitosis, domain by domain, is a telling progress report on the commitment process dynamically advancing.

4.5 The mitotic domains will facilitate molecular studies of morphogenesis.

4.6 Does cell–cell communication influence cycle 14 mitotic pattern?

4.7 Mitotic domains are the first visible manifestations of prior interaction between maternal gene products and early zygotic pattern genes.

During cycle 14, membranes form between the blastoderm nuclei segregating them into separate cells (Fullilove and Jacobson, 1971; Turner and Mahowald, 1976). Cellularization thus produces an embryo that is organized as an epithelial monolayer surrounding an elliptical yolk cell, which is a syncytium of polyploid yolk nuclei. The pole cells sit outside this epithelial layer at the posterior end. Gastrulation commences immediately following cellularization, and transforms the epithelial monolayer into the multilayered body of the larva. During gastrulation, a complex mitotic pattern occurs which is quite unlike the metachronous pattern observed for cycles 10–13 (Poulson, 1950; Sonnenblick, 1950; Madhavan and Schneiderman, 1977; Turner and Mahowald, 1977; Foe and Alberts, 1983; Hartenstein and Campos-Ortega, 1985).

Here, I present a detailed atlas of this mitotic pattern. By correlating the locations of groups of synchronously dividing cells with respect to anteroposterior and dorso-ventral landmarks, I show that mitotic pattern provides a precise array of genetic and topographic markers on the surface of the early embryo. I believe this atlas of mitotic pattern to be, as well, an atlas of cell commitment states, since at least some cell groups identified by mitotic synchrology exhibit cell biological properties and larval fates distinct from those of cell groups on different mitotic schedules.

2 Materials and methods

2.1 Egg collection and staging

Population cages of laying Drosophila melanogaster (Oregon R) flies were maintained and their eggs harvested essentially as described by Elgin and Miller (1978). Eggs were harvested after 1 h collections, aged to the desired stage, and freed from their chorions ('dechorionated') by immersion and gentle swirling in a 50% solution of Chlorox (active ingredient sodium hypochlorite) for 90 s, followed by extensive rinsing.
The rinse solution, used also to flush eggs from collection plates, was 0.12 M-NaCl, 0.04 % Triton X-100.

2.2 Fixation and staining of whole embryos

Preparation for observing microtubules and engrafted protein on the embryo's surface

Embryos were permeabilized, fixed, and the vitelline membranes removed en masse as described by Karr and Alberts (1986), except that the fixation time was extended to 20 min and the devitellinization in methanol–EGTA was carried out at room temperature. During the permeabilization step, taxol (to stabilize microtubules) was added for 2 min prior to fixation. Prior to staining, fixed devitellinized embryos were rinsed for about 1 h in PBS solution (137 mm-NaCl, 2.7 mm-KCl, 1.5 mm-KH2PO4, 8.0 mm-Na2HPO4, adjusted to pH 7.3 at 20°C) and then preincubated in PBS containing 1 % bovine serum albumin, 0.1 % Triton X-100, and 2 % normal goat serum (PBTG), for 2 h. All antibody incubations and rinses were carried out in PBTG with continual but gentle rotation, to ensure mixing. Embryos were next incubated with monoclonal mouse anti-β-tubulin IgG (from Amersham) and with affinity-purified polyclonal rabbit anti-engrailed protein on the embryo's surface (from Jackson Immuno Research Laboratories). Finally the embryos were rinsed in PBS and mounted in Mowiol-88 (Biotechnology Associates Inc., Birmingham Alabama) saturated with propylgallate. The embryos were slightly flattened, to flatten under the weight of 22 x 50 mm coverslips to increase the in-focus surface. These are semiformaldehyde preparations.

Preparation of embryos for observing mitosis beneath the embryo's surface or in the cephalic fold

Some domains, or parts of domains, divide after imagination, within the cephalic fold, or obscured beneath the extended germ band. In my preparations, microtubules in the interior of the embryo do not stain well. I suspect that this is because, to obtain satisfactory preparations, microtubules must be stabilized with taxol prior to formaldehyde fixation, yet, prior to fixation, taxol does not penetrate well below the surface layer of cells. Thus to visualize mitosis in internal domains, I also stain my preparations to reveal nuclei. While other DNA-specific fluorescent stains can be used for this purpose, I prefer chromomycin A3 (Sigma), which preferentially stains GC-rich DNA sequences. Because it does not stain centromeric heterochromatin, which has a similar condensed configuration during both interphase and mitosis, chromomycin-stained preparations are particularly easy to score for cell cycle phase. Antibody-stained embryos, prepared as described above, were transferred, following the final PBTG rinse, into a solution of PBS containing 10 mm-magnesium and 20 μg ml⁻¹ chromomycin overnight, and then transferred without rinsing to a mounting medium of Fluromount G (Biotechnology Associates Inc., Birmingham Alabama) saturated with propylgallate. The embryos were slightly flattened, as described above, and then sealed under the coverslip using nail varnish. Since chromomycin is viewed with the same filter as fluorescein, rhodamine-conjugated goat anti-mouse IgG was used as the secondary antibody to visualize the mouse anti-β-tubulin in chromomycin-stained preparations.

Exactly the same fixation and antibody-staining methods for microtubules just described above for Drosophila were used to prepare and photograph the blastoderm cells of Calliphora embryos shown in Fig. 4. I stained the nuclei in Calliphora by immersing the antibody-stained whole embryos in PBS to which I added 1 μg ml⁻¹ Hoechst dye 33258 for 4 min. I mounted the fixed and stained embryos in either Mowiol-88 or Fluormount G.

2.3 Fluorescence photomicroscopy of whole-mount embryos

Embryos were examined using a Zeiss standard microscope equipped with an Epi-illumination system. Fluorescein and chromomycin fluorescence were observed using a number 09 Zeiss filter set, rhodamine with a number 15 filter set, and Hoechst 33258 with an 01 filter set. Zeiss Plan-Neofluor 25/0.8 oil, glycerol, water immersion, Plan-Neofluor 40/0.9 oil, glycerol, water immersion, Planapochromat 63/1.4 oil immersion and Neofluor 100/1.3 oil immersion objectives were used. The images were recorded on Kodak 2415 technical pan film which was developed with Kodak D-19.

2.4 Determining the temporal sequence of developmental events at 25°C

The critical concerns here were three.

1. To observe and film living embryo preparations with high-resolution and high-magnification optics,
2. to supply abundant oxygen, deprivation of which dramatically alters developmental rate, and
3. to maintain the developing embryos at a constant and accurately known temperature, variation of which dramatically alters developmental rate.

For each preparation, an embryo one to two h after egg deposition was manually dechorionated by rolling on double-sided scotch tape, transferred with a dissecting needle to the center of a hydrophobic petriperm culture dish with gas-permeable bottom (Heraeus petriperm plates), oriented, and covered with a drop of Halocarbon oil (series 11-21 Halocarbon Products Corporation, 82 Burlers Court, Hackensack, New Jersey). No. 1 coverslip shims were placed on either side of the oriented embryo, and a third coverslip (No 1.5) was positioned over the embryo such that this glass was supported by the shims to prevent the embryo being crushed; the membrane surface of the petriperm plate permits free exchange of gases to the embryo and is optically clear enough for high-resolution viewing. Single embryos in these preparations develop normally and hatch.

Mitotic and morphogenetic events were timed (data for Fig. 2) from time-lapse video-tape recordings made through the phototube of a Zeiss standard microscope using a Dage-MTI 67 M Newvicon TV camera interfaced with a Gyrir model TLC-2051 time-lapse recorder onto 1/2 inch video tape. The taped images were played back through a Lenco model PMM-935 monitor. Transmitted-light bright-field optics using Plan-Neofluor 25/0.8, Plan-neofluor 40/0.9, or Plan 100/1.25 Zeiss objectives were employed. Nomarski optics cannot be used satisfactorily with petriperm membranes which are birefringent. These methods accounted for items 1 and 2 above.

To account for item 3, a homemade operational-amplifier negative-feedback circuit monitored the temperature registered by a small thermistor located in mid air near the microscope stage and, by frequently switching on and off a fan-forced, electrical, whole-room heater, clamped the temperature of the entire room and microscope at any preset target value. Because of the large thermal ‘inertia’ of the room and microscope, the output of the heater in brief pulses caused only very slight oscillations of temperature in the fan-
stirred room air. As verified by mercury thermometers, by a
thermocouple probe mounted on the microscope stage, and
by the thermistor sensor (with a response time of seconds),
this circuit held the temperature of everything in the vicinity
of the embryo to within ±0.2°C of any reasonable target value
− here 25°C.

All developmental times in this paper were measured at
25°C, and referenced from the start of interphase 14 as
time = 0 min. The embryonic nuclei both exit mitosis 13 and
begin interphase 14 in a metachronous wave that moves from
the poles to the equator in about two min. The start of mitosis
14 was taken as the time when the wave had traversed half the
distance from anterior pole to equator. In live embryos, cells
preparing for mitosis are discernible because they round up
immediately prior to division. At high magnification, metaphase
cells can be identified by the disappearance of their
nuclear envelopes. In cells that divide parallel to the embry-
onic surface, cytokinesis is also clearly visible. A cycle 14 cell
takes about 5 min (at 25°C) from the time it rounds up to
complete cytokinesis. ‘Domain mitosis times’ given in the left
panel of Fig. 2 are when the first cells in a domain undergo
cytokinesis.

36 video time-lapse recordings were made and analyzed.
Each one showed only one view (dorsal or lateral or ventral),
and only about one-third of the egg length, and therefore
provided information on only a few of the mitotic domains or
morphogenetic movements. I selected a set of 24 developmen-

tal events whose time of occurrence I could assess from time-
lapse tape recordings. Only a subset of these occurred in any
given recording. I estimated as accurately as I could the clock
time (from the Gyr’s time-date generator) at which each
observable event occurred. Because each event is an arbi-

trarily chosen milestone in a continuously changing process,
every event’s estimated time could be in error by several
minutes. Moreover, different embryos develop on schedules
that differ enough to advance or delay event times by as much
as 7 min over the 203 min that elapse from the start of cycle 10
(at ~46 min) until the stage that Fig. 14 depicts (at 157 min).
This variability is not due to temperature variation or vari-
ations in oxygen supply. Indeed, some embryos, that start off
at a slower pace than average, accelerate and finish at a faster
pace, and vice versa.

One way to compute average event times from such a set of
data is to arbitrarily declare a particular event (such as the
start of cycle 14) as ‘the key event’, and to measure the time of
all other events for each embryo relative to the time when its
‘key’ event happens. If one does this and computes average

event times for an ensemble of embryos, the sequence of
average event times one obtains unfortunately depends upon
which event is chosen as the ‘key’. Using the start of cycle 14
as the ‘key’ yields a different sequence of average event times
than using the start of cephalic fold formation does. Moreover
any ‘key’ event one uses will be missing from some video

tapes, rendering them useless. Given the variability between
embryos, there seems no a priori reason to select one
particular event as the ‘key’. Instead of biasing event times by
selecting an arbitrary ‘key’ event, I used a nonlinear optimiza-
tion computer program, written by Garrett Odell, that
computes, from the kind of raw data set described above,
‘best estimate’ event times in a way that treats all events
equally, avoiding artifacts caused by assigning special signi-
ficance to any particular event. This program, in effect,
calculates those best-estimate average event times that mini-
rize the accumulated variances around those averages as fol-

1. assign to each embryo’s (incomplete) set of event times a

single ‘time-shift’ parameter, adjustment of which shifts
that embryo’s sequence of times back and forth along the
time axis;

2. given any set of time-shift parameters, compute the

average time of each of the 24 events I observed;

3. compute the value of an ‘error function’ which is a
certain weighted sum of all the nonlinearly measured
errors* by which each individual event time differs from
the average time (from step 2) for that event;

4. iteratively adjust the set of embryo time-shift parameters
until that set of time-shift parameters is found which
produces the minimum possible value of the error
function of step 3;

5. after the iterations of step 4 converge, the ‘best estimate’
average event times are the averages of step 2, shifted to
put the average time of the start of cycle 14 at 0 min.

All domain mitosis and developmental event times
reported in this paper were derived in this fashion and have
standard deviations of about ±2 min. In addition, this pro-


cedure produces estimates of the durations of the 25°C
syncytial division cycles 10, 11, 12, and 13: (7-6 min, 9-7 min,
12-6 min, and 16-0 min respectively) that differ slightly from
those in Foe and Alberts (1983). The differences are probably
due more to slight oxygen deprivation in the earlier studies
than to differences in the data reduction procedures.

2.5 Construction of the mitotic domains atlas

The mitotic domain maps shown in Fig. 1 and Fig. 3 are hand
drawings, constructed by studying many hundreds of nega-
tives of fixed and stained embryos. These two maps depict the
position of the domains at two stages − at about 75 min (25°C),
when mitosis has begun in only the first six domains, and at
about 160 min, when Δ4M, whose cells are the last to divide,
is about one-third through its mitosis. Only a subset of the
domains is in mitosis at any one time and the sequence in
which cells divide was reconstructed from analyzing fixed anti-
tubulin-stained embryos which reveal cell cycle phase

(Fig. 4). Double-staining revealed the relative positions of the
engrailed stripes and the various mitotic domains. The micro-


graphs in the ‘D’ panels of Figs 6-14 are double exposures,
each made from photographing an embryo double-stained to
visualize both tubulin and en protein, with different fluor-

ochrome labels as described above; the signal-to-noise ratio in
these double exposures is obviously lower than for either
fluorochrome alone. In constructing the two maps, I make the
assumption that a domain occupies, in the future and the past,
the same position relative to an engrailed stripe that it has at
the time of its mitosis. Large scale changes in positions of the
various domains were confirmed from time-lapse recordings
of live embryos. The extensive rearrangements that occur in
the procephalon make it difficult to follow unmarked cells, so
the positions of the head domains in Fig. 3 are approxi-
mations. The maps show the embryos as if completely
flattened − for example the dorsal and ventral midlines are
both visible in lateral view. While I have tried to make the
maps topologically correct, they are not metrically accurate,
so the micrographs, not the atlas drawings, should be used to
determine linear distances on the embryo surface and the
relative size of domains.

*Discrete events whose times can be sharply estimated
carry higher weight than ‘events’ that span several minutes.
Mitotic domains in Drosophila embryos

3 Results

3.1 Synopsis of the paper and new nomenclature

In Drosophila embryos fixed and stained with antibodies against tubulin, the configuration of the microtubules in each cell reveals the phase of the nuclear division cycle. By studying many embryos, stained in this way, I have determined the sequence in which cells in different regions of the embryo undergo mitosis. Time-lapse movies of live embryos, filmed so as to reveal mitoses (see Methods), confirm the results obtained from these studies of fixed anti-tubulin-stained embryos.

Following cellularization, and after gastrulation has begun, the embryonic cells enter mitosis in a highly reproducible sequence, detailed in Section 3.2 below. That is, discrete groups of cells enter mitosis nearly synchronously, but out of synchrony with the surrounding cells; I refer to each such group of nearly synchronously dividing cells as a 'mitotic domain'. Except for those mitotic domains that symmetrically straddle the dorsal or ventral midlines, each domain on one side of the embryo has a mirror-image partner on the other side, separate from it, but mitotically synchronized. On each side of the bilaterally symmetric embryo most mitotic domains comprise only adjacent cells, but a few consist of disjoint cell clusters metamerically repeated.

The general shape of individual domains is constant from embryo to embryo, even though many domains have a complex and irregular shape. In Figs 1 and 3, I schematize the organization of the mitotic domains as projected onto embryos of two different developmental stages. In these Figs different colors distinguish different domains and show the unity of the various domains with disjoint members. A single embryo, stained to reveal mitosis, would never resemble either Fig. 1 or Fig. 3. This is because there is, obviously, no single time when the cells of all the mitotic domains are dividing simultaneously. Thus Fig. 1 and Fig. 3 are abstractions.

In each cluster of contiguous cells that forms an entire mitotic domain, or in each separated member of disjoint domains, mitosis appears to start in one or in a small number of cells and to spread, wavelike in all directions from these first cells, until the mitotic wave stops at the domain's boundary. Thus, the boundaries are the places in the blastoderm cell sheet with steep discontinuities in cell cycle phase. Except for the slight metachrony of the 13th mitotic wave that sweeps from the poles to the equator, all blastoderm nuclei begin cycle 14 at the same time, at about 130 min after egg deposition at 25°C (Campos-Ortega and Hartenstein, 1985). Except for the slight metachrony associated with the waves mentioned above, all cells within a mitotic domain terminate their 14th cycle at the same time. Thus all cells in a given mitotic domain share approximately the same duration of the 14th interphase and this duration is different for cells in other domains. If the cycle 14 cells are classified according to their interphase length, mitosis partitions the blastoderm into more than 25 cell types. In the Figures and text, I time all events from the start of interphase 14 at 25°C. Mitotic domain 1, the first to complete interphase 14 and to enter mitosis, does so at about 70 min on this scale, while the cells of mitotic domain 25 do not enter their 14th mitosis until about 115 min. The mitotic domains exhibit considerable variation in size. For example, prior to a 14th division, mitotic domain 10 consists of roughly 800 contiguous cells, while mitotic domain 25 consists of 14 cell pairs - just 28 single cells - each occupying disjoint but precisely specified positions on the embryo surface. Differences in the duration of cycle 15 further partitions some of the cycle 14 mitotic domains into subdomains (my unpublished studies).

I give each mitotic domain a number according to its relative time of entry into mitosis. This also orders domains according to increasing durations of cycle 14 (see Figs 1 and 3). To provide an unambiguous nomenclature that includes the division cycle as well as sequence, I combine these sequential domain numbers with a subscript that identifies the nuclear cycle in question. For example, $\delta_1s_9$ names the ninth cluster of cells to begin their 14th mitoses, and is an abbreviation for 'mitotic domain 9 of cycle 14'. To abbreviate 'domain', I borrow the Greek 'd' symbol '3' from its use in mathematics where it denotes 'partial differentiation'. Because it is difficult to study the mitotic patterns inside the cephalic fold, I may have failed to discover all of the mitotic domains in that region. If subsequent studies reveal new mitotic domains, those domains will need sequence-number names that indicate their proper place in the temporal mitosis schedule of domains already identified. The obvious name choice would be to use decimal numbers such as, for example, $\delta_{1s}0.5$ to name a domain that begins mitosis after $\delta_{1s}9$ but before $\delta_{1s}10$. Asynchrony during mitosis 15 apparently partitions $\delta_{1s}6$ into two subdomains, for which I suggest the designations $\delta_{1s}6\rightarrow\delta_{1s}1$ and $\delta_{1s}6\rightarrow\delta_{1s}2$, respectively. This nomenclature, if generalized to other domains, would imply clonal relationships between the domains of cycle 15 and 14 - in this case that the cells of $\delta_{1s}6\rightarrow\delta_{1s}1$ and $\delta_{1s}6\rightarrow\delta_{1s}2$ are descendents of those in $\delta_{1s}6$. While, in some regions of the embryo, the positions of the cycle 15 domains suggest this, I have not yet rigorously tested this proposition.

The embryo contains regions where cells never divide again, and others in which the cells divide asynchronously during long intervals spanning 40 min or more. These regions are not, by definition, 'domains of synchronously dividing cells'. Nevertheless, on the basis of their morphological properties, described in Section 3.4 below, I shall treat each such region as a unified group of cells - a domain. I designate these domains not with numbers, but with letters (see Figs 1 and 3). Cells in domains A and B do not divide during the period I studied, and Technau and Campos-Ortega (1986) report that cells in the amnioserosa (my domain A) remain in their 14th interphase throughout embryogenesis. In the cells of ventral domains N and M (abbreviated $\delta_{1s}M$ and $\delta_{1s}N$), cell cycle length varies from 103 to 140 min, and 140 to 190 min, respectively. In section 3.2 below, I describe in detail these letter-designated
domains as well as the number-designated domains of synchronously dividing cells.

In Section 3.3, I describe the position of the mitotic domains of cycle 14 with respect to the pattern of accumulation of the protein encoded by the engrailed (en) gene. Location of certain domains in register with particular en stripes identifies those domains as primordial segments. I classify some serially repeated cell clusters as constituting members of a common domain because they divide at the same time and because they occupy homologous positions in the presumptive segments of the embryo as seen by their alignment with respect to the en stripes. To name a particular member of a metamerically reiterated domain, I append a superscript to specify the parasegment or parasegments to which the cells in question belong (see the parasegmental numbering system introduced by Martinez-Arias and Lawrence, 1985). Thus $\partial_{41}^{25}$, names the anteriormost members of $\partial_{12}^{25}$ - the cell pair that occurs in the mandibular segment of the embryo, which is designated parasegment 0 (see Fig. 3C). In Section 3.3, I also describe the dorsalventral position of the domains, measured by cell count from the ventral midline along the embryo's circumference.

Fate studies by others have shown that cells in the positions occupied by specific domains predictably give rise to specific structures. Table 1 lists the fates I infer from these studies for many of the mitotic domains of cycle 14.

In addition to the same cell cycle length, the constituent cells of many domains share specific attributes - cell morphology, spindle orientation, morphogenetic movement behaviors - that distinguish them from cells in neighboring domains. Section 3.4 gives examples of these attributes. It is clear that specialized cell behaviors in certain mitotic domains organize the transformation of the blastoderm into the multilayered body plan of the developing larva. In many cases, cells manifest these characteristic behaviors prior to the mitoses by which I identify them as members of a particular domain. On these observations rests my conclusion that the partitioning of the blastoderm into distinct mitotic domains reveals patterned differences in cell commitment.

In Section 3.5, I describe the morphogenetic movements that progressively transform embryonic organization during the hour immediately following cellularization; Readers should bear in mind that, though they are described in separate sections, cell divisions and morphogenetic movements occur concurrently. Fig. 1 depicts diagrammatically the positions that the cells in each mitotic domain occupy at the time that the first embryonic cells enter mitosis 14, even though, at that time, only six domains - $\partial_{141}, \partial_{142}, \partial_{143}, \partial_{144}, \partial_{145}$, and $\partial_{146}$ - have actually initiated the mitoses that define them. Fig. 3 is an analogous diagram showing the positions of the domains about 85 min later, after many intervening morphogenetic movements, and after most cells have undergone their 14th mitoses. At the stage depicted in Fig. 3, only one domain - $\partial_{14}M$ - is still dividing. The flow of the morphogenetic movements continuously alters the positions of the cells that make up the various domains. In Fig. 2, I present a time line that summarizes the schedule of mitosis and the major morphogenetic movements that occur while the mitotic domains are undergoing their 14th division. In order to emphasize the dynamic nature of the embryo during this interval, I schematize in Fig. 2 the way four mitotic domains ($\partial_{44}, \partial_{47}, \partial_{410}, \partial_{414}$) change their shapes and positions.

The morphogenetic movements of gastrulation and germ band elongation involve rapid and extensive mechanical deformations of the blastoderm cell sheet. Since the mitoses that define the domains occur on this deforming cell sheet, the shape of each mitotic domain - both before and after it divides - must change significantly with time, and the relative positions of the many domains must likewise change. For a given domain, any embryo-to-embryo variation of its time of mitosis relative to its morphogenetic-movement stage would cause significant variation in the shape of its dividing patch of cells as revealed by anti-tubulin staining. I do not see such variation. The tight coupling between the mitotic and blastoderm-deformation schedules suggests that a common 'clock' controls both cell cycle timing and establishment of the cell properties that drive the morphogenetic movements, or that interaction between the various domains plays some causal role in driving gastrulation movements. The mechanism that synchronizes mitotic timing with the morphogenetic deformation schedule cannot involve the former causing the latter because, as discussed in Section 4.5, halting all mitoses does not seriously perturb gastrulation movements.

3.2 A complex pattern of mitotic domains arises during the 14th nuclear cycle and partitions the gastrulating embryo

All the micrographs in this article are of fixed whole-mount embryos, stained with fluorescently labeled antibodies to exhibit the configuration of microtubules (see Methods). To facilitate interpretation of these anti-tubulin-stained preparations, Fig. 4 displays side-by-side, and at high magnification, the organization of the microtubules and of the nuclear DNA at various stages of the cell cycle. At metaphase and anaphase, the microtubules form primarily bipolar mitotic spindles, while at interphase they form a network in the cytoplasm surrounding the nucleus of blastoderm cells. The prophase and telophase configurations of the microtubules are also distinct. Thus, the anti-tubulin staining, by discriminating between cells that are preparing for, engaging in, or just completing mitosis, enabled me to deduce the temporal sequence in which different cell groups divide.

The blastoderm nuclei complete their 13th mitosis (Fig. 5) and enter interphase 14 metachronously (Foe and Alberts, 1983). From the start of nuclear cycle 14 until the onset of gastrulation about 55 min later, no mitotic activity occurs in the blastoderm. Thus, at the inception of gastrulation all of the blastoderm cells are.
in interphase of cycle 14 (Fig. 6), having all spent nearly identical periods in that cycle. During the subsequent two hours, most of the cells pass through the mitosis of their 14th cycle, with groups of cells in different regions of the embryos dividing at different times. Figs 6–14 are micrographs of fixed and stained embryos, arranged in chronological sequence. Fig. 6 presents four views of embryos 65 min after the start of interphase 14, while Fig. 14 presents the same four views about 95 min later. In each Figure (6–14), A, B, C, and D are dorsal, ventral, and lateral views respectively, of embryos stained to reveal only tubulin, while D shows lateral-view embryos additionally stained to reveal the distribution of en protein. In each Figure, the four panels are of different individual embryos, so that minor heterogeneity in age exists between panels A, B, C, and D. With anti-tubulin staining, a domain is most dramatically visible when fixation catches its cells in metaphase or anaphase (Fig. 4). However, one can frequently distinguish domains even after mitosis, because cycle 15 cells are smaller than undivided cycle 14 cells. Comparisons of the diagrams of Figs 1 and 3 with the micrographs in Figs 6–14 will help the reader identify the various embryonic areas listed in the following subsection.

My fixation and anti-tubulin-staining methods do not illuminate the microtubules of cells in the embryo’s interior. To counteract this difficulty in studying internal domains in fixed embryos, I stained the embryos with the fluorescent DNA-specific stain chromomycin, and used differences in chromosome state (see Fig. 4) to delineate cell cycle phase (see Methods). I established the times at which the various cell groups on the embryo surface enter mitosis from time-lapse video recordings of live embryos, all events being timed from the beginning of interphase of cycle 14 at 25°C (see Methods). I deduced the approximate time of mitosis of the internal domains, viewed only in fixed preparations, by correlating the cycle phase of their nuclei with those of domains on the embryonic surface whose division times I know from studies of live embryos. Fig. 2 presents the schedule of cell divisions along a time line that also depicts the major morphological events of gastrulation.

A catalog of the twenty-five domains of synchronously dividing cells

In this subsection, I list the domains of synchronous mitosis, in the order in which they divide, describing each domain’s characteristic attributes. Figures 7–14 show the location of every domain during its mitosis except for domains that divide within the embryo or within the cephalic fold. In this list, I describe the locations of these domains during their internal mitoses. I describe the trajectory of each domain that moves significantly during the time interval encompassing gastrulation, germ band elongation, and the cycle 14 mitoses – the time interval whose endpoints Figs 1 and 3 portray. The following two subsections similarly list the two domains of asynchronous mitosis and the two domains in which cells do not divide.

Mitotic domain δ161. This is a relatively large domain, located dorsolaterally at the anterior of the embryo. Mitosis in this earliest domain begins at about 70 min. The first cells to enter mitosis do so near the center of the domain (Fig. 7A) and from them a mitotic wave radiates across the domain. Fig. 8A and C show clearly the metasynchrony that results from the mitotic wave in δ161. Mitosis begins simultaneously on both the right and the left halves of the embryo; thus two cell clusters, with identical cell cycle durations, occupy mirror-symmetric positions in each half embryo (Figs 7A and 8A). Unless noted otherwise, all the domains described below also occur as pairs. Whether paired or not, every domain is bilaterally symmetric.

Mitotic domain δ162. This domain includes ventral cells immediately anterior to the cephalic fold (Figs 7B, 8B) and a contiguous stripe of cells that extends dorsally along the anterior face of the cephalic fold. The internal cells of this and of the other domains described below are visible in fixed, chromomycin-stained embryos (see Methods), but not in the surface views of anti-tubulin-stained embryos presented here. Section 3.5 below describes in detail the cephalic fold and other morphological features of the early embryo used as landmarks in this catalog of domains.

Mitotic domain δ163. This domain is unpaired and straddles the dorsal midline near the anterior tip of the embryo. A mitotic wave spreads from a single site near the center of this domain to meet laterally the two mitotic waves that originate in the pair of δ161 domains (see Fig. 8A). Invagination of δ168, between 100 and 110 min, pulls this domain around the anterior tip of the egg to the ventral surface (Compare Figs 1 and 3).

Mitotic domain δ164. This domain occupies the posterior tip of the germ band (Figs 8A and C, and 9A and C). Mitoses occur in it just when germ band elongation starts. Amnioproteodeal invagination and germ band elongation carry it from its ventral location in the just-cellularized blastoderm to a dorsal-anterior position – anterior in the egg – at full germ band elongation (see the right panel of Fig. 2). A slit, that superficially appears to be continuous with the ventral furrow, separates the left and right halves of this domain (Fig. 8A). This slit, which is structurally distinct from the ventral furrow, probably marks the site of the future anal opening (see Turner and Mahowald, 1976; and Discussion).

Mitotic domain δ165. When they divide, the cells that constitute this large domain lie on the dorsolateral surface immediately anterior to the cephalic fold (Fig. 8A and C) and on the contiguous anterior face of the cephalic fold.

Mitotic domain δ166. When the cells of this domain divide, most of the domain lies on the dorsolateral surface, just posterior to the cephalic fold (Fig. 8C). The posterior margin of the domain abuts the fourth
engrailed stripe (Figs 8D, 9D). The domain's anterior margin lies inside the cephalic fold on its posterior face. At this stage, $\partial_{145}$ and $\partial_{146}$ lie opposite each other across the cephalic fold (see Fig. 1). They are not, however, contiguous on the embryo's surface since $\partial_{147}$ lies between them inside the cephalic fold. As the cephalic fold partially opens at about 105 min, the anterior margin of $\partial_{146}$ shifts backwards to lie well behind the cephalic fold (compare Figs 1 and 3).

**Mitotic domain $\partial_{147}$.** This domain lies between $\partial_{145}$ and $\partial_{146}$, and its mitoses occur while the domain is located wholly within the cephalic fold. Thus, none of the micrographs of embryonic surfaces displayed here show $\partial_{147}$ cells in mitosis (chromomycin-stained preparations are not shown). The domain appears to be of about the same size as $\partial_{145}$. The right panel of Fig. 2 shows schematically how this domain first enters the cephalic fold when this fold forms, and then leaves it when this fold partially opens at about 105 min (also compare Figs 1 and 3).

**Mitotic domain $\partial_{148}$.** This unpaired domain is located immediately ahead of the 'T' at the anterior end of the ventral furrow. It straddles the ventral midline (Figs 8B, 9B). Many, though not all, of the $\partial_{148}$ cells divide along axes normal to the embryonic surface, leaving daughter cells both on the surface and in a new layer immediately below it. The high magnification view of this domain in Fig. 18C shows the resulting distinctive end-on orientation of mitotic spindles in $\partial_{148}$ cells. The cell group that remains on the surface invaginates at 100–110 min, during interphase of their 15th cycle (Fig. 18E and F).

**Mitotic domain $\partial_{149}$.** This is a large domain located anterior to the cephalic fold, adjacent and ventral to $\partial_{145}$. $\partial_{149}$ shares with $\partial_{148}$ the distinguishing feature that its cells divide along axes perpendicular to the embryonic surface, leaving one-half of its daughter cells on the embryonic surface and the other half in a new layer inside the presumptive embryonic head. No other domains have this characteristic. While most of the cells in $\partial_{148}$ do this, all of the cells in $\partial_{149}$ divide in this orientation. Fig. 9B and C shows the $\partial_{149}$ spindle microtubules thus oriented. Fig. 17B gives a high-magnification view.

**Mitotic domain $\partial_{1410}$.** This is the largest of the mitotic domains. Prior to mitosis 14 it consists of a 16-cell-wide ribbon of about 800 contiguous cells on the ventral surface of the blastoderm, straddling the midline. Between 55 and 65 min, the cells that make up this ribbon undergo apical constrictions and invaginate, creating the ventral furrow. The cells of the ventral furrow are the primordium of the mesoderm. The $\partial_{1410}$ cells divide after they are internalized, at about 80 min (see Fig. 2). Within this largest domain, minor heterogeneity in nuclear cycle phase does occur, but I do not observe a conspicuous pattern; therefore I designate this large cell group as a single mitotic domain at cycle 14. Following their division, the $\partial_{1410}$ cells spread posteriorly under $\partial_{144}$, which is behind their original boundary (compare Figs 1 and 3).

**Mitotic domain $\partial_{1411}$.** A little after 80 min, mitosis begins simultaneously at five dorsolateral sites on each side of the embryo (Fig. 9B and C). From these sites, mitotic waves spread eventually to include all the cells that lie in a lateral strip directly posterior to $\partial_{146}$ (Fig. 10B and C). Because mitosis begins simultaneously at multiple sites within this lateral region, I infer either that it comprises multiple distinct mitotic domains that just happen to have identical cycle lengths, or that it comprises a domain with reiterated members (as do certain other domains described below). Pending means to distinguish these possibilities, I represent this region – $\partial_{1411}$ – as if composed of reiterated members, each member likely spanning a longitudinal repeat distance of two parasegments. However, because cell clusters with very similar division times occur adjacent to each other, and the mitotic waves initiated from these multiple sites converge, I could not distinguish with much precision the boundaries between the presumed reiterated members of this domain. (It is worth noting, however, that the slow-dividing parts of this region often coincide with the even-numbered stripes of engrailed protein expression; data not shown). In Figs 1 and 3, a single color fills the entire region and dashed lines show tentative boundaries between the presumed reiterated members of $\partial_{1411}$.

**Mitotic domain $\partial_{1412}$.** This small domain consists of a row one to two cells wide, located immediately anterior to $\partial_{144}$ (shown at high magnification in Fig. 19D). $\partial_{1412}$ is coincident with the 14th engrailed stripe, which marks the posterior of a presumptive eighth abdominal segment (shown diagrammatically in Fig. 1; engrailed staining of $\partial_{1412}$ not shown).

**Mitotic domain $\partial_{1413}$.** Beneath the posterior tip of the elongating germ band, a pouch-like invagination forms. Domains $\partial_{1412}$ and $\partial_{1413}$ constitute this pouch, which holds the germ line precursors or pole cells (shown diagrammatically in Fig. 1). The cells of $\partial_{1413}$ form a ring around the pouch opening. This unpaired domain undergoes mitosis while hidden beneath the elongating germ band.

**Mitotic domain $\partial_{1414}$.** In each half-embryo, this domain consists of a row one to two cells wide immediately lateral to the ventral furrow, and apparently running almost the entire length of the germ band (Fig. 10A and B). The two rows of cells that make up this domain lie far apart on opposite sides of $\partial_{1410}$ in the blastoderm-stage embryo, but come together at the ventral midline when $\partial_{1410}$ invaginates to form the ventral furrow (see right panel of Fig. 2). Poulson (1950) called the cells of $\partial_{1414}$ the 'mesectoderm' because they constitute a boundary between the internalized ventral furrow cells, which are the mesodermal
primordia, and the cells that remain at the surface, which are the ectodermal primordia. Posteriorly, the mesectoderm, \( \partial_{414} \), and the internalized mesoderm, \( \partial_{410} \), reach, but do not extend into, \( \partial_{424} \), which forms the posterior tip of the elongating germ band (see Fig. 10A and, at high magnification, Fig. 19D). In Figs 1 and 3, I represent \( \partial_{414} \) reaching precisely as far anterior as the mesoderm, but I am uncertain of the anterior boundary of \( \partial_{414} \). This is because, anterior and contiguous to \( \partial_{414} \), there appears to be another narrow domain, \( \partial_{415} \), which, like \( \partial_{414} \), bounds territories of invaginating cells. Since these two territories abut and divide at nearly the same time, it is possible that I have designated as two domains what is actually only one, or have incorrectly located the boundary between them. The long row of cells that bounds each side of the ventral furrow uniquely expresses the protein and RNA encoded by the single minded gene (Thomas et al. 1988; Crews et al. 1988). I do not know where the anterior or posterior boundary of the single minded expression domain lies, but its boundaries may help establish the boundaries of \( \partial_{414} \).

Mitotic domain \( \partial_{415} \). The territory designated as \( \partial_{415} \) is about one cell wide and, as Fig. 1 shows, separates domains 8 and 23 from domains 2, 9, 1, and 3 by completely surrounding domains 8 and 23 (Fig. 10B and, at high magnification, Fig. 18D). It is possible that this complex territory is not really a unique domain, but results simply from the collision of mitotic waves that originate in \( \partial_{418} \) and \( \partial_{419} \) and terminate slowly at the margins of these domains. I believe \( \partial_{415} \) is a legitimate domain, however, because its mitoses are not oriented perpendicular to the embryo's surface the way divisions in adjacent domains are. Further, as mentioned above, I am uncertain of the anterior boundary of \( \partial_{414} \) which abuts \( \partial_{415} \).

Mitotic domain \( \partial_{416} \). In each half embryo, \( \partial_{416} \) comprises 7 small metamerically reiterated clusters of cells (Fig. 10A, B, and C). These are located within the engrailed stripes that mark the posterior of presumptive abdominal segments 1-7, i.e., en stripes 7-13 (Fig. 10D and, diagrammatically, in Figs 1 and 3). The posterior of the eighth abdominal segment is occupied by a different domain, \( \partial_{412} \).

Mitotic domain \( \partial_{417} \). Soon after the \( \partial_{416} \) cell clusters enter mitosis, mitosis begins again at a similar number of new sites, each immediately posterior to one of the \( \partial_{416} \) sites (data not shown). While the reiterated members of \( \partial_{416} \) occur inside the abdominal engrailed stripes, those of \( \partial_{417} \) occur in the interstripe region. Figs 1 and 3 reflect my impression that the first abdominal segment lacks \( \partial_{417} \) cells, but this is not certain because of the close proximity of \( \partial_{412} \) in this same segment. The presumptive thoracic and gnathal segments definitely lack domains 16 and 17.

Mitotic domain \( \partial_{418} \). This unpaired chalice-shaped domain straddles the dorsal midline, between mitotic domains 1, 3 and 20 (Fig. 10A). By 135 min, well into their 15th interphase, the cells of \( \partial_{418} \) become squamous (Fig. 13A).

Mitotic domain \( \partial_{419} \). All along the length of the germ band, this long narrow domain, one to two cells wide, separates domain A and \( \partial_{411} \). Fig. 11C and D show a few late-dividing cells of this domain.

Mitotic domain \( \partial_{420} \). This is an unpaired domain on the dorsal midline lying just posterior to \( \partial_{418} \), and between the paired members of domain B (shown prior to division in Fig. 10A). When mitosis occurs in this domain at about 105 min, it is mostly hidden inside the cephalic fold (Fig. 11A and diagrammatically in Fig. 3D). I presume \( \partial_{420} \) abuts domain A, but the cephalic fold obscures the presumed contact between them. Like the cells of domain A and \( \partial_{418} \), the cells of \( \partial_{420} \) become squamous (Fig. 14A).

Mitotic domain \( \partial_{421} \). When the cephalic fold begins to open ventrally, cells in the ventrolateral region, denoted as \( \partial_{421} \) in Figs 1 and 3, begin to divide; division in this territory is prolonged and asynchronous (described below). Embedded amongst the asynchronously dividing and ingressing cells of \( \partial_{421} \) are the reiterated clusters of \( \partial_{421} \), that divide with a synchrony typical of other mitotic domains. Mitosis starts from six sites in each half-embryo (Fig. 11B), one in each presumptive gnathal and thoracic segment. Because mitosis in \( \partial_{421} \) and \( \partial_{421} \) are practically simultaneous, I have not been able to determine with certainty how the boundaries of \( \partial_{421} \) align with the stripes of engrailed expression. Figs 1 and 3 show my best guess. Also, it is possible that an analogue of \( \partial_{421} \) with a slightly different mitotic schedule exists in the presumptive abdominal segments (not indicated in Figs 1 and 3). Further studies of this issue are in progress.

Mitotic domain \( \partial_{422} \). This is the large, unpaired, internal domain which, together with \( \partial_{423} \), makes up the pouch-like invagination beneath the posterior tip of the elongating germ band (shown diagrammatically in Figs 1 and 3). Mitosis in this internal domain occurs at the same time that the cephalic fold opens ventrally – the stage shown in Fig. 11.

Mitotic domain \( \partial_{423} \). This is a small unpaired domain which, in the just-cellularized blastoderm, occupies the anterior tip of the embryo (see Fig. 1). Invagination of \( \partial_{423} \) between 100 and 110 min pulls this domain to the ventral surface and, during this translocation, the \( \partial_{423} \) cells divide (shown at high magnification in Fig. 18E). Mitosis in adjacent regions additionally transforms the shape of the anterior tip; once on the ventral surface, the \( \partial_{423} \) cells invaginate, at about 130 min, to form the stomodeum (Fig. 18G). By 150 min, the cells of \( \partial_{43} \) begin to form the clypeolabral lobe, a protrusion which covers, ventrally, the invagination formed by \( \partial_{423} \) (Fig. 18H and, diagrammatically, Fig. 3).

Mitotic domain \( \partial_{424} \). This is a very small late-dividing
domain located in the presumptive head, between domains 2, 5 and 9. When the first domains are entering mitosis, \( \delta_{142} \), is located on the lip of the cephalic fold, but by the time the cells of this domain divide, the opening up of the cephalic fold ventrally has repositioned \( \delta_{142} \) further forward (compare Figs 1 and 3). Fig. 18F shows the cells of this domain dividing.

**Mitotic domain \( \delta_{1425} \).** At about 115 min, the first cells in the ventral territory immediately adjacent to \( \delta_{1414} \), the mesectoderm, divide. These first-dividing cells constitute \( \delta_{1425} \), whose metamerically reiterated members are located amongst the cells of domain M that begin division about 25 min later and will divide asynchronously, as described below. \( \delta_{1425} \) is organized, in each half-embryo, as 14 single cells. When these cells divide, they are spaced with an 8-cell longitudinal periodicity along the ventral surface of the germ band (Fig. 12A, B, and C). Studies of the *engrailed* expression pattern (Section 3.3) show that, at 115 min in the extended germ band stage embryo, an 8-cell-wide repeat corresponds to 1 segment, while at 65 min, prior to elongation, each segment is organized as a three- to four-cell-wide repeat (compare Fig. 6D and Fig. 12D). \( \delta_{1425} \) cells lie immediately anterior to each of the *engrailed* stripes 1–14 (see Fig. 12D and Section 3.3, below). Frequently, there are two closely apposed \( \delta_{1425} \) cells, rather than a single one, in each hemisegment. When that occurs, both cells lie anterior to the en stripe (shown at high magnification in the insert to Fig. 12D).

**A catalog of two domains of late-dividing cells with lingering asynchronous mitoses**

**Mitotic domain \( \delta_{42N} \).** Domain \( \delta_{42N} \) is the ventrolateral region that surrounds \( \delta_{416} \), \( \delta_{417} \), and \( \delta_{4121} \) (see Figs 1 and 3). Mitotic figures begin to appear in \( \delta_{42N} \) at about 100 min, after mitosis has concluded in \( \delta_{416} \) and \( \delta_{417} \), but while \( \delta_{4121} \) cells are still dividing. Mitosis starts simultaneously at multiple sites within domain \( \delta_{42N} \) (Fig. 11A and C), proceeds at a high rate between 105 and 135 min, and then drops off abruptly as shown in the left panel of Fig. 2. It is striking that new mitotic figures continue to appear throughout this large domain for nearly 40 min (see for example Figs 12B and 13B). Cells in the dorsal half of the domain tend to divide before those in the ventral half, creating the biphasic division pattern shown in the left panel of Fig. 2. The late-dividing cells that are scattered throughout region \( \delta_{42N} \) do not exhibit conspicuous bilateral symmetry (compare right and left sides of the embryo in Figs 12B and 13B). It is difficult to tell whether the early mitoses are also unpatterned because they are partly contemporaneous with, and hard to distinguish from, the bilaterally and metamERICALLY patterned mitosis in \( \delta_{4121} \). During this period of prolonged mitotic activity, two waves of presumptive neuroblasts ingress, i.e. leave the blastoderm monolayer individually, from \( \delta_{412N} \) (described in Section 3.5). The first wave of \( \delta_{412N} \) neuroblasts divide soon after they leave the embryo surface (i.e. between 105 and 115 min). I have not studied the mitotic schedule of the second wave.

**Mitotic domain \( \delta_{44M} \).** At about 140 min, after mitosis in \( \delta_{44N} \) subsides, cells begin to divide in \( \delta_{44M} \). This is the region surrounding the segmentally reiterated \( \delta_{4425} \) cells, whose mitoses occur synchronously at 115 min (Figs 1 and 3). Unlike that of \( \delta_{4425} \), mitosis in \( \delta_{44M} \) seems to occur without any (obvious) bilateral symmetry (compare, for example, the left and right halves of \( \delta_{44M} \) in Fig. 1A and B). Mitosis in \( \delta_{44M} \) spans at least 40 min; the rate of mitosis is initially slow, builds to an intense rate between about 160 and 180 min, then tapers off, as indicated in the left panel of Fig. 2. Well prior to their mitosis, at about 100 min, the cells of \( \delta_{44M} \) assume a distinct morphology, elongating perpendicularly to the long axis of the embryo (shown at high magnification in Fig. 16). They maintain this shape until they divide, but not thereafter. Their mitotic spindles, like those of \( \delta_{4425} \), tend to be oriented parallel to the long axis of the cells. Two waves of neuroblast ingress occur from this region prior to any mitosis in \( \delta_{44M} \), and a third wave occurs during the period of cell division (described in Section 3.5). The \( \delta_{44M} \) cells that ingress in the first two waves do not divide prior to ingress; I have not studied the division pattern of the \( \delta_{44M} \) neuroblasts.

**A catalog of two domains of nondividing cells**

**Domain A.** Domain A becomes the amnioserosa. At blastoderm and prior to germ band elongation, domain A is a wide band of cells, straddling the dorsal midline and spanning the length of the germ band. Germ band elongation buckles and folds this domain (compare Figs 8C and 9C and, diagrammatically, Figs 1 and 3). Domain A cells do not divide during the embryonic period described here, nor apparently thereafter (Technau and Campos-Ortega, 1986). By about 90 min, these cells have become squamous; the relatively large size of their nuclei suggests that they are polyploid (shown at high magnification in Fig. 16).

**Domain B.** Domain B is a paired domain that occupies a wedge-shaped area on the dorsolateral surface of the presumptive head (shown at high magnification in Fig. 17A and B). Since I have not observed mitosis in these cells during the two hours following the start of cycle 14, I have provisionally designated them as nondividing. Cells may ingress from domain B.

**Some examples of cycle 15 domains**

Immediately after the \( \delta_{4425} \) cells divide, and prior to the start of mitosis in \( \delta_{44M} \) and its conclusion in \( \delta_{44N} \), cells in many other domains complete their 15th cycle (examples indicated in Figs 13 and 14). Study of chromycin-stained embryos reveals that the first domain to complete its 15th cell cycle is \( \delta_{4410} \), whose cells begin mitosis 15 at about 115 min (see Fig. 2). Data and analysis of the domains of cycle 15 will be presented elsewhere, in preparation for which I here use the cycle 15 subdomain nomenclature defined in Section 3.1. Fig. 13 reveals \( \delta_{46} \rightarrow \delta_{45} \), a subdivision of \( \delta_{46} \) that mitosis 15 creates.
These 14 segments constitute the germ band. The tail region (tl) lies posterior to the last domains that would lie beyond the horizon in micrographs of whole mounts. Orientations, color codes, and labels are as in Fig. 1. Note that intervening morphogenetic movements have repositioned these 14 segments. The lumen of the amnioproctodeal invagination (api) holds the pole cells (pc). Dashed outlines, instead of solid pattern and sequence of mitotic-domain division times. The right panel lists key developmental events spread along the same time axis, and cartoons the progression of blastoderm deformations during gastrulation and germ band elongation. The composite time axes, identical in both panels, measures minutes from the start of the 14th interphase at 25°C. The time axes also indicate the developmental 'stages', numbered 5 through 10, of Bowes (1975 and 1982), as modified by Hartenstein and Campos-Ortega (1985) and Wieschaus and Nüsslein-Volhard, (1986); horizontal dotted lines mark these stage boundaries.

Left panel: The numbers, fanned out, and N & M reference the mitotic domains shown in colors in Figs 1 and 3. Lines run from the time (on the 25°C time axis) when division starts in a particular domain, through the number identifying that mitotic domain, to end at a dot showing the location of that domain in a cartoon version of one of the panels of Fig. 1. Mitoses occur for extended periods in domains N and M, and the curves spanning 103–170 min at the bottom of the panel are histograms that graph roughly estimated rates of mitoses in domains N and M. The mitotic rate is at its peak value in domain M, for example, at about 165 min.

Right panel: Lines connect selected developmental events during the 170 min following the start of cycle 14 to the times when they occur. A cartoon sequence shows the deformations and motions imposed by gastrulation and germ band elongation upon four selected mitotic domains: 4, 7, 10, and 14. In the five cartooned embryos, arrows indicate the deformational flow of the blastoderm cell sheet during gastrulation and germ band elongation. These deformations carry mitotic domains along, as indicated by the representations of the trajectories of the four domains:

- Domain 4, which forms the posterior tip of the germ band, is stippled. Germ band elongation carries it around the posterior pole (the right end) of the egg. As indicated in the left panel, its cells divide at the very start of stage 8. Thereafter, continuing elongation of the germ band pushes it under the amnioserosa and out of sight, between 95 and 105 min (at 25°C).
- Domain 7, shown hatched with horizontal lines, forms parts of the anterior and posterior walls of the cephalic fold, so it disappears from view when that fold forms, as shown in the stage 6–7 embryo, and its cells divide early in stage 8 while hidden from view. When, at the end of stage 8, the cephalic fold partially opens, domain 7 emerges posterior to the fold and forms the maxillary lobe during stage 10.
- Domain 10, the presumptive mesoderm, invaginates during stage 6, forming the ventral furrow (see Fig. 15). Its cells divide early in stage 8.
- Invagination of domain 10 draws the rest of the blastoderm cell sheet toward the ventral midline, as indicated by the flow arrows in the stage 5 embryo. This brings the paired strips that constitute domain 14, hatched with diagonal lines, into contact at the ventral midline. Domain 14 cells divide, also during midstage 8, while germ band elongation is doubling the domain's length. The arrows in the stage 10 embryo indicate that this domain is eventually internalized, to form part of the ventral nervous system.

Fig. 3. Atlas of the mitotic domains projected onto an embryo at full germ band elongation. The stage depicted is about that shown in the micrographs of Fig. 14 (155–160 min), after most of the embryonic cells have completed mitosis 14. Embryo orientations, color codes, and labels are as in Fig. 1. Note that intervening morphogenetic movements have repositioned a number of domains relative to their earlier locations shown in Fig. 1. For example, domain 4 has moved farther forward along the dorsal midline and is partially hidden under domain A; the cephalic fold has opened ventrally, revealing the two most anterior of the presumptive gnathal segments, mandibular (mm) and maxillary (mx); domain 23 has invaginated to form the stomodeum (sto). Figs 1 and 3, hand-drawn, are topologically accurate but take metric liberties in order to depict domains that would lie beyond the horizon in micrographs of whole mounts.
Stages

1. Cellularization of the blastoderm
2. Gastrulation
3. Rapid germ band elongation
4. Slow germ band elongation
5. Gnathal and clypeolabral lobe formation

Domain mitosis times (min from start of cycle 14 at 25°C).
Stages min from start of cycle 14 at 25°C.

- Inward growing membranes reach base of nuclei.
- Cell membranes reach full depth.
- Transplantation experiments imply cell fates are determined.
- The cephalic fold and ventral furrow start to form.
- The ventral furrow closes & the depression carrying pole cells shifts dorsally. Buckling of amnioserosa creates 2 dorsal folds.
- Antibodies reveal the first 14 engrailed stripes.
- The posterior midgut invaginates internalizing pole cells: 65–73 min.
- First cells enter mitosis 14.
- Germ band elongation begins.
- Polar mitosis of domain 9 internalizes brain neuroblasts: 80–90 min.
- The amnioserosa becomes squamous and the dorsal folds disappear: 95–100 min.
- Germ band elongation carries the telson out of sight under the amnioserosa: 95–105 min.
- Domain M cells change shape: 100–105 min.
- Domain 8 invaginates: 100–110 min.
- The ventral portion of the cephalic fold opens.
- First wave of ventral neuroblast ingress complete.
- Antibodies reveal a 15–th engrailed stripe across the telson (105 min) and four head patches appear sequentially: 105–140 min.
- First cells enter mitosis 15.
- The clypeolabral lobe begins to form.
- The clypeolabral lobe is well formed; the gnathal lobes begin to form.
- The mesectodermal cells ingress after 170 min.
Fig. 5. Embryo fixed and stained for microtubules during mitosis of cycle 13 (5 min before the start of interphase 14). The syncytial blastoderm nuclei divide with near synchrony. Anti-tubulin staining reveals the bipolar nuclear spindles that surround each metaphase nucleus. Note the scale bar; all embryos in Figs 5–15 are shown at this same magnification.

Fig. 4. Five distinguishable states of microtubule and chromatin organization by which to assay cell cycle phase. These high-magnification views are intended as an aid in identifying cell cycle phase in the whole-mount preparations of Figs 5–19. In this paper I use both the state of chromosome condensation and microtubule organization: anti-tubulin staining (left panels) to detect division of cells on the surface of the embryo, and Hoechst 33258 (or chromomycin) staining of DNA (right panels) to detect division of cells in the embryo's interior (see Methods). The cells shown here are not of Drosophila but of the blowfly, Calliphora vomitoria. Both are advanced diptera, with essentially identical microtubule and DNA organization; I show Calliphora cells because, being approximately 25 times greater in volume than Drosophila cells, they are better subjects for high magnification micrographs. They were prepared for visualization in precisely the same way as the Drosophila whole mounts shown throughout (Methods). In each of the five left(tubulin)/right(DNA) pairs, the cells on the left are the same as those on the right. In these whole mount preparations, we view nuclei end-on from the apical (outer) side of the columnar blastoderm cells. During interphase and prophase the centrosomes, from which microtubules radiate, always lie toward the apical caps of the cells. In the top two left panels, the microscope is focused closer to the apical cap of the cell, with all of the nucleus below the plane of focus, to optimally visualize centrosomes and microtubular organization. In all the remaining panels, the microscope is focused on the equator of the nucleus roughly half way between the apical and basal ends of the cells (to optimally visualize chromatin structure). The five pairs of micrographs show five cell cycle phases: inter, pro, meta, ana, and telo. In each left panel, a dual arrow connects a paired set of centrosomes. Magnification 1400x.

- **Inter**(phase). This shows cells at the end of interphase 14. (Their appearance is similar throughout interphase except that during early interphase the centrosomes are unreplicated, and immediately following replication the centrosomes are close together.) The centrosomes that replicated in S-phase are now separating in preparation for mitosis. The abundant microtubules that radiate from these centrosomes fill virtually all of the cytoplasm, forming a 'basket' around the nucleus (Edgar et al., 1987). In the left panel, with all of the nucleus beneath the plane of focus, this basket appears as a mat spanning the apical cytoplasm, but at the midnucleus focal plane of the right panel, the tubulin stain would illuminate a ring of microtubules, lying in the thin zone of cytoplasm surrounding the nucleus. Since the micrographs in Figs 6–18 are usually focused so as to reveal mitotic spindles, which lie beneath the apical ends of interphase cells, most of the interphase cells exhibit that 'ring' configuration. This high-magnification figure does not exhibit the 'rings' because they are obvious in the other micrographs (see, for example, Fig. 6). The DNA, though beginning to condense, is still mostly in its interphase configuration (right panel).

- **Pro**(phase). The centrosomes are now fully separated, occupying opposite sides of the nuclei. The microtubules that radiate from the centrosomes still extend throughout the cytoplasm. At the midnucleus focal plane, the microtubular 'rings' described above would be thickened by diametrically opposite bright crescents: the centrosomes from which the stained microtubules radiate. In each cell, the DNA is condensed into chromosomes, which occur tangled within the intact nuclear membrane.

- **Meta**(phase). All of the cells in the field are in interphase except for two metaphase cells, in which virtually all of the microtubules are organized into bipolar spindles. This leaves most of the cytoplasm clear black, apparently free of tubulin stain. The chromosomes are fully condensed and aligned on the metaphase plate at the spindle's midplane. The dual arrow connecting paired centrosomes runs down one spindle's axis in the left panel.

- **Ana**(phase). Only a subtle visible change occurs in the microtubule configuration as cells progress from metaphase to anaphase: when the sister chromatids separate, bursting the metaphase plate, the spindle microtubules splay apart. From left to right, the three cells are in early, mid, and late anaphase, showing the progressing movement of chromatids along the spindle microtubules towards the centrosomes.

- **Telo**(phase). In the left panel, the dual arrow connects two previously paired centrosomes, which now lie in sister cells about to separate. The two bright tubulin spots near the center of this double-ended arrow mark the spindle's central remnants as cytokinesis severs the midbody. By mistaking these midbody remnants for centrosomes, it might be possible to confuse each of the two new (half-size) sister cells in telophase with cycle 15 cells in prophase. Note, however, that telophase sisters occur always in pairs, with the midbody remnants always contiguous, and always brighter than the centrosomes at the opposite ends of the cells. As spindle microtubules begin to break down, the microtubular baskets are already starting to form around the two new nuclei.
Fig. 6. 65 min embryo. In this Fig. and in Figs 7–14. A is a dorsal, B is a ventral, and C is a lateral view of the surface of fixed embryos stained for anti-tubulin; D is a lateral view of the surface of a fixed embryo stained with anti-tubulin and with antibody against the *engrailed* protein, photographed so as to reveal both stains (see Methods). Each embryo’s anterior is to the left and, in the lateral views (C) and (D), the dorsal surface is up. The anti-tubulin staining reveals the ring shaped network of tubulin in the cytoplasm that surrounds each interphase nucleus (see caption of Fig. 4). The cephalic fold (cf)
has just formed. The mesoderm has just invaginated and the lips of the resulting ventral furrow (vf) mark the ventral midline. When the shallow depression carrying the pole cells (pc) moves forward from its earlier location at the posterior of the blastoderm, the anterior dorsal fold (adf) and the posterior dorsal fold (pdf) crease the dorsal surface as it buckles. 

en
grailed (en) protein stripes appear (arrowheads in D), but have not reached full or uniform expression at this time. Damage during mounting tore the dorsal side in embryo D.
Fig. 7. 70 min embryo. Domains 1 and 2 are entering mitosis (cells in prophase and metaphase). Here, and in the following figures, numbers mark mitotic domains – numbers that indicate the order in which domains enter their 14th mitosis. Compare the micrographs with the schematic drawings in Fig. 1 and Fig. 3 for aid in locating specific domains. The catalogue of domains in Results Section 3.2 describes each domain, and Section 3.5 describes the morphogenetic movements. The deep posterior dorsal fold (pdf) and the shallow anterior dorsal fold penetrate further into the dorsal side of the embryo as the posterior tip of the embryo begins to move forward along the dorsal surface. In these fixed embryos, from which the
enveloping vitelline membranes have been removed, the posterior fold has opened under the pressure of the coverslip (see mounting technique in Methods). Damage during mounting caused the tears on the dorsal side in embryo D. The cells that flank the lips of the ventral furrow (vf) are domain 14 cells not yet in mitosis; they appear stretched. (T) marks a secondary focus of invagination at the anterior tip of the ventral furrow (see text).
Fig. 8. 75 min embryo. The cells of domain 2 are now mostly in telophase, as are the cells in the center of domains 1 and 3. Domains 4, 5, and 6 have just entered metaphase and a few cells in domains 8 and 9 are in prophase. When the cells of domain 8 round up and divide, this obliterates the 'T' cleft at the front of the ventral furrow (compare with Fig. 7). Wavelike heterogeneity in nuclear cycle phase is visible across the domains and is especially apparent in large domains (see for example domain 1). The amnioproctodeal invagination (api) has formed, internalizing the pole cells. The posterior tip
of the germ band, marked by domain 4, has begun to move forward along the dorsal surface of the egg. The posterior dorsal fold (pdf) is deep. The most posterior of the fourteen *engrailed* stripes (en-14) lies just anterior to the anterior margin of domain 4. The first two *engrailed* stripes are hidden from view inside the cephalic fold (cf). The posterior margin of domain 6 is coincident with the anterior edge of (en-4). The coincidence of these domain boundaries with the specific *engrailed* margins is also visible in the 80 min embryos.
Fig. 9. 80 min embryo. Domain 2 cells are in cycle 15 interphase, as are the cells in the centers of domains 1 and 3. Cells in domains 4, 5 and 6 are mostly in telophase, and cells in domains 8 and 9 are in metaphase. All the mitotic spindles in domains 9, and many in domain 8, are oriented perpendicular to the embryo surface, unlike those in any other domain; see Figs 17B and 18C for high magnification views of these domains. On each side of the embryo, domain 11 comprises multiple clusters of dividing cells; at this time the cells in the center of each cluster are in metaphase, indicated by the fans of arrows. The cells in domain 12, a small domain posterior to domain 4, are in prophase in panel C; domain 12 is better illustrated at
a slightly later stage and at high magnification in Fig. 19D. A few cells in domain 14, which flanks the midline, are also beginning to divide. Domain 7 cells are also dividing at the stage shown here, but they are not visible in these surface views since they lie within the cephalic fold. Invisible here, but also dividing at about this time are the cells of domain 10, that ventral ribbon of cells that invaginated earlier to form the ventral furrow. Domain A folds up accordion-like in front of the tip of the extending germ band further deepening the anterior and posterior dorsal folds, \(adf\) and \(pdf\).
Fig. 10. 90 min embryo. Cells in domains 1–8 are now all in interphase of cycle 15. The radially oriented cells of domain 9 are mostly in telophase. Mitotic waves originating in the 5 parts of domain 11 have merged so that the entire lateral surface is composed of mitotic cells. Heterogeneity in phase is clearly visible in this area; most cells are in telophase, though the regions the waves reached last are still in metaphase. Cells in domains 14 are in anaphase or telophase, and cells in 15 and 16 are predominantly in metaphase. Domain 18 cells are in prophase. Domain 13 cells are completing their mitosis at this stage, but are located out of sight, in the embryo's interior. On each side of the embryo, domain 16 comprises 7 clusters.
Domain 4, at the tip of the forward elongating germ band, has almost reached the level of the cephalic fold (cf). The morphology of domain A cells now begins to differ from that at earlier stages as these cells begin their transition from columnar to squamous. Neither domains 14 nor 16 penetrate domain 4, whose small cycle 15 cells are all in interphase; by 100 min (Fig. 11) domain 4 is no longer visible, having been pushed beneath domain A by the elongating germ band. Domain 16 members occur inside the abdominal *engrailed* stripes, seen here in the seventh, eighth, and ninth stripes (en-7), (en-8), and (en-9) (panel D).
Domains 1–18 are in interphase 15. Domain 19, the narrow strip of cells, between the already-divided cells of domain 11 and domain A, have mostly divided; a few anaphase stragglers remain. Domain 20 is pulled into the cephalic fold (Fig. 1) where its cells divide hidden from view. At this stage (100 min), mitosis starts in domain N proper, where mitotic figures continue to appear in a ‘salt and pepper’ fashion for about 40 min (Figs 2, 12, and 13). Domain 21 comprises six metamerically reiterated members on each side within domain N, one in each of the presumptive
gnathal and thoracic segments (105 min). Cells of the large internal domain 22 start mitosis (see Fig. 3). Between 100 and 110 min, the cells of domain 8 invaginate (arrow). Fig. 18E and F show the invagination of domain 8 better and at high magnification. At this stage, the cephalic fold (cf) unfolds ventrally (in progress in panel C (100 min), just open in panel B (105 min), and shown at high magnification in Fig. 18E and F). By 100 min, the posterior tip of the elongating germ band vanishes beneath the amnioserosa (domain A), whose cells are now fully squamous and thus nearly transparent. The dorsal folds have disappeared.
Fig. 12. **115 min embryo.** Mitosis continues at scattered sites in domain N. In each half embryo, all cells in domain M, the double row flanking domain 14, elongate perpendicular to the midline. This starts at about 100 min (shown at high magnification in Fig. 16). In each half embryo, domain 25 comprises 14 separate cells (or occasionally cell pairs) within domain M, with a 7- to 8-cell spacing along the germ band. The domain 25 cells (small arrows), usually divide in the direction domain M cells have elongated (see magnified insert in panel B); much later, the domain M cells divide in this same direction (see Fig. 14A and B). The ventral part of the cephalic fold is now fully unfolded (note ventral midline in
panel B), so that all 14 stripes of early engrailed expression, including those of the gnathal segments which the cephalic fold used to conceal, are now visible on the ventral embryonic surface (compare Figs 1 and 3). Each cell of domain 25 occurs immediately anterior to an engrailed stripe (panel D and second en stripe in insert). By this stage, domain 24, a small late-dividing domain in the procephalon, completes division (shown better at high magnification in Fig. 18F). By this stage, new loci of engrailed expression appear at several sites in the procephalon (en-P1), (en-P2), and (en-P3) and in the tail region, where a 15th stripe bisects domain 4 (not visible here).
Fig. 13. 130–135 min embryo. Mitosis continues at scattered sites in domain N. Occasional mitotic figures now begin to appear in domain M. At about this time cells, that in cycle 14 made up domains 6 and 11, begin their 15th mitoses (cycle 15 mitotic cell clusters are labeled by *). The boundaries of these dorsolateral cycle 15 domains coincide with boundaries of engrailed stripes. The area labeled (6-1), corresponding to the posterior part of domain 6, is the first of these domains to begin mitosis 15 (shown starting mitosis in panels B and C (130 min), and with the entire domain in mitosis in D (135 min)).
Cells in the procephalon are entering mitosis 15 as well (*); I mark the three *engrailed* stripes in the procephalon visible at this stage 'en-P1', 'en-P2', and 'en-P3'. The anterior boundary of *en-P2* coincides with the anterior margin of one of these cycle-15 procephalic mitotic domains (panel D). Just prior to this stage, domain 23 invaginates to form the stomodeum. Here (sto) marks its site of invagination. Posterior to this site, another hole marks the site into which domain 8 invaginated even earlier. The two invaginations are shown at high magnification in Fig. 18E, F, and G.
Fig. 14. 155–160 min embryo. Mitosis 14 is now proceeding at a high rate, but asynchronously, in domain M (see Fig. 2). The dorsolateral surface of the embryo has largely completed its 15th mitosis, though numerous cells are still dividing (labelled (*)). New regions of mitosis 15 have appeared in the head (*). Extensive cell division and morphogenetic movements are reshaping the anterior tip of the embryo, continuing processes begun earlier (see Figs 11–13). This process
began earlier at about 130 min (see Fig. 13). The cells of domains 18 and 20, still in interphase 15, are squamous. Reorganization of domain 3 has formed the clypeolabrum, (clyp), a lobe which protrudes below the stomodeum (sto). See Fig. 18 for a high-magnification view. Panel D shows the 15th engrailed stripe which subdivides domain 4, covered by the amnioserosa (A). During mounting, the embryo shown in panel D was damaged in the region between its fourth and fifth engrailed stripes.
Fig. 15. Ventral furrow formation visualized in a 55 min embryo. Between 55 and 65 min, a ribbon of cells along the ventral midline of the embryo invaginates. These invaginated cells will divide synchronously, as mitotic domain 10, to form the mesodermal primordium. Just prior to invagination, the diameter of the apical tips of the domain 10 cells constrict (arrows). Since the circumference of the embryo in its rigid vitelline membrane does not change, constriction of these cells must stretch other cells, and this stretching appears to be concentrated in the immediate neighbors of the constricting cells. A minute or so later, these stretched cells will constrict their apices (data from time-lapse video recordings, see Methods). These sequential apical constrictions apparently cause the globally coherent invagination of the mesodermal plate (Odell et al. 1981).

Fig. 16. A 105 min embryo illustrates that cells of some domains have already developed distinct morphologies. This is a middorsal view with the procephalon to the right, the germ band to the left, and the amnioserosa sandwiched between. Domain A is transformed into the thin membrane-like amnioserosa, because its cells become squamous. In the germ band, the cells of domain M, from about 100 min until the time at which they divide, appear stretched perpendicular to the ventral midline (compare with the adjacent cells of domain N and the double row of domain 14 cells, which straddle the ventral midline). In the procephalon, domain B cells are larger than their divided neighbors in domain 18. Magnification 880x.
Fig. 17. Radially oriented mitosis in domain 9. (A) a 75 min and (B) an 85 min embryo. In the procephalon, the cells of domain 9 divide along axes perpendicular to the embryo surface. In A, cells in the center of domain 9 are entering prophase while, in B, most of the cells of this domain are in anaphase. These anaphase cells have spindles oriented at right angles to those of the adjacent domains (compare the end-on view of spindles of domain 9 in panel B with those of domains 5 and 6 in panel A). The cells of domain 2, already divided, and the nondividing cells of domain B are visible in both panels.
Ventral views showing early development of the anterior gut primordia. Panels A, B, C, D, E, F, G, and H are of embryos aged about 65, 70, 80, 90, 105, 115, 130, and 150 min respectively.

(A) By 65 min, a ribbon of cells along the ventral midline (domain 10) completes invagination, thereby internalizing the mesodermal primordium. The lips of the resulting ventral furrow (vf) mark the ventral midline. The cephalic fold (cf) is forming; having begun at the sides of the embryo, it has not quite reached the ventral midline.

(B) By 70 min, domain 2 cells are in metaphase. The (T) at the anterior end of the ventral furrow is created by a group of cells in this region (which also belong to domain 10) that invaginate slightly later than the ventral furrow proper.

(C) At about 80 min, domain 8 cells obliterate this T-shaped invagination when they round up to divide. Many of the cells in domain 8 divide along axes perpendicular to the embryo surface (see arrow).

(D) Following their division at 80 min, until they invaginate between 100 and 110 min, the domain 8 cells occur as a roughly circular
plate anterior to domain 2. At 90 min, the time of this panel, they are encircled by actively dividing cells – domain 15. Domain 14 cells seen here are mostly in telophase.

(E) Between 100 and 110 min, the domain 8 cells invaginate. This invagination is shown in progress in (E) and complete in (F). The domain 23 cells, which divide while the invagination of domain 8 occurs, are brought from the anterior tip to the ventral surface by that invagination. Also, while domain 8 invaginates, the ventral part of the cephalic fold unfolds – compare panels D, E, and F.

(F) At about 115 min, domain 24, a small cluster of cells anterior to the cephalic fold, divides.

(G) At about 130 min, the cells of domain 23 invaginate. The stomodeum (sto) is the small cup-like invagination formed by domain 23. Just posterior to this invagination lies an orifice that marks the site where domain 8 invaginated earlier. Cells around the stomodeal invagination, most probably belonging to domain 1, undergo their 15th mitosis at this time.

(H) By 150 min, the domain 3 cells have begun to reorganize themselves into the clypeolabral lobe (clyp).
Fig. 19. Dorsal views showing the amnioproctodeal invagination and early germ band elongation. Panels A, B, C, D are at about 65, 70, 75, and 85 min, respectively. In the cellular blastoderm, a shallow depression at the posterior pole holds the pole cells (pc). This depression, which lies within domain 22, shifts dorsally (panel A) when the mesoderm invaginates at about 65 min. The entire domain invaginates (panel B) when its cells constrict apically (in panel A see region where arrow marks domain 22). This invagination carries the pole cells, still visible in panel B, into the interior of the embryo. By about 75 min (panel C), the pole cells have disappeared from sight inside this “amnioproctodeal” invagination (api), and the posterior tip of the germ band has come forward to form a lid above it. Mitosis in internalized domain 22 occurs much later, at 105 min.

Domain 4 constitutes the tip of the elongating germ band. Panel B shows a few of its cells ending their 14th interphase as their centrosomes separate in preparation for mitosis. In panel C, all of the domain 4 cells are in mitosis, and by 85 min (panel D), they are in interphase of cycle 15. The rapid phase of germ band elongation (see Fig. 2) starts in panel B and continues through panel D.

In panel D, the cells of domain 11, in telophase, have nearly concluded mitosis and the domain 12 cells are in metaphase. Domain 14 cells are all in mitosis. Note that domain 14 does not extend into domain 4, nor does domain M, nor do the cells of domains 16 or 17, dividing in panel D (domains 16 or 17, are not labelled but can be identified by analogy with Fig. 10A). Magnification 340x.
3.3 A given domain occupies, in every embryo, the same anteroposterior position with respect to en-stained stripes, and the same dorsoventral position with respect to the ventral midline.

Many of the domain boundaries coincide precisely with the boundaries of en-stained stripes.

To determine whether mitotic domains occupy fixed positions anteroposteriorly, I have correlated domain positions with the regions of en-stained (en) protein expression. en protein, which I detect by antibody staining, begins to accumulate in the blastoderm in a striped pattern between 60 and 65 min after the start of cycle 14 (DiNardo et al. 1985). By the time cycle 14 mitotic activity begins at about 70 min, en protein forms 14 circumferential stripes aligned at regular intervals over the posterior two-thirds of the embryo (see the time line in Fig. 2). The en protein marks the nuclei of cells that will give rise to the posterior third of each gnathal, thoracic, and abdominal segment (DiNardo et al. 1985). In embryos double-stained with antibodies against both tubulin and en protein, I visualize both antigens with secondary antibodies labelled with different fluorochromes (see Methods). In Figs 6–14, the D panels display micrographs of staged embryos in which double exposures reveal simultaneously the location of both the en and tubulin proteins. Initially, prior to germ band elongation, the en stripes are about one cell wide and occur within a three- to four-cell wide segmental repeat (Fig. 6D). The cell rearrangements of germ band elongation (see Section 3.5) produce en stripes that, in regions that have not yet undergone division, span two to three cells and occur within a seven- to eight-cell-wide segmental repeat (Fig. 6D). The cell rearrangements of germ band elongation (see Section 3.5) produce en stripes that, in regions that have not yet undergone division, span two to three cells and occur within a seven- to eight-cell-wide segmental repeat (Fig. 6D). The cell rearrangements of germ band elongation (see Section 3.5) produce en stripes that, in regions that have not yet undergone division, span two to three cells and occur within a seven- to eight-cell-wide segmental repeat (Fig. 6D). The cell rearrangements of germ band elongation (see Section 3.5) produce en stripes that, in regions that have not yet undergone division, span two to three cells and occur within a seven- to eight-cell-wide segmental repeat (Fig. 6D).

- The 14 pairs of cells that constitute $\delta_{14}25$ occur on the ventral surface, immediately anterior to each of the 14 en stripes. When, as occasionally happens, two adjacent cells, rather than a single cell, divide together in $\delta_{14}25$, both cells are anterior to the en stripe (Fig. 12D and insert). Thus $\delta_{14}25$ occurs immediately anterior to the parasegment boundaries (the boundaries between the anterior and posterior parts of each segment; see the terminology of Martinez-Arias and Lawrence, 1985), in segments 1–14.
- On the ventral surface, domain M, identified by the distinctive shape of its cells (see Section 3.4), extends from the anterior boundary of the first en stripe (the anterior margin of parasegment 1) to the posterior boundary of the 14th the anterior margin en stripe, which is the anterior margin of $\delta_{14}4$ (data not shown).
- The 7 paired cell clusters that make up $\delta_{14}16$ lie within en stripes 7 through 13, that is, in the posterior parts of presumptive abdominal segments 1 through 7 (Fig. 10D).
- The 7 paired cell clusters that make up $\delta_{14}17$ occupy the interstripe region of presumptive abdominal segments 2 through 8. Abdominal segment 1 probably does not have a member of this domain (data not shown).
- The boundary between $\delta_{14}11$ and $\delta_{14}6$ coincides with the anterior boundary of the fourth en stripe (Figs 8D and 9D). Thus the boundary between $\delta_{14}6$ and $\delta_{14}11$ is the parasegmental boundary in the first thoracic segment. There are three presumptive gnathal segments. The first and second en stripes demarcate the parasegmental boundaries in the two most anterior of these (DiNardo et al. 1985). From the time when the cephalic fold forms at around 65 min, until it opens ventrally at around 105 min, the cephalic fold encloses en stripes 1 through 3 (compare Figs 1C and 3C). This unfortunately obscures the relative positions of these stripes with respect to the boundaries between $\delta_{14}7$ and $\delta_{14}6$ and $\delta_{14}5$.
- $\delta_{14}12$ coincides with the most posterior of the 14 en stripes that appear during early embryogenesis. Fig. 8D reveals that the 14th en stripe lies just anterior to $\delta_{14}4$, and Fig. 19D reveals the localization of $\delta_{14}12$ immediately anterior to $\delta_{14}4$. The coincidence of $\delta_{14}12$ with this en stripe, though not shown directly, can be inferred. The 14th en stripe marks the posterior cells of a presumptive 8th abdominal segment. Later in development, at about 105 min, a 15th en stripe appears and sub-divides $\delta_{14}4$.
- In the germ band (i.e. the conspicuously metameric posterior two-thirds of the embryo), on the dorsolateral surface, in the region occupied by $\delta_{14}6$, $\delta_{14}7$ and $\delta_{14}11$, virtually all of the mitotic domains of cycle 15 are aligned with respect to the en stripes. One example of this is $\delta_{15}6$–$\delta_{15}1$ which, as shown in Fig. 13D, extends from the front of en stripe 3 to the front of stripe 4. In the procephalon, the nonmetameric anterior of the embryo, a specific but noncircumferential pattern of en-staining develops after the anterior cells have completed their 14th mitosis. Here again, certain of the cycle 15 domains line up with respect to the en-staining cells. Because of the extensive cell rearrangements in the presumptive head, it is difficult to follow precisely the borders of the cycle 14 domains and thus to identify the lineage (and therefore the nomenclature) of the cycle 15 domains in this region. Fig. 13D shows a domain that is aligned with a patch of en expression. Description of the cycle 15 mitotic domain pattern and the alignment of these domains with respect to the en-staining will be the subject of future studies.

The dorsoventral position of mitotic domains is constant embryo to embryo

The mitotic domains reveal a dorsoventral partitioning of the embryo not reflected in the en stripes. Viewed from ventral to dorsal around the embryo circumferential...
ence, the dorsal and ventral boundaries of the mitotic domains mark out a pattern of longitudinal strips on the surface of the germ band (shown diagrammatically in Figs 1 and 3). The width of each of these strips changes as germ band elongation proceeds and as cells divide. The ventral-to-dorsal widths listed below are given as a cell count just prior to mitosis 14 in the strip in question. Some imprecision in determining strip widths results because the blastoderm cells occur in a hexagonal close-packed array and boundaries between domains are therefore not straight lines. The list following enumerates these strips in ventral-to-dorsal order.

1. On the ventralmost surface of the blastoderm, \( \delta_{4,10} \) is a strip about 8 cells wide in each half embryo (thus about 16 cells wide, overall). This domain invaginates soon after cellularization. This strip has \( \delta_{4,8} \) as an anterior counterpart and \( \delta_{4,22} \) as its posterior counterpart, both of which also invaginate.

2. A longitudinal strip one cell wide in each half embryo, \( \delta_{4,14} \), occupies the blastoderm surface just lateral to \( \delta_{4,10} \). Invagination of \( \delta_{4,10} \), which comprises the ventralmost cells, brings the two members of \( \delta_{4,14} \) together at the ventral midline. Topologically \( \delta_{4,15} \) and \( \delta_{4,13} \) may represent the anterior and posterior equivalents of \( \delta_{4,14} \), bounding domains that invaginate.

3. On the ventrolateral surface of the gastrula, dorsal to the strip of \( \delta_{4,14} \) cells, lies a longitudinal strip of cells made up of \( \delta_{4,22} \) and \( \delta_{4,25} \). This strip extends from the anterior boundary of the 1st en stripe to the posterior boundary of the 14th stripe. There is no equivalent of this strip either anterior or posterior to these boundaries. Many neuroblasts leave \( \delta_{4,22} \) prior to mitosis, reducing its width; just prior to mitosis, \( \delta_{4,22} \) is only 1 cell wide behind the parasegmental boundary in the first abdominal segment and, on average, almost two cells wide forward of this boundary.

4. Adjacent and dorsal to \( \delta_{4,22} \), \( \delta_{4,23} \) is a strip 6-7 cells wide within which lie \( \delta_{4,21}, \delta_{4,26}, \) and \( \delta_{4,17} \). \( \delta_{4,23} \) extends to the posterior tip of the embryo but reaches no further forward than the front of the 1st en stripe.

5. Immediately dorsal to \( \delta_{4,23} \) lies a strip about eight cells wide which includes \( \delta_{4,11}, \delta_{4,6}, \) and presumably \( \delta_{4,7} \). After forming the ventral furrow, \( \delta_{4,10} \) cells precisely underlie the ventral and ventrolateral strips (2), (3), and (4), described above, but do not extend under this strip, (5). The early cell rearrangements anterior to the cephalic fold make it difficult to determine the relationship of strip (5) to the head domains but \( \delta_{4,5}, \delta_{4,1}, \) and \( \delta_{4,3} \) may represent procephalic counterparts.

6. \( \delta_{4,19} \) is a narrow strip, 1-2 cells wide, between \( \delta_{4,11} \) and domain A.

7. Domain A is an expanse of nondividing cells lying along the dorsal midline over the presumptive germ band. \( \delta_{4,18} \) and \( \delta_{4,20} \) occupy a similar dorsal position in the anterior of the embryo.

3.4 Cells of specific mitotic domains exhibit characteristic cell biological behaviors and give rise to specific structures

It is striking that in some of the mitotic domains the cells all exhibit unique traits that are distinct from those of cells in adjacent domains. In many cases these traits are morphogenetically important. The following examples illustrate the kinds of different characteristics that distinguish different mitotic domains. Additional distinguishing characteristics will likely be found in the coming years.

All cells in \( \delta_{4,10} \) (Fig. 15), \( \delta_{4,22} \) (Fig. 19A and B), and \( \delta_{4,8} \) (Fig. 18E and F) invaginate abruptly by infolding as coherent sheets of cells. These three domains invaginate at different times. \( \delta_{4,10} \) and \( \delta_{4,22} \) invaginate while their constituent cells are in interphase 14, that is before the division by which I identify each group as a specific mitotic domain, while \( \delta_{4,8} \) invaginates during interphase 15. As they invaginate, the apical ends of these cells decrease in diameter (see Fig. 15 and Fig. 19A), changing their shape from tall columnar to wedge-shaped, with the apices facing the embryo exterior (Poulson, 1950; Turner and Mahowald, 1977; Odell et al. 1981). Early in this process, apical constriction is restricted to small clusters of cells in \( \delta_{4,10} \), and apical constriction in some cells appears to stretch the apices of adjacent cells prior to their subsequent constriction (illustrated in Fig. 15). \( \delta_{4,10} \) is the primordial mesoderm, \( \delta_{4,22} \) is the posterior midgut primordium, and \( \delta_{4,8} \) may be the anterior midgut primordium, though that is uncertain. Table 1 lists the fates I infer, from the sources cited, of these and other domains.

From ventral furrow closure (65 min), during their mitosis (about 90 min), and until they are internalized (230-260 min), the cells of \( \delta_{4,14} \) occupy two continuous rows, each primarily one cell wide, on either side of the ventral midline (panel B in Figs 10, 11, 12, 13, and 14. For a high magnification view, see Fig. 16). During the 20-25 min between the time when the ventral furrow invaginates and the \( \delta_{4,14} \) cells divide, the \( \delta_{4,14} \) cells appear stretched, as \( \delta_{4,10} \) cells do just prior to their apical constriction (see for example, Fig. 7B). The \( \delta_{4,14} \) cells, however, do not undergo apical constrictions and do not invaginate. Much later, while still in interphase of their 15th cell cycle, all the cells of \( \delta_{4,14} \), but not those of neighboring domains, slowly sink into the embryo's interior where they will contribute to the ventral nerve cord.

The cells of \( \delta_{4,8} \) and \( \delta_{4,9} \) also generate daughters destined for the embryo interior, but these cells arrive in the interior by yet another mechanism – an oriented division. During mitosis of cycle 14, the cells of these domains divide along axes perpendicular to the surface of the embryo (Figs 17B and 18C), unlike the cells of all other domains which divide along axes parallel to that surface. As a consequence of this oriented division, the daughters of \( \delta_{4,8} \) form two parallel sheets. The superficial daughters of \( \delta_{4,8} \) subsequently invaginate, during their 15th interphase (Fig. 18E and F); these together with their sisters likely constitute the primordium of the
The morphogenetic movements of gastrulation come the extraembryonic amnioserosa while the dermal invagination takes less than 10 min and is shown). anterior tip = 100 % egg length.

The cells of domain A have become squamous (Fig. 16), while those of domain M have elongated perpendicular to the anteroposterior axis of the embryo (Fig. 12B and, at high magnification, Fig. 16). The squamous cells become the extraembryonic amnioserosa while the elongated cells produce both neuroblasts and ventral epidermal precursor cells. The cells of some mitotic domains adopt unusual shapes. For example, by about 105 min, the cells of domain A have become squamous (Fig. 16), and those of domain M have elongated perpendicular to the anteroposterior axis of the embryo (Fig. 12B and, at high magnification, Fig. 16). The squamous cells become the extraembryonic amnioserosa while the elongated cells produce both neuroblasts and ventral epidermal precursor cells.

3.5 The morphogenetic movements of gastrulation require the specialized function of various mitotic domains

The midline cleft produced by permanent invagination of the mesoderm, and the transient cephalic fold, create useful visual landmarks during germ band elongation

The morphogenetic movements of gastrulation begin about 55 min into cycle 14. A midventral band of cells, about 16 cells wide and 50–60 cells long, invaginates (Fig. 15) to form an internalized tube of cells, called the ventral furrow, that is the primordial mesoderm (Sonn enblick, 1950; Pouls on, 1950; Turner and Mahowald, 1977; Fullilove and Jacobson, 1978). This quick mesodermal invagination takes less than 10 min and is complete by 65 min. It leaves a cleft along the ventral midline (Fig. 6B) which extends from about 15 % to 80 % egg length*. Later, all of the internalized cells will divide together as a single domain, 31410 (data not shown).

The 'cephalic fold', also called the 'head fold' or the 'cephalic furrow', forms concurrently with the ventral furrow. Formation of the cephalic fold starts first on the lateral surfaces of the embryo, at about 65 % egg length, and spreads from there both dorsally and ventrally, encircling the embryo with an oblique crease (Fig. 6A–C). Time-lapse video tapes show that this is a transient fold which, during the course of the early developmental period described herein, first deepens and later partially unfolds (shown in the process of opening in Fig. 11C). The early unfolding of the cephalic fold reaches completion only on the ventral surface. Fig. 18D, E and F are high magnification micrographs displaying embryos just prior to, during, and just after, the ventral unfolding of the cephalic fold. All of 3147, the posterior of 3145 and 3142, and the anterior parts of 314M, 314N, and 3146 together form the walls of this fold during the period from 65 to 105 min; after furrow opening, they lie outside of it (shown diagrammatically in Figs 1 and 3). As the cephalic fold opens ventrally, it deepens dorsally, pulling 31420 into the fold. As shown by Underwood et al. (1980), the changing makeup of the cephalic fold accounts for conflicting data on the location of the presumptive gnathal and thoracic segments with respect to this fold (compare, for example, Poulson, 1950, to Turner and Mahowald, 1977). The cephalic fold forms while all the cells involved are in interphase of cycle 14. Since cells divide while incorporated into the walls of the fold, it is difficult to monitor the boundaries of the mitotic domains inside the cephalic fold (see Methods). Late in embryogenesis the cephalic fold disappears completely (not shown).

As the embryo elongates more than two-fold; the amnioserosa folds up ahead of the extending germ band

I use the term 'germ band' to denote the conspicuously metameric portion of the embryo, as distinguished from the procephalon which lies anterior to the cephalic fold, and from the specialized tail region at the posterior tip. At about 70 min, the germ band begins to elongate axially and to narrow laterally (Turner and Mahowald, 1977). This elongation involves both the surface cells and the presumptive mesoderm within. The elongating germ band arches inside the rounded posterior end of the confining vitelline membrane, pushing the pole cells and the posterior tip of the embryo forward along the dorsal surface of the egg. At any given time during germ band elongation, the germ band exhibits a bent shape; the bend stays fixed at the egg's posterior pole while germ band cells flow through the bend, i.e. different cells occupy the bend at different times. Figs 6 through 14 show successive stages of this elongation. By about 90 min, the posterior tip (3144) has extended forward to the cephalic fold as the result of an increase in the germ band's midline length (Fig. 10A). Further elongation carries the tail region away from the surface and out of sight, tucking it in behind the cephalic fold (see Fig. 11A). Fig. 2 depicts the trajectory of 3144; also compare Figs 1D and 3D. At the conclusion of its elongation, the germ band has bent in half with about one-half of its length lying along the egg's dorsal surface, between the cephalic fold and the posterior pole, while one-half lies along the ventral surface, between the cephalic fold and the posterior pole.

The amnioserosa is a tissue made up of nondividing squamous cells (shown at high magnification in Fig. 16). It develops from the dorsal cells of the blastoderm (domain A in Figs 1 and 3). As the germ band moves the posterior tip of the embryo forward along the dorsal surface of the egg, it appears to crush the flexible tissue of the amnioserosa against the unyielding tissue anterior to the cephalic fold (see for example Figs 8C and 9C); beginning early in the process of germ band
### Table 1. Fates of the mitotic domains

<table>
<thead>
<tr>
<th>Domain</th>
<th>Embryonic primordium</th>
<th>Larval structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The anterior of domain 1 fuses with domain 3 to form the clypeolabral lobe; the posterior contributes to the procephalic lobe</td>
<td>fused with mitotic domain 3 generates the labrum and the roof of the preoral cavity (atrium) with its epistomal sclerite and labral sense organs; the posterior probably forms part of dorsal bridge</td>
</tr>
<tr>
<td>2</td>
<td>anterior part of the domain forms the hypopharyngeal lobe; the posterior part probably forms the mandibular lobe</td>
<td>floor of the pharynx, including the pharyngeal ridges (T-ribs) and the hypopharyngeal organ; lateral walls of the pharynx including the lateralgrate, ventral arm and the dorso-lateral papilla of the maxillary sense organ</td>
</tr>
<tr>
<td>3</td>
<td>fuses with domain 1 to form the clypeolabral lobe</td>
<td>fused with mitotic domain 3 generates the labrum and the roof of the preoral cavity (atrium) with its epistomal sclerite and labral sense organs</td>
</tr>
<tr>
<td>4</td>
<td>posterior tip of the germ band</td>
<td>probably the anal pads; domain 4 fuses with embryonic segment a8 and the posterior parts of domains II and N to form the specialized larval tail region</td>
</tr>
<tr>
<td>5</td>
<td>part of procephalic lobe</td>
<td>roof of the pharynx including the dorsal arm, the vertical plate, part of the dorsal bridge, and possibly the dorsal surface of the head (the anterior lobe) carrying the dorso-medial papilla of the maxillary sense organ, and the antennal sense organ</td>
</tr>
<tr>
<td>6</td>
<td>dorsal ectoderm extending from t1; the anterior margin of this domain is a guess, since it is located out of sight inside the cephalic fold</td>
<td>floor of the preoral cavity including the hypostomal sclerites, the bridge of the H-piece, labial sense organ, lower-lip organ and labial black dot; also the epidermis and part of the peripheral nervous system of t1p</td>
</tr>
<tr>
<td>7</td>
<td>the maxillary lobe; the anterior and posterior margins of this domain are located out of sight inside the cephalic fold</td>
<td>surface of the head surrounding the larval orifice including the maxillary cirri, mouth hooks, ectostomal sclerites, lateral bar of the H-piece, ventral organ and central papilla of the maxillary sense organ</td>
</tr>
<tr>
<td>8</td>
<td>probably the anterior midgut primordium</td>
<td>if anterior midgut, then the epithelial lining of the gastric caeca and of the anterior portion of the ventriculus</td>
</tr>
<tr>
<td>9</td>
<td>superficial daughters – part of procephalic lobe; deep daughters – brain neuroblasts</td>
<td>larval fate of superficial daughters unknown; the internalized daughters presumably form the cephalic complex</td>
</tr>
<tr>
<td>10</td>
<td>mesodermal primordium</td>
<td>the connective tissues, the somatic and splanchnic muscles of both the head and body, the fat bodies, the circulatory system including the blood, and all parts of the gonads except the germ cells</td>
</tr>
<tr>
<td>11</td>
<td>dorsal ectoderm of t1p-a8; the posterior margin of this domain is not visible in the stages examined and may extend beyond a8</td>
<td>dorsal epidermis and part of the peripheral nervous system cells of t1p-a8p; the trachea and probably both anterior and posterior spiracles are formed from this domain. The dorsal epidermis produces a complex array of fine hairs distinguishing dorsal from ventral cuticle</td>
</tr>
<tr>
<td>12</td>
<td>ventral ectoderm of a8p</td>
<td>possibly the genital disk</td>
</tr>
<tr>
<td>13</td>
<td>proctoderm or hindgut primordium</td>
<td>anus, hindgut, and malpighian tubules</td>
</tr>
<tr>
<td>14</td>
<td>mesectoderm, also called midline cells</td>
<td>midline neurons of the central nervous system and gial or peripheral sheath cells</td>
</tr>
<tr>
<td>15</td>
<td>unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>16</td>
<td>unidentifed part of ventral ectoderm reiterated in a1p-a7p</td>
<td>unknown</td>
</tr>
<tr>
<td>17</td>
<td>unidentifed part of ventral ectoderm reiterated in a2p-a6</td>
<td>unknown</td>
</tr>
<tr>
<td>18</td>
<td>dorsal surface of procephalic lobe</td>
<td>dorsal midline following dorsal closure</td>
</tr>
<tr>
<td>19</td>
<td>dorsalmost ectoderm of the germ band</td>
<td>therefore probably the dorsal pouch</td>
</tr>
<tr>
<td>20</td>
<td>probably dorsal ridge</td>
<td>unknown</td>
</tr>
<tr>
<td>21</td>
<td>unidentified part of the ventral ectoderm marking parasegment boundaries, reiterated in g1-t3</td>
<td>epithelial lining of the posterior portion of the ventriculus</td>
</tr>
<tr>
<td>22</td>
<td>posterior midgut primordium</td>
<td>pharynx, esophagus, and proventriculus</td>
</tr>
<tr>
<td>23</td>
<td>stomodeum or foregut primordium</td>
<td>unknown</td>
</tr>
<tr>
<td>24</td>
<td>unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>25</td>
<td>unidentified part of ventral ectoderm reiterated in g1-a8</td>
<td>unknown</td>
</tr>
<tr>
<td>M</td>
<td>ventral ectoderm of g1p-a8p; SI, SII, and SIII neuroblasts are intermingled with epidermoblasts in this domain</td>
<td>ventral nerve cord neurons and ventral epidermis of g1p-a8p</td>
</tr>
</tbody>
</table>
Table 1. continued

<table>
<thead>
<tr>
<th>Domain</th>
<th>Embryonic primordium</th>
<th>Larval structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>ventral ectoderm of g1p—beyond a8(2). Here SI and SII neuroblasts are intermingled with epidermblasts (2, 9)</td>
<td>neurons of both the ventral nerve cord and the peripheral nervous system, and ventral epidermis cells, from g1p—a8(2); the sensory cones of the tail region probably originate from the posterior part of this domain (5). The ventral epidermis of r1—a8 produces denticle bands in the anterior of each presumptive segment, distinguishing ventral from dorsal cuticle</td>
</tr>
<tr>
<td>A</td>
<td>amnioserosa(1)(6)</td>
<td>possibly pericardial wall (9)</td>
</tr>
<tr>
<td>B</td>
<td>possibly neurogenic ectoderm (1)(4)</td>
<td>possibly proto- and deutocerebrum (1)(4)</td>
</tr>
</tbody>
</table>

Superscript numbers in parentheses reference evidence supporting the fate designations of the mitotic domains. 'a', 't' and 'g' denote abdominal, thoracic or gnathal segment identity. Numbers 1–8 used with these letters denote the sequential number of serially reiterated segments; subscripts 'a' and 'p' used in conjunction with these numbers denote the anterior or posterior part of a segment; the anti-engrailed antibody specifically stains only the posterior of each segment (DiNardo et al. 1985; Kornberg et al. 1985). For example, Δ3p designates the posterior part of the third thoracic segment, which is the part of that segment that stains with anti-engrailed antibody.

2. DiNardo et al. 1985; fate is inferred from the position of the domain with respect to engraved stripes.
13. Tentative conclusions based on the present study.

elongation, two folds develop—a shallow, 'anterior dorsal fold' and a deep 'posterior dorsal fold' (Turner and Mahowald, 1977; shown in Fig. 6C and later stages). These folds cross the amnioserosa and extend into the tissue lateral to it. By 100–105 min, the amnioserosa cells have become squamous and the two dorsal folds have disappeared (see for example Fig. 12C). At full germ band elongation, the amnioserosa ends up sandwiched between the dorsal and ventral halves of the extended, bent, germ band. The changing position of the amnioserosa is shown schematically in Figs 1 and 3, which represent embryos at the start of, and at, full germ band extension.

**Formation of the posterior midgut and proctodeum carry the pole cells inside the embryo**

Prior to germ band elongation, the pole cells lie clustered at the posterior end of the egg in a shallow depression formed by 31422 blastoderm cells. This depression, and the pole cells within it, move to the dorsal surface of the egg (shown at high magnification in Figs 19A and B). As the posterior tip of the elongating germ band pushes toward the cephalic fold, the depression containing the pole cells invaginates, forming a pouch that holds the pole cells. Two mitotic domains constitute the walls of this invagination: the major part corresponds to 31422 and is the posterior midgut primordium, while the cells ringing the opening, 31413, form the proctodeum or hindgut primordium. Figs 1D and 3D show their positions diagrammatically in sagittal section. In Fig. 19C and D, the elongating germ band conceals 31422 and 31413 which lie beneath it.

**Formation of the anterior gut tube occurs piecewise**

At about 55 min, the ventral furrow begins to form by invagination of a ribbon of ventral cells. Immediately anterior to this ribbon of cells is a roughly square block of cells which invaginates about 5 min later creating a 'T' at the anterior end of the ventral furrow (compare Figs 18A and B, also Figs 6B and 7B). Immediately anterior to this invaginated area is Δ48, whose cells will divide along axes perpendicular to the embryonic surface at about 80 min (Fig. 18C), giving rise to two closely adhering layers of daughter cells. Later, between 100 and 110 min the superficial layer of Δ48 cells invaginates (compare Fig. 18D, E and F), presumably carrying its adhering layer of sister cells deeper into the embryo as diagrammed in Fig. 3D. This invagination of Δ48 cells draws adjacent surface domains towards the site of invagination; for example Δ43 cells move from their original dorsal position to the anterior tip of the embryo (compare Figs 1 and 3).

As Δ48 invaginates, the ventral portion of the cephalic fold begins to open (compare Fig. 18E with F), moving Δ42 and the invaginating 3148 forward with respect to the remaining (dorsal) portion of the cephalic fold. Figs 1C and 3C, which indicate segments, conveniently illustrate these repositionings.

While Δ48 invaginates, the cells of Δ423 enter mitosis 14 (Fig. 18E) and, 20–25 min later, first Δ43 and then Δ443 cells enter mitosis 15 (Fig. 18G). At about the time Δ43 cells divide, the Δ423 cells invaginate (Fig. 18G). Next, the Δ43 cells rearrange and begin forming the cytoplasmal lobe (Fig. 18H).

It is widely believed that the anterior midgut arises from invaginating cells at the anterior 'T' of the ventral furrow (Poulson, 1950; Turner and Mahowald, 1977; Campos-Ortega and Hartenstein, 1985). As shown schematically in Fig. 1, I observe that all the cells that invaginate with the ventral furrow, including those that form the 'T' at the anterior end of that furrow, divide together as a single mitotic domain, Δ410. A small group of internal cells with distinct mitotic properties does occur just anterior to the ventral furrow, and these are the Δ448 daughters internalized by an oriented
division. I suggest that the anterior midgut, which is an endodermal tissue, arises from these internalized \( \partial_{48} \) cells, rather than from anterior cells of \( \partial_{10} \), and that the "T" of cells at the anterior of the ventral furrow form the mesoderm of the nonmetameric part of the presumptive head. Since the surface cells of \( \partial_{48} \) also enter the embryo interior -- these by invagination -- this portion of the domain may also contribute to the anterior endoderm. These suggestions need experimental confirmation.

\( \partial_{423} \), initially at the anterior tip of the blastoderm, forms the stomodeal (or foregut) primordium. In the terms of classical embryology, this, like the proctodeal (or hindgut) primordium, is an ectodermal tissue. My time-lapse studies show that, following the invagination of \( \partial_{423} \), the cells of the surrounding domains begin slowly to involute into the embryo's interior at the site where \( \partial_{423} \) invaginated earlier. Involution continues for several hours and, when it is complete, most of the domains previously anterior to (or lying within) the cephalic fold will have moved inside the developing head. In this way, the ectodermal cells of the embryonic head, which initially develop on the exterior, come to lie inside the embryo, in the anterior part of the gut tube, where they secrete the chitinous mouth parts of the larva (see Table 1). This involution presumably places the \( \partial_{423} \) cells further down the gut tube as the most internal of the ectodermal parts of the foregut. The ectodermal part of the gut tube will subsequently connect to the anterior midgut and this to the posterior midgut and the latter to the hind gut.

Oriented division puts the brain primordium inside the developing head; other brain neuroblasts may arrive by ingression

Between 80 and 90 min the \( \partial_{49} \) cells divide. As previously stated, each cell of this large domain divides along an axis perpendicular to the embryonic surface (shown at high magnification in Fig. 17B), leaving one of its daughters on the surface and placing the other inside the embryo. Existing fate maps indicate that the internal daughter cells contribute to the embryonic brain while the superficial daughters may give rise to ectodermal structures of the head (Table 1).

Domain B also occupies a region that existing fate maps assign to the brain (Table 1). Extensive cell rearrangements in the presumptive head made it difficult to keep track of this domain for the entire three hours discussed here; it is clear, however, that the cells of domain B do not divide prior to 130 min. If neuroblasts are leaving this region, it is individually by migration, while still in interphase 14. Thus, cell division in domain B is occurring, if at all, only after the cells leave the embryo surface.

Ventral cord neuroblasts ingress

Cells on the ventral surface of the germ band, in the domains labelled M and N in Figs 1 and 3, undergo their 14th mitosis with less synchrony than do those of the other mitotic domains (see Section 3.2 and Fig. 2). During the course of germ band elongation, large numbers of these cells ingress to generate neuroblasts. The cells that remain on the surface give rise to the ventral epidermal cells, which secrete the ventral part of the larval cuticle (Table 1). Hartenstein and Campos-Ortega (1984) report that neuroblast ingestion occurs in 3 waves over a period of 2-2.5 h. The first wave of ingestion internalizes cells from \( \partial_{4M} \) and from both margins of \( \partial_{14N} \) between 100 and 110 min. The second wave internalizes cells from the ventral margin of \( \partial_{4N} \) and from \( \partial_{4M} \), probably between about 115 min and 135 min. The third wave of ingestion removes more cells from \( \partial_{4M} \) during the long period when cells from this domain are dividing (see Fig. 2).

Though domains M and N resemble each other in exhibiting a less patterned mitotic activity, I distinguished them because they divide during different intervals (103-140 min and 140-190 min, respectively) and exhibit distinct cell morphologies. At about 100 min, the cells in domain \( \partial_{4M} \) begin to elongate along axes parallel to the embryo surface (and at right angles to the ventral midline), while the adjacent columnar cells of \( \partial_{4N} \) remain oriented perpendicular to the embryonic surface (Fig. 16). The elongation of domain M cells occurs coincidently with the first wave of neuroblast ingestion and is no longer evident after the domain completes mitosis.

4 Discussion

4.1 Mitotic domains occupy fixed positions on the embryo's surface

From studies of fixed and fuchsin-stained Drosophila embryos, Hartenstein and Campos-Ortega (1985) concluded that, soon after gastrulation starts, mitotic waves begin at five loci* and spread from these loci at different speeds in different directions. From my more detailed studies of anti-tubulin-stained fixed embryos and of videotapes of live embryos, I come to conclusions that are in only partial agreement with theirs. I too observe that the pattern of mitosis following interphase of cycle 14 is discontinuous, complex, bilaterally symmetric, and strikingly reproducible between individuals. I conclude, however, that the overall pattern is best described not as five waves with complex dynamics, but rather as a large number of discrete mitotic domains, each composed of cells that divide in close synchrony. Mitosis, triggered from one or a small number of sites within each domain, radiates rapidly as a wave across the domain, and stops at the domain boundary.

In nuclear cycle 14 at least 25 mitotic domains exist (displayed diagrammatically in Figs 1 and 3). Most of these occur as pairs, located with mirror-image symmetry, while several are also segmentally repeated. Thus, during germ band elongation, I identify not 5, but over 50 distinct mitotic trigger sites in each half of the embryo.

Since all the somatic nuclei of the blastoderm begin

* These loci are equivalent in location to what I designate \( \partial_{44}, \partial_{44}, \partial_{45}, \partial_{46}, \partial_{410} \).
Mitotic domains in Drosophila embryos

interphase of cycle 14 at nearly the same time, the mitotic domains delineate regions of specific cell cycle length. \( \delta_{1,4} \) (which is the designation for domain 1 of cycle 14) has the shortest cycle length of the cycle 14 mitotic domains – 70 min at 25°C. \( \delta_{4,25} \) cells spend 115 min in cycle 14, and cells in domain M, a domain whose cells divide in a less synchronous fashion, spend up to 190 min in cycle 14. Cells in at least one region, domain A, never divide but remain in interphase of cycle 14 throughout embryogenesis. Though the differences in cell cycle length between many of the domains are small, the sequence with which the various domains divide is invariant embryo-to-embryo (data summarized in the left panel of Fig. 2).

The *engrailed* (*en*) gene is expressed in all the cells of the posterior of each segment and needed in those cells for the establishment of the anterior–posterior parasegmental boundary (Morata and Lawrence, 1975). Localized *en* expression is first detectable in the young embryo during cycle 14, appearing in the posterior region of each presumptive segment and, because it persists there through subsequent stages, it provides a powerful molecular marker of metameric pattern in developing embryos (DiNardo et al. 1985; Kornberg et al. 1985).

Across the surface of the germ band, that central part of the embryo that will produce the conspicuously segmented body of the animal, 14 stripes of *en* protein expression appear during cycle 14, soon after cellularization is complete (DiNardo et al. 1985). About 40 min later, a 15th *en* stripe appears in the tail region, and patches of expression appear in the procephalon. These two regions, one at either end of the embryo, produce the less obviously segmented parts of the body plan. In the germ band, the boundaries of the *en* stripes coincide with the anteroposterior boundaries of certain of the cycle 14 mitotic domains. A dramatic example is \( \delta_{4,25} \); each isolated cell of the 14 bilaterally arranged cell pairs that make up this domain sits immediately anterior to one of the 14 *en* stripes (Fig. 12D and, diagrammatically, in Figs 1C and 3C). Section 3.3 of Results describes additional examples. Many of the mitotic domains of cycle 15 also line up conspicuously with the *en* stripes. Fig. 13 and its legend describe two examples, one in the germ band and one in the procephalon.

The dorsal and ventral boundaries of the mitotic domains, measured by cell count from the ventral midline, are also constant from embryo to embryo. Figs 1 and 3 summarize these data which I detail in Results section 3.3.

4.2 Mitotic domains mark embryonic primordia

Earlier investigations of normal development, and of development following tissue ablation and transplantation, indicated which larval structures normally arise from which specific regions of the embryo; these studies resulted in the construction of 'fate maps'. In Table 1, I attribute to each mitotic domain the fate of the cells in this region according to this earlier body of work (references are listed in Table 1). However, since the domains partition the embryo more finely and with greater precision than these earlier fate studies, the correlation between the domains and the fate maps are tentative. In order to work out a fate map in greater detail, I have initiated studies in which I mark and follow the trajectory of cells in specific domains. This is possible for domains whose cells divide while located on the embryonic surface because, in living embryos, I can discern dividing cells when they round up to divide (see Methods).

While I do not yet know the fate of cells in every domain, it is clear from the alignment of domains with the *engrailed* stripes, and from the global patterns the domains create, that they prefigure the body plan of the developing embryo. The anterior and posterior ends of the embryo are distinct from each other and from the germ band, and within the germ band small differences in dorsal-ventral partitioning distinguish the presumptive gnathal, thoracic, and abdominal segments. Here I discuss the organization at the posterior and anterior ends of the embryo – and the dorsal–ventral partitioning. As explained above, the location of certain domains in register with particular *en* stripes identifies those domains as primordia of specific segments. On the basis of a reiterated mitotic pattern, the 8th appears to be the most posterior of the abdominal segments laid down prior to gastrulation. (Note that the 14th *en* stripe marks the posterior of the presumptive 8th abdominal segment; see Figs 1 and 3.) There has been controversy as to whether the embryo, posterior to abdominal segment 8, is segmented and, if so, how many segments exist there (Matsuda, 1976). The studies of Jürgens (1987) and Hartenstein and Campos-Ortega (1984) indicate the absence of ventral primordia from the 'tail region'. I agree with these conclusions. The mitotic schedule of the tail region is quite different from the segmentally reiterated pattern anterior to it. The posterior part of the 8th abdominal segment differs from the other abdominal segments. Instead of \( \delta_{4,16} \), another domain, \( \delta_{4,12} \), is present. None of \( \delta_{4,10}, \delta_{4,14}, \delta_{4,15} \), \( \delta_{25}, \delta_{4,16} \) or \( \delta_{4,17} \) extend to the posterior tip. Instead, a single domain – \( \delta_{4,4} \) – replaces them. \( \delta_{4,4} \) does extend along the dorsal side of \( \delta_{4,4} \). Possibly \( \delta_{4,11} \) does too, but when the cells of \( \delta_{4,11} \) divide, the extending germ band obscures the posterior part of \( \delta_{4,11} \) so I am uncertain about the posterior reach of this dorsal domain. The domains at the posterior tip of the germ band are illustrated in Fig. 19 and schematically in Figs 1 and 3. According to the fate map designations of the domains (Table 1) the posterior tip of the germ band would thus seem to lack:

- the mesodermal primordia, and hence a coelomic cavity,
- a major part of the ventral ectoderm, and
- hence corresponding parts of the segmental ganglia.

The omission of these crucial parts at gastrulation, and their replacement by a new primordium, \( \delta_{4,4} \), would seem to indicate the early repression of segmentation genes in the tail.
However, at about 105 min, well after the first 14 en stripes have subdivided the conspicuously segmented germ band, a 15th en stripe does appear in the tail region, and this suggests segmentation. Also, Jürgens (1987) reports data on segmentation and homeotic mutations that suggest that the tail region evolved through the fusion of ancestral posterior segments. The cuticle patterns of homeotic mutants support the conclusion that the tail has lost extreme dorsal and ventral cuticle patterns of homeotic mutants. Homeotic genes to transform lateral primordia while lateral primordia remain in it for the ectopic expression of homeotic genes to transform.

There is a similar controversy as to whether the anterior region of the embryo, the procephalon, is segmented and, if so, how many segments it might include (reviewed by Rempel, 1975). The procephalon comprises twelve domains – 3141, 3142, 3143, 3145, 3148, 3149, 31415, 31418, 31420, 31423, 31424 and B. These, together with the gnathal segments, give rise to the embryonic head. Jürgens et al. (1986) studied the effects that UV ablation in this region had on the development of the cuticular structures of the larva. They ablated cells of blastoderm-stage, and fully extended germ-band-stage embryos. Unfortunately, extensive morphogenetic movements separate the two stages on which they experimented, and the mitotic domains I describe appear during the interval between those stages. Thus it is difficult to judge which domains they ablated. I have, nevertheless, attempted to correlate the cycle 14 domains with the sites they irradiated by following the movements of specific domains of cells into the extended germ band stage. Table 1 lists my preliminary conclusions.

The development of the procephalon differs from that of the obviously segmented germ band – differs in ways similar to the discrepancies in the tail region. The procephalon also lacks the ventral mitotic domains which give rise to the ventral nervous system and the ventral hypoderm in the germ band (Figs 1 and 3 and Table 1). As in the tail region, the procephalon cells begin to develop patterned en expression only after 100 min, during interphase 15. While the anterior and posterior regions differ in similar ways from the segmented germ band, they differ from each other dramatically as regards mitotic schedule; the procephalon is exceedingly more complex than the tail region. While many of the 12 domains of the procephalon show no conspicuous resemblance to domains in either the germ band or the posterior tip, there is, dorsally, an apparent echo of segmentation, namely:

- Three domains in the procephalon – 3143, 3141 and 3145,
- Two domains in the gnathal head region – 3146 and 3147,
- The five metamerically reiterated members of 31411 in the thorax and abdomen.

Dorsalventral differences in mitotic pattern delineate boundaries between cells with different tissue fates. Prior to ventral furrow formation, domains 31410, 3148, 31410, 31422, and 31413 constitute the ventralmost surface of the blastoderm. Gastrulation movements internalize all of these domains which I believe to be the primordia of foregut, anterior midgut, mesoderm, posterior midgut and hindgut, respectively (see Table 1 and Results Section 3.5 for details and references on cell fates). On the blastoderm, immediately dorsal to these internalized domains, lies 31414 which, following invagination of the mesodermal primordia, occupies the midline of the early gastrula. 31414 gives rise to the ‘midline cells’ of the ventral nervous system. On the ventrolateral surface of the gastrula, next to this narrow strip, is 314M which surrounds 31425, and immediately dorsal to these is 314N, which surrounds 31421, 31416, and 31417. From these six domains will come the cells that generate the ventral nervous system, and the ventral epidermal cells that later secrete the ventral cuticle with its characteristic denticle belts. Certain precursors of the peripheral nervous system also arise from these ventral domains. Domains 31411, 3146, 3147, 3145, 3141, and 3143 together constitute the dorsolateral surface of the gastrula; in the germ band the cells of 31411 generate the remainder of the peripheral nervous system and the cuticle-secreting cells of the dorsal hypoderm including the tracheal pits. In the head, cells from 3147, 3146, 3145, 3141, and 3143 will secrete the specialized cuticle of the mouthparts, and generate specialized sensory organs. Domain A constitutes the dorsal surface of the blastoderm. This domain gives rise to the amnioserosa, a membrane-like layer of nondividing cells that covers the dorsal surface of the developing germ band; late in embryogenesis the lateral surface of the embryo grows over the amnioserosa leaving the domain A cells inside where they apparently contribute to the pericardial wall. When dorsal closure is complete, 31419 will occupy the dorsal midline.

4.3 Mitotic domains may turn out to be phylogenetically general; the techniques for visualizing them certainly are

Many authors have attempted to classify morphological structures in terms of evolutionary derivation. Matsuda (1965) and Rempel (1975), among others, argue that evidence for such evolutionary derivation should be based primarily on embryological data. The mitotic domains appear to constitute the earliest embryonic primordia so far recognized. Visualizing mitotic figures using fluorescently labeled secondary antibodies to antitubulin is a phylogenetically general method. Thus, if mitotic domains turn out to be phylogenetically general, they will provide a short cut method for investigating and comparing the early development of diverse organisms – for assessing homology; and they may provide a basis for extrapolating to other organisms the concepts of Drosophila development accumulating so
quickly now as a consequence of the recent investment of many thousands of person-years to produce the collection of mutant stocks, cloned genes, *in situ* hybridization probes, and antibodies now available for studies of pattern formation in *Drosophila*.

4.4 Patterned mitosis, domain by domain, is a telling progress report on the commitment process dynamically advancing

Elucidation of the complex stereotyped pattern of nuclear division in the early *Drosophila* embryo raises a number of questions central to developmental biology. Mitotic synchrony in each domain seems to be indicative of some deeper underlying property, yet to be determined, that the cells of any one mitotic domain share and that distinguishes them from the cells of adjacent domains. This property may be the same that results in the commitment of cells to distinct developmental pathways. I observe that some cycle 14 mitotic domains are distinct in that their cells all exhibit specific cell biological traits: in certain domains the orientation of the mitotic spindles is fixed; in others cell shape is distinctive; in still others all the cells of the domain participate together in a specific morphogenetic movement such as invagination. It is striking that many domains exhibit these distinctive properties before the mitoses by which I identify the cells as members of distinct mitotic domains.

The complexity and reproducibility in pattern of mitosis, the extraordinary bilateral symmetry of this pattern, and the fact that the cells that make up certain of the domains share morphogenetic traits different from the cells in neighboring domains, leads me to believe that divergence of cell cycle length is a consequence of cells having embarked along distinct developmental pathways. Because distinctive traits are evident in late interphase, I believe that this divergence is already well underway by the G₂ phase of cell cycle 14.

It is during cycle 14, the same in which this complex mitotic pattern first appears, that Illmensee (1978), Simcox and Sang (1983), and Technau (1987) demonstrated, by transplantation experiments, the existence of some determined cell types. Their experiments imply that nuclei, that began cycle 14 totipotent, are ‘determined’ cells less than one hour later – determined in the sense that transplantation to ectopic locations can no longer divert them from their chosen developmental program. To make this transition, a cell must convert an early gene expression state, that is easily guided toward various destinations, into an eventual gene expression state that is irreversible in the face of significant perturbation. Surely, this transition cannot be instantaneous. The spatiotemporal pattern by which segmentation gene expression waxes and wanes, revealed recently by work in many labs, suggests that *Drosophila* cells acquire ‘determination’ during a process that spans about one hour. Towards the end of this period, gastrulation and germ band elongation commence. To bring about these morphogenetic rearrangements, different patches of cells must act differently. I believe that the differently specialized actions of different mitotic domains, which must result from differences in gene expression states, will turn out to be consequences of determination in progress, possibly not yet irreversible. That is, given the fast pace of early *Drosophila* development, cells may begin to act differentially even before they acquire an irreversible gene expression state. I believe that the mitotic domains will turn out to forecast domains of eventual cell determination. Obviously, rigorous experimental testing will be necessary to correlate the time when cells become determined as members of each domain with the time when the same cells become robustly determined according to the classical tests of commitment – retaining their identity in the face of transplantation to foreign environments.

4.5 The mitotic domains will facilitate molecular studies of morphogenesis

The complex morphogenetic movements that take place in the early embryo result because of region-specific specialization in cell function. In *Drosophila*, soon after cellularization in mid-to-late cycle 14, the simple cell sheet of the blastoderm begins reorganizing itself into the more complex three dimensional structure of the gastrula. I observe that at least some of the mitotic domains function as morphogenetic units in this reorganization. For example, all of the cells in a particular domain may undergo a change in cell shape, or undergo an oriented mitosis, or form an invaginating cell sheet. The screening of *Drosophila* embryos for spatially regulated transcription patterns (such as by O’Kane and Gehring, 1987), is likely to identify genes whose expression coincides with individual mitotic domains. Some of these genes will be those that organize the cell biological machinery responsible for the morphogenetic behavior characteristic of that domain. This opens an exciting research direction: if we can assess the changes in the mitotic domain atlas, and changes in action and interaction of domains in the many mutant *Drosophila* embryos now available, in which known early-acting genes are missing or aberrant, then we will attach specific genetic handles to specific developmentally important cellular functions.

It is very important to note that embryos that lack the product of the gene called *string* do not divide following mitosis 13. Following cellularization in cycle 14, *string* embryos nevertheless undergo a surprising degree of morphogenesis and tissue differentiation (my unpublished observations). This fact proves that mitosis *per se* is not requisite for commitment. What then is the reason for the apparently tight choreography of the cycle 14 mitotic domains – both their mitotic schedule and the synchrony of this schedule with respect to the morphogenetic movement schedule? My guess is that this choreography is essential to confine mitoses to times when cells are ‘off duty’ morphogenetically. The total reorganization of the cytoskeleton during mitosis probably precludes a mitotic cell from contributing usefully to morphogenetic mechanics. Thus, mitosis in a particular domain at the wrong moment could be catastrophic, whereas no division at all (as in *string* mutants) may have no dramatic consequence for the
spatiotemporal patterning of mitosis, whose most
observable side effect is this paper's mitotic domain atlas, may serve only to keep this cell-generation task from interfering with various actions cells must perform in order to build the larval body.

4.6 Does cell–cell communication influence cycle 14 mitotic pattern?

Though different mitotic domains exhibit different cell cycle lengths on average, it is striking that inside each domain, one, or a small number of cells, enters mitosis ahead of their neighbors. Prior to cellularization, in division cycles 10 to 13, mitotic waves normally start near the two poles of the embryo and spread across the entire embryonic surface (Foe and Alberts, 1983). Following cellularization in cycle 14, mitosis spreads, apparently similarly, wave-like within each mitotic domain. But, whatever mechanism propagates the signal to divide either does not cross domain boundaries, or else cells outside a dividing domain cannot respond to that signal. If, indeed, a mitotic signal propagates, the propagation mechanism through the cycle 13 syncytium is likely different from the mechanism by which the newly formed cycle 14 cells communicate. Gap junctions and other communication channels are known to develop rapidly following cellularization (Rickoll and Counce, 1980). Recent studies on molluscs show that restrictions in cell communication arise concurrently with the establishment of distinct cell fates (Serras and van den Biggelaar, 1987). Such restrictions in intercellular communication might concomitantly act to subdivide large morphogenetic fields into smaller ones by restricting the flow of certain molecules important for morphogenesis. In light of the uninhibited interaction between the early cycle 14 syncytial nuclei, followed by the apparent complex spatial and temporal pattern of cell–cell communication that the existence of patterned mitotic waves suggest, the Drosophila blastoderm would now seem an ideal system for probing experimentally the role of embryonic cell–cell interaction.

4.7 Mitotic domains are the first visible manifestations of prior interaction between maternal gene products and early zygotic pattern genes

One central question of developmental biology that this paper does not address is how each cell in the embryo selects, from the totipotent menu of available choices, those several specific genes for its 'first main course': gastrulation. What is the mechanism by which cells in different regions of the embryo make different choices of gene combinations to express? During the hour preceding division in the first mitotic domain, a complex network of many zygotically transcribed genes, that respond to maternally installed gene products, and that interact with each other, paint upon the newly cellularized blastoderm cell sheet a patchwork pattern of gene product accumulation. Many different genes participate: several sequential tiers of segmentation genes, dorsal-ventral genes, homeotic genes, etc. (reviewed in Akam, 1987; Anderson, 1987). There is evidence that considerable 'cross-talk' occurs between these classes of genes, i.e. some segmentation genes influence the patterning of some dorsal–ventral genes, and vice versa (Carroll et al. 1987). Some of these genes (e.g. hairy) are transiently expressed, serving, apparently, only to orchestrate the expression pattern of other genes, then turn off (Carroll et al. 1988). Others must be present for the entire duration of a particular morphogenetic activity, for example engrailed during segmentation (DiNardo et al. 1985).

If the accumulating product of each early pattern gene contributed to the cells that expressed it a unique color, then, just prior to gastrulation, the blastoderm sheet of cells, each cell with a mix of colors, would appear clothed in an exceedingly complex plaid. By mechanisms not yet understood, this tapestry of gene products prefigures the larva's body. How does the blastoderm cell sheet translate this two-dimensional map of patterned protein expression into the three-dimensionally complex larval body, which is the same cell sheet dramatically deformed, folded, and rearranged? Coherent cell action is required – I mean mechanical morphogenetic action by different groups of cells performing mechanically different tasks. It must be that the special combination of genes that cells in a particular group express impels them to act at a particular time in a particular way. I believe that the various specialized actions that certain mitotic domains exhibit represent the earliest detectable morphogenetic consequences of the dynamic genetic determination process.

Probably because my observation techniques have limited resolution, and because I do not know exactly what to look for, I cannot detect visually that every mitotic domain does something unique and interesting, so I am extrapolating, from the fact that some domains do, to the hypothesis that every domain represents a group of cells, with at least temporarily common gene expression states, collectively performing some purposeful larval construction task. Preliminary studies of mutant embryos confirm the genetic control of these domains by early-acting genes (my work in progress; see also Zusman and Wieschaus, 1988). The challenge this presents is to discover which are the important genes (colors) that endow each interesting square of the plaid with its instructions. Cataloging the morphogenetic performance of various domains produces a list of consequences, and cataloging gene expression patterns will produce a corresponding list of potential causes. In this way the mitotic domains will hopefully provide a useful new tool with which to study the genetic control of embryogenesis, perhaps uniquely useful because they provide so early a glimpse of the emerging pattern and its morphogenetic consequences.

In summary, the atlas of mitotic domains this paper presents raises challenging questions. If a mitotic
domain represents a group of cells newly committed toward some particular larval fate, then that commitment may involve (or consist entirely of) new expression of a gene, or combination of genes, unique to that domain. If this is true in general, then each mitotic domain represents four questions in search of molecular answers.

1. What mechanism orchestrates mitotic synchrony in each domain?
2. What specific combination of gene products, among all those expressed within a domain, uniquely defines that domain?
3. What combinations of prior gene expression initiates expression of those domain-specific genes?
4. What cellular actions do those domain-specific genes orchestrate?

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