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Pattern formation in the *Drosophila* embryo

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Three plausible hypotheses about developmental commitments in the *Drosophila* embryo propose that: (1) a micromosaic of localized determinants in the egg trigger somatic commitments; (2) monotonic anterior–posterior and dorsal–ventral gradients in the egg specify positions by a series of threshold values; (3) sequential subdivision of the early embryo into 'anterior' or 'posterior' 'middle' or 'end', 'dorsal' or 'ventral', 'odd' or 'even' compartmental domains encodes the somatic commitment in each region in a combinatorial epigenetic code. Evidence in favour of such a combinatorial code includes its capacity to account for major features of transdetermination and for many single and coordinated homoeotic transformations. In particular, both these metaplasias often cause transformations between ectodermal tissues such as antenna and genitalia, whose anlagen lie far apart on the blastoderm fate map. This phenomenon is not naturally explained by monotonic gradient models. In contrast, not only transformation between distant regions of the fate map, but also the observed geometries of compartmental boundaries on the wing, and probable ones in the early embryo, are naturally explained by reaction–diffusion models. These systems form a discrete succession of differently shaped monotonic and non-monotonic eigenfunction gradient patterns of the same morphogens, as the tissue containing the chemical system changes in size and shape, or in other parameters. The successive mirror symmetries in non-monotonic gradients predict that distant regions of the embryo make similar developmental commitments, and also predict specific classes of pattern mutants forming mirror symmetric structures along the embryo on a variety of length scales. Finally, reaction diffusion systems spontaneously generate transverse gradients of the underlying chemicals when more than one eigenfunction is amplified at once, and therefore specify two-dimensional positional information within domains.

Although it is attractive, no feature of the combinatorial code hypothesis is verified. Current data relating to whether the sequential formation of compartmental boundaries actually reflects the commitment of the two isolated 'polyclones' to alternative fates, whether any genes act continuously to maintain disc commitments, and whether homoeotic mutants actually 'switch' disc determined states, are assessed.

Drosophila fate map and metaplasias

Over the past decades of investigation, the fundamental questions of developmental biology have remained those of cellular differentiation and pattern formation. Insights with respect to both these problems have been gained in the past several years in studies of *Drosophila melanogaster*. My aim will be to review briefly the kinds of data that initially led to the formulation of current hypotheses, discuss new experiments that bear on them, and describe new efforts to extend the present models.

Drosophila is a holometabolous insect, with egg, larva, pupa and adult stages (Gehring & Nöthiger 1973). The egg is about 500 μm long. During 13 cleavage divisions, nuclei form a syncytium. By the 9th division, nuclei migrate to the cortex of the egg. After the 13th, cell

membranes extend from the oolemma down, then beneath each nucleus to create the cellular blastoderm, which is a two-dimensional ellipsoidal sheet of about 6500 cells (Turner & Mahowald 1976). Shortly after the blastoderm stage, at 3.5–4.0 h at 25 °C, gastrulation, germ band extension and retraction ensue, following which the segmented embryo is visible (Turner & Mahowald 1977). After hatching, at about 24 h the larva undergoes three instars in five days, pupates and emerges as an adult several days later (Turner & Mahowald 1977, 1979).

The adult ectoderm of *Drosophila* derives during metamorphosis from different two-dimensional hollow sacks of cells, the imaginal discs, and groups of cells forming histoblasts (Gehring & Nöthiger 1973; Gehring 1976). There are 12 bilaterally symmetric pairs of major imaginal discs, and a single fused genital disc. Each is known to carry a tissue heritable commitment to form a specific adult cuticular region during metamorphosis; eye–antenna, prothorax, mesothorax and wing, metathorax, pro-, meso- or metathoracic leg, genital. The adult abdomen derives from the histoblasts.

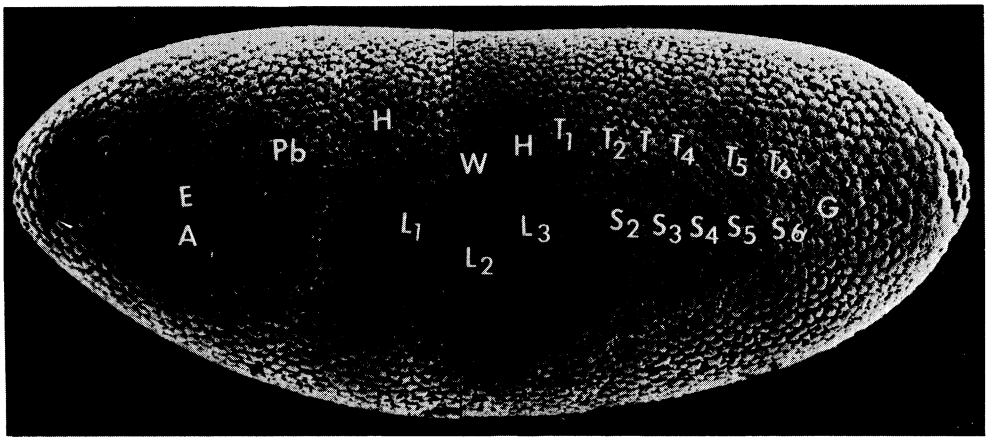

FIGURE 1. Fate map of blastoderm projected onto scanning electron micrograph (magn. ×600) of a *Drosophila* egg just after completion of blastoderm and onset of cephalic furrow formation. Pole cells visible at posterior pole. A, antenna; E, eye, Pb, proboscis; H, humerus; W, wing–thorax; H, haltere; L1–L3, prothoracic, mesothoracic and metathoracic legs; T1–T6, first to sixth abdominal tergites; S2–S6, second to sixth abdominal sternites; G, genital.

From the use of gynandromorphs (Garcia-Bellido & Merriam 1969; Hotta & Benzer 1972; Janning 1978; Merriam 1978), and direct experiments based on microdeletion (Bownes & Sang 1974*b*), microcautery (Bownes & Sang 1974*a*; Bownes 1975), microturbulence (Bedian *et al.* 1981) and localized ultraviolet irradiation (Lohs-Schardin *et al.* 1979*a*, *b*), a detailed fate map of the anlagen for adult ectodermal structures on the blastoderm has been constructed (figure 1). Adult ectodermal derivatives are formed from bilateral bands of cells extending along the lateral equator of the egg from about 20% egg length to about 80% egg length, measured from the posterior pole. At the bastoderm stage, the sagittal midline contains about 85 cells from pole to pole, and one segment's anlagen comprises a length of about 3.5 cells along this axis (Schubiger & Newman 1981).

The existence of a blastoderm fate map poses in *Drosophila* the problem of positional information in embryogenesis. What processes in the egg cue nuclei in diverse areas to adopt different developmental fates? Three current hypotheses are discussed below. The first major point to stress is that the fate map is two-dimensional, and homologous to the adult: head, thorax, abdomen and genitalia map from anterior to posterior on the egg and adult. One critical

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feature of this homology is that the two major metaplasias in *Drosophila*, homoeotic mutants and transdetermination, do not respect it in any simple way.

Transdetermination, analysed extensively since 1966 (Hadorn 1966; Gehring 1967, 1976), is observed when imaginal discs or disc fragments are cultured in adults and then injected into larvae for metamorphosis. At moderate frequencies a disc normally destined for one fate forms normal adult derivatives of another disc. There are now good grounds to believe that transdetermination can arise in more than one cell at a time, that it is an all-or-none phenomenon, that the new determined state is as fully heritable as the old, and that transdetermination does not reflect somatic mutational events, but heritable alterations in relative stable epigenetic states. Figure 2 summarizes the patterns of transdeterminations seen when fragments of each type of disc are cultured. The major features are that any disc can transdetermine into only a few of the other possible disc types in a single step, that sequences of transdetermination are observed, that most steps are reversible with asymmetric frequencies, and that single-step transdeterminations can occur between discs whose anlagen are distant from one another on the blastoderm fate map. I here emphasize the last property. Genital discs can transdetermine directly to antenna, which lies at the opposite end of the egg, yet cannot transdetermine directly to mesothorax, which lies between antenna and genital on the fate map.

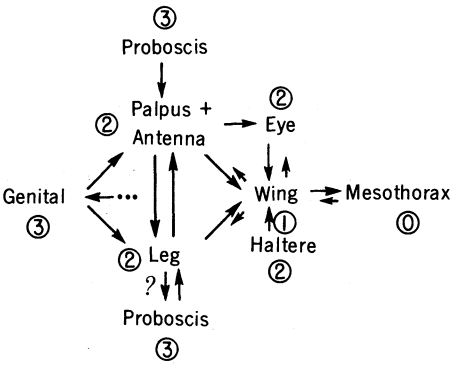

FIGURE 2. Observed transdertermination steps from each disc. Lengths of arrows represent the relative probabilities of transitions. Circled numbers are the minimum number of transdetermination steps to mesothorax. Each step toward mesothorax is more probable than its inverse.

Homoeotic mutants exhibit many of the properties shown in transdetermination; in particular, many cause transformations between non-neighbouring domains on the blastoderm fate map (reviewed by Ouweneel 1976). Among the critical features of homoeotic mutants are the following. (1) Most observed transdetermination steps are also known homoeotic conversions. (2) Most, but not all, homoeotic mutants convert any single affected tissue into a single different tissue. (3) A number of homoeotic mutants coordinately transform two tissues into two different tissues. For example, the dominant mutant *Nasobemia* can coordinately convert antenna to mesothoracic leg (Gehring 1966), and eye to wing (Stepshin & Ginter 1972); strong alleles of *engrailed* coordinately transform posterior compartments of wing, metathorax, legs, and head to mirror-image anterior compartments (Morata & Lawrence 1978; Lawrence & Morata 1979). (4) Finally, a significant number of homoeotic mutants cause transformation between distant regions on the fate map. *Tumorous head* (*tuh1,3*) transforms head to distal abdomen and genitalia (Postlethwait *et al.* 1972); *lethal(3)III–10* transforms genitalia to antenna or leg (Shearn *et al.* 1971); *Nasobemia* transforms antenna to mesothoracic leg, jumping past prothoracic structures.

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Other homoeotic mutants cause transformations between adjacent regions. For example, *Hexaptera* and most members of the *bithorax* complex (Lewis 1978) cause transformations between adjacent thoracic or abdominal segments.

These well known facts point to a fundamental tension, which must be adequately addressed by any hypothesis about *Drosophila* development. Those tissues that transform into one another in transdetermination or homoeotic mutants must, in some fundamental sense, have 'neighbouring', developmental programmes. The fact that transdetermination and homoeotic mutants can jump large distances across the fate map implies that distant regions can have neighbouring developmental programmes. Neighbourliness of programmes does not map simply onto the neighbourliness of anlagen on the two-dimensional fate map. One useful strategy in constructing alternative hypotheses about positional information processes in the *Drosophila* embryo is to seek those that may naturally explain both kinds of neighbourliness.

Models of positional information in the *Drosophila* egg

Currently, there are three alternative types of hypothesis concerning positional information in the *Drosophila* embryo: micromosaic models, monotonic gradient models, and models based on the sequential formation of monotonic and then non-monotonic gradients.

Micromosaic models are based on the plausible hypothesis that determinants for specific anlagen are prelocalized in the egg cortex before fertilization, and instruct nuclei that migrate to the cortex to adopt particular developmental fates. There is unambiguous evidence for prelocalized determinants of germ cell formation in the posterior pole plasm. Transplantation of pole plasm to the anterior pole or lateral flanks of genetically marked host eggs induced pole cell formation by anterior or lateral nuclei (Illmensee & Mahowald 1976). No evidence supports the hypothesis that specific prelocalized determinants instruct somatic nuclei to adopt fates restricted to single segments. In particular, attempts to find maternal effect mutants with this property have failed (Rice & Garen 1975). Although the micromosaic models cannot be formally excluded, they seem highly unlikely at present.

A number of workers have proposed that monotonic gradients of one or a few substances underlie anterior–posterior and dorsal–ventral axes in *Drosophila* development (Sander 1975; Meinhardt 1977; Deak 1980; Slack 1980). Evidence in favour of this class of models has been carefully reviewed by Sander (1977, 1980). Monotonic gradient models, as a class, assume that a series of successively higher threshold values of the anterior–posterior or dorsal–ventral gradient variables, trigger nuclei (or cells) at different positions along the anterior–posterior or dorsal–ventral axis of the egg to adopt successive segmental or dorsal–ventral assignments. Monotonic gradient models have impressive simplicity and explanatory strength. It is now understood that chemical reaction–diffusion systems are capable of generating the requisite monotonic gradients in the embryo. Monotonic gradients explain two major classes of data in insect embryos. (1) In *Euscelis*, displacement of a particulate posterior mass to defined positions along the longitudinal egg axis yields expected mirror symmetrical reversals of segments with respect to the mass (Sander 1975). (2) Ligation of Drosophila eggs at different stages of cleavage results in formation of the anteriormost and posteriormost segments of first instar larvae, with a gap of missing segments centred around the location of the ligation (Schubiger & Wood 1977; Schubiger *et al.* 1977; Vogel 1977). As the time of ligation approaches the cellular blastoderm stage, the gap narrows to a single segment. Ligation in the anterior half egg shifts the fate of

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cells in the posterior half to more posterior segments, while the posterior half forms fewer, but larger, segments. Disruption of the bilayer membrane that forms across the egg during ligation, and isolates the two half-eggs, restores normal segmentation, suggesting that interaction between the half-eggs is necessary to establish middle segments (reviewed in Schubiger & Newman 1981).

To account for the gap phenomenon, Meinhardt (1977) has proposed that ligation creates a diffusive barrier. The axial gradient becomes flatter in the anterior and posterior isolated half-eggs, and steep across the ligation barrier. Flattening of the gradients in each half-egg yields fewer, broader segments, preservation of the most anterior and posterior segments, and a gap of missing segments.

Despite these strengths, monotonic gradient models have several weaknesses. One fundamental failure of this class of models is that they can provide no natural explanation for the fact that homoeotic mutants and transdetermination jump large distances across the fate map. It is not obvious why genital and antenna, at opposite ends of the fate map, might have neighbouring developmental programmes. Other difficulties with this class of models will be discussed below.

The combinatorial code hypothesis

Several bodies of concepts and experiments suggest that in metazoans and insects, and in *Drosophila* in particular, different tissues might encode different developmental commitments by unique combinations of states in a number of functionally independent genetic subsystems.

1. The most general ideas derive from current data on overlapping patterns of gene expression in different cell types. Estimates of the number of genes in complex metazoans ranges from about 5000 to 6500 (Lewin 1974; Levy *et al.* 1977) in *Drosophila* to about 50000 in mammals and higher plants (Hastie & Bishop 1976). A typical mammalian cell appears to coordinate the expression of about 15000–25000 distinct mRNA sequences in its polysomal fraction. In mammals and higher plants, the differences in patterns of gene expression in different cell types ranges from about 5% to 40%, and corresponds to thousands of sequences (Hastie & Bishop 1976; Axel *et al.* 1976; Kamalay & Goldberg 1980; Levy & McCarthy 1975; Chikaraishi *et al.* 1978). Different cell types typically display overlapping patterns of gene expression, with a large core of ubiquitous shared sequences. The existence of such overlapping patterns raises the possibility of relatively independent, parallel programmes of gene expression. Recent evidence in favour of this general hypothesis has become available in analyses of patterns of protein synthesis with the use of two-dimensional gel electrophoresis. For example, compared with normal myeloid cell lines, leukaemic sublines shown alterations in the patterns of synthesis of 92 or more proteins among 450 resolvable spots, in independent, parallel, and partly overlapping sets (Lieberman *et al.* 1980).

2. Gierer (1973) was the first worker to propose a general combinatorial scheme to encode differentiated states in metazoans. He pointed out the enormous regulatory economy achieved in combinatorial systems, since a few master genes in alternative states of activity could in principle encode a large number of alternative commitments, and coordinately regulate large batteries of genes. Much the same general idea lies behind the well known Britten–Davidson formal models of gene regulation (Britten & Davidson 1969; Davidson & Britten 1979), and also emerges naturally in considerations of the expected structure and dynamics of large genetic regulatory systems (Kauffman 1974).

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3. The initial application of a combinatorial model specifically to *Drosophila* development derived from the union of four simple ideas (Kauffman 1973). First, developmental commitments in imaginal discs occur between heritable alternatives. Presumably these heritable alternatives are carried in some genomic machinery, for example as alternative stable states of coupled gene activities like that seen in the bistable C_1–cro feedback loop in λ phage (Neubauer & Calef 1970; Thomas 1978). If each developmental commitment is a choice between only a few alternatives, minimally two, then encoding a large number of commitments requires a number of different gene feedback loop systems. It follows that any committed state must necessarily be encoded by combinations of states of the underlying genetic loops. Secondly, the hypothesis that imaginal disc determined states are encoded by combinations of states of several master gene systems immediately accounts for the existence of allowed and forbidden one-step transdeterminations, and sequences of transdeterminations, as one change, and multiple changes in the underlying combinations of master gene states. Thirdly, if most homoeotic mutants act by switching an underlying master gene system from one to another stable state, as temperature-sensitive mutants of C_1 do in the C_1–cro loop (Neubauer & Calef 1970; Thomas 1978; Furth 1980), then most homeotic mutant conversions should be observed transdetermination one-step transitions. Conversely, if in transdetermination any of the underlying master gene systems might 'wobble' to its alternative state, but any homoeotic mutant affects only a single gene system, then, as observed, the pattern of single step transdetermination steps from any disc should be broader than, but inclusive of, each homoeotic transformation from that disc. Fourthly, if disc-determined states are encoded by combinations of underlying genetic subsystems, then, typically, one state of each underlying master gene system will occur in one subset of discs, and its alternative state will occur in the complementary subset of discs. A combinatorial code model inherently predicts the existence of classes of mutants coordinately affecting subsets of discs, and, more strongly, classes of mutants coordinately affecting complementary subsets of discs. The existence of eight classes of mutants affecting four complementary subsets of discs supplied early independent evidence in favour of a combinatorial model. Applied to homoeotic mutants, a combinatorial model predicts that some homoeotic mutants will cause a coordinated transformation of more than one disc, or encoded region, at once. As noted above, examples include *Nasobemia*, which coordinately transforms eye to wing and antenna to mesothoracic leg; and strong alleles of *engrailed*, which coordinately transform posterior wing, metathoracic leg, and head compartments to mirror-image anterior compartments.

4. Sequential compartmentalization during imaginal disc development, discovered by Garcia-Bellido *et al.* (1976), has supplied a further important body of evidence in favour of a combinatorial model in *Drosophilia* development. Sequential formation of compartmental boundaries of clone restriction in the wing disc (Garcia-Bellido 1975; Garcia-Bellido *et al.* 1976), leg discs (Steiner 1976), genital disc (Dubendorfer 1977), and, perhaps, eye–antenna disc (Baker 1978; Morata & Lawrence 1978), have been thoroughly discussed. In figure 3 I project the known boundaries on the wing and thorax onto the fate map of the third-instar wing–thorax disc. Under the assumption that a compartmental boundary reflects a developmental commitment, the geometries of the compartmental boundaries suggest that a terminal wing disc compartment is characterized by a unique combination of alternative commitments, e.g. 'anterior' not 'posterior'; 'dorsal' not 'ventral'; 'wing' not 'thorax'; 'proximal' not 'distal'. I shall discuss more critically below the evidence bearing on the interpretation that compartmental boundaries in fact reflect development commitments.

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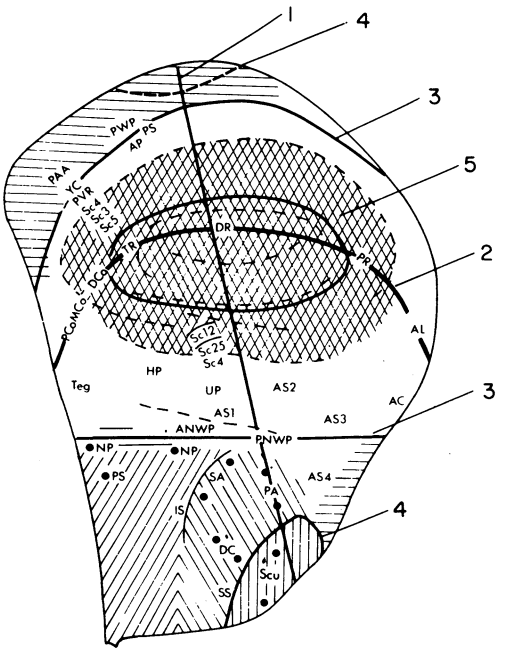

FIGURE 3. Five compartmental boundaries that arise successively on the growing wing-thorax disc: (1) anterior–posterior; (2) ventral–dorsal; (3) wing (midregion of disc)–thorax (two end regions of disc); (4) scutum–scutellar boundaries; (5) proximal–distal wing boundary. Fate map from Bryant (1975). Abbreviations for wing disc pattern elements: PST, presutural bristle; NP, notopleural bristles; SA, supraalar bristles; PA, postalar bristles; DC, dorsocentral bristles; Scu, scutellar bristles; ANWP, PNWP, anterior and posterior notal wing processes; Teg, tegula; HP, humeral plate; UP, unnamed plate; AS1–AS4, first to fourth axillary sclerites; Pco, Mco, Dco, proximal, medial, and distal costa; TR, triple bristle row (anterior wing margin); DR, double bristle row (distal wing margin); PR, posterior row of hairs; Sc4, Sc25, Sc12, groups of sensilla companiformia on the proximal dorsal radius; AL, alar lobe; AC, axillary cord; PAA, prealar apophysis; YC, yellow club; PVR, proximal ventral radius; PWP, pleural wing process; PS, pleural sclerite; AP, axillary pouch, Sc4, Sc3, Sc5, groups of sensilla campaniformia on the proximal ventral radius.

SEQUENTIAL COMPARTMENTAL MODEL APPLIED TO THE EARLY EMBRYO

The sequential formation of compartmental boundaries in imaginal discs raises the possibility that similar compartmental events might sequentially subdivide the early embryo. Before discussing the current evidence for such sequential events, I describe a particular hypothetical sequence, which offers a natural explanation of the relation between neighbouring positions on the fate map and the neighbouring character of developmental programmes in regions which can be distant from one another on the fate map. In figure 4 I show a sequence of four compartmental events. The first divides the egg into 'anterior' and 'posterior' halves. The second, by analogy with the two boundaries on the wing disc dividing the disc into a central wing region and end thoracic regions, divides the early egg into two distal 'end' regions and a central 'middle' region. The third event creates a single dorsal ventral boundary creating 'dorsal' and 'ventral' egg regions. The fourth event simultaneously forms four compartmental boundaries creating 'even' and 'odd' subdomains. Each of these alternative compartmental commitments is assumed to be maintained by different master gene systems in one of two alternative states (1 or 0), such that a specific domain, e.g. 'anterior', 'middle', 'dorsal', 'odd', corresponds to a specific disc, here the mesothoracic disc.

The explanatory strengths of this model have been discussed more fully elsewhere (Kauffman

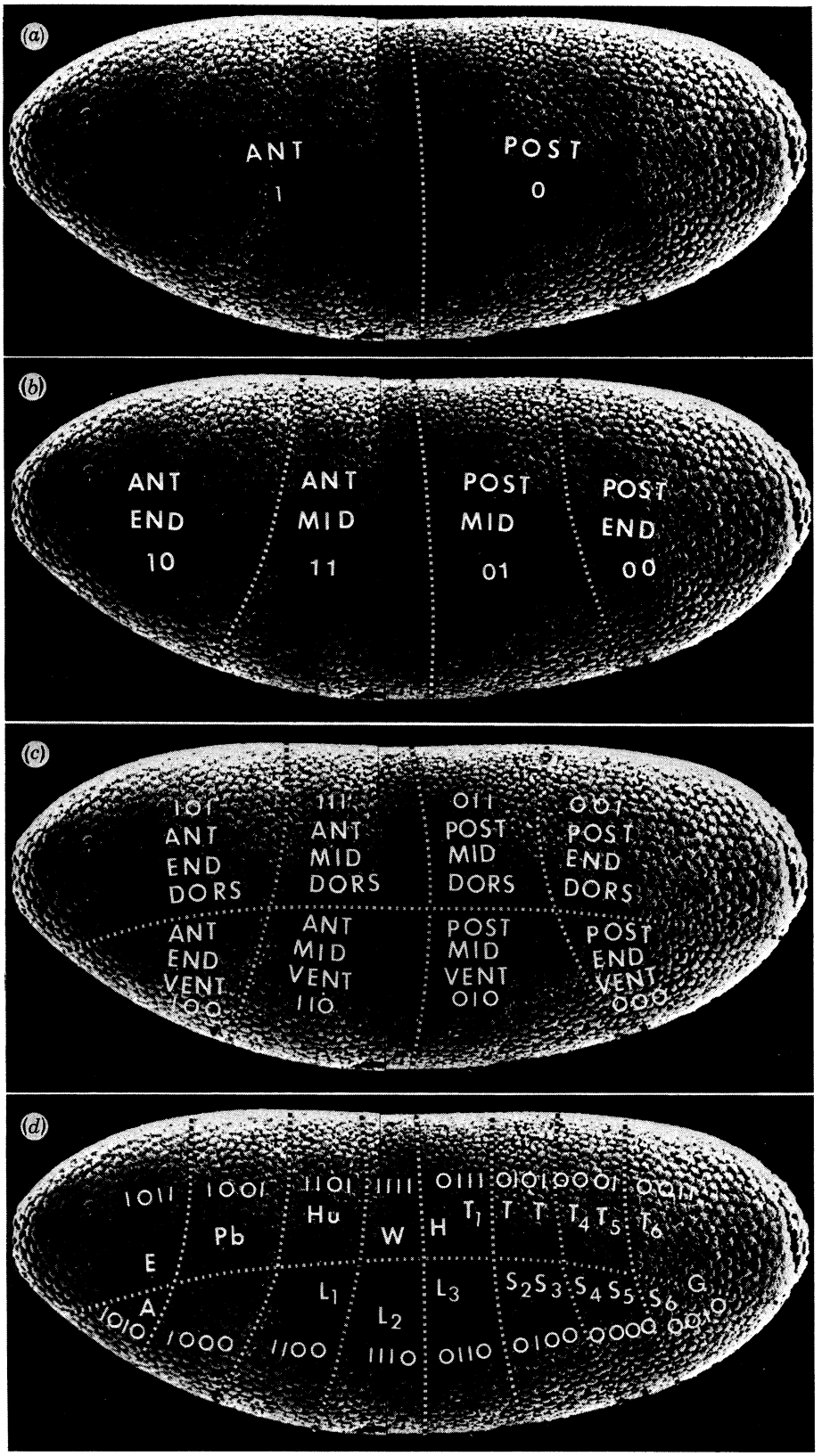

FIGURE 4. Four hypothetical compartmental events successively dividing the egg. (*a*) The first divides the egg into anterior–posterior half-egg domains; (*b*) a second event forms a pair of compartmental boundaries defining end and middle compartments; (*c*) the third event creates a dorsal–ventral boundary; (*d*) the fourth event creates four compartmental boundaries defining even and odd compartments. Each terminal compartment carries a record of the alternative commitments taken during its formation, e.g. 'anterior', 'middle', 'odd', 'dorsal' (1111) = mesothorax. The combinations of alternative commitments constitute an epigenetic code.

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1973; Kauffman *et al.* 1978) and are briefly summarized here in the model's capacity to account for most observed patterns of transdetermination, and many known homoeotic mutants (tables 1 and 2). For example, the model correctly predicts that haltere should transdetermine to wing more frequently than to antenna, since both transitions share one decision alteration, but the haltere to antenna transition requires two further switch changes. Mutation of the 'end' decision (0) to the middle (1) state in antenna and eye convert antenna to mesothoracic leg, and eye to wing, as seen in *Nasobemia*. Mutation of the first 'anterior' decision (1) to the posterior state, converts head to genital, at the other end of the fate map, as observed in *tuh1,3*.

TABLE 1. PREDICTED RELATIVE TRANSDETERMINATION FREQUENCIES DERIVED FROM THE CHEMICAL WAVE MODEL APPLIED TO THE BLASTODERM

($L_{1,2} \rightarrow A > L_{1,2} \rightarrow G$ means that the model predicts that transdetermination from the first or second leg to antenna is greater than to genital. Abbreviations are explained in the legend of figure 1. T, true; F, false.)

prediction	status	prediction	status	prediction	status
$H \rightarrow W > H \rightarrow A$	T	$A \rightarrow W > A \rightarrow H$	T	$L \rightarrow W > L \rightarrow E$	T
$H \rightarrow W > H \rightarrow L_{1,2}$	T	$A \rightarrow L > A \rightarrow W$	F	$L_{1,2} \rightarrow W > L_{1,2} \rightarrow H$	T
$H \rightarrow W > H \rightarrow E$	T	$A \rightarrow Pb > G \rightarrow Pb$	?	$L > A > L \rightarrow E$	T
$H \rightarrow W > H \rightarrow Pb$	T	$A \rightarrow E > A \rightarrow W$	F	$L_{1,2} \rightarrow A > L_{1,2} \rightarrow G$	T
$W \rightarrow A > H \rightarrow A$	T	$A \rightarrow G > L_{1,2} \rightarrow G$	T	$L_2 \rightarrow G > L_3 \rightarrow A$	?
$W \rightarrow E > H \rightarrow E$	T	$A \rightarrow E > E \rightarrow A$	T	$L_1 \rightarrow Pb > L_1 \rightarrow G$	?
$W \rightarrow L_{1,2} > H \rightarrow L_{1,2}$	T	$A \rightarrow L_2 > L_2 \rightarrow A$	?T	$G \rightarrow A > G \rightarrow Pb$	T
$W \rightarrow L > W \rightarrow A$	T	$E \rightarrow W > E \rightarrow H$	T	$G \rightarrow A > G \rightarrow W$	T
$W \rightarrow L > W \rightarrow G$	T	$E \rightarrow A > E \rightarrow G$	T	$G \rightarrow L_{2,3} > G \rightarrow W$	?T
$W \rightarrow A > W \rightarrow G$	T	$E \rightarrow A > E \rightarrow L$	T	$G \rightarrow A > G \rightarrow L_{1,2}$	?T
$W \rightarrow E > W \rightarrow Pb$	T	$E \rightarrow W > E \rightarrow L$	T	$G \rightarrow A > A \rightarrow G$	T
$W \rightarrow E > W \rightarrow G$	T			$G \rightarrow L > L \rightarrow G$	T
$W \rightarrow E > W \rightarrow A$	?			$G \rightarrow H > G \rightarrow W$	?F

A fundamental property of any combinatorial model is that it supplies a natural measure of how close neighbours two developmental programmes are, in terms of the number of 'decisions' that must be changed to convert one to another. The point that I wish to stress here is that the success of this particular combinatorial scheme lies in the fact that the hypothetical sequence of compartmental events allows both adjacent and also distant domains on the blastoderm to have neighbouring developmental programmes. For example, head and genital, encoded as ('anterior' 'end' 'dorsal' 'odd') and ('posterior' 'end' 'dorsal' 'odd') respectively, differ only in the initial 'anterior'–'posterior' decision. Further the particular sequence and geometries of compartmental boundaries make clear suggestions about the geometries of the underlying positional system that generates compartmental boundaries. In a subsequent section I shall show briefly that reaction–diffusion models of the type that can generate monotonic gradients also generate a succession of monotonic and non-monotonic gradients that appear to have the proper geometries to trigger sequential compartmental events, and have the surprising added property of generating transverse gradients that can supply two-dimensional positional information within each compartment.

CURRENT STATUS OF DATA ON THE SEQUENTIAL COMPARTMENTAL COMBINATORIAL CODE MODEL

The hypothesis about *Drosophila* development that emerges from considerations of transdetermination, many homoeotic mutants and sequential compartmentalization is this: during

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TABLE 2. OBSERVED HOMOEOTIC TRANSFORMATION AND THE CODE CHANGES REQUIRED FOR THE CODE SCHEME IN FIGURE 4*d*

mutant	symbol	transformation	coordination	code change	switches required	references
Antennapedia[1]	Antp	antenna → leg 2	—	1001→1101	1	*a*, *c*
Pointed wing	Pw	antenna → wing	—	1001→1111	2	*c*
Nasobemia	Ns	antenna → leg 2	parallel	1001→1101	1	*e*
		eye → wing		1011→1111	1	*f*
dachsous	ds	tarsus → arista	—	1101→1001	1	*c*
Opthalmoptera[2]	Opt^G	eye → wing	—	1011→1111	1	*c*
Hexaptera	Hx	prothorax → mesothorax	—	1011→1111	1	*c*
podoptera	pod	wing → leg		1111→1101	1	*c*
tetraltera[3]	tet	wing → haltere	—	1111→0111	1	*c*
Contrabithorax	Cbx	wing → haltere	parallel	1111→0111	1	*c*
		leg 2 → leg 3		1101→0101	1	*d*
Ultrabithorax	Ubx	haltere → wing	parallel	0111→1111	1	*d*
		leg 3 → leg 2		0101→1101	1	*c*
tumorous head	tuh 1,3	eye → genital	parallel	1011→0011	1	*g*
		antenna → genital	divergent	1001→0001	1	*c*
		antenna → leg		1001→1101	1	*g*
lethal (3) III–10	l(3)III–10	haltere → wing	parallel	0111→1111	1	*b*
lethal(3)XVI–18	l(3)XVI–18	genital → antenna	divergent	0001→1001	1	*c*
		genital → leg		0001→0101	1	*b*
lethal(3)703	l(3)703	antenna → leg	parallel	1001→1101	1	*b*
lethal(3)1803R	l(3)1803R	genital → leg	divergent	0001→0101	1	*c*
		genital → antenna		0000→1001	1	*c*
		haltere → wing	parallel	0111→1111	1	*c*
proboscipedia	pb	proboscis → antenna	divergent	1000→1001	1	*a*
		proboscis → leg		1000→1100	1	*c*
extrasexcombs[4]	ecs	leg 2 → leg 1	convergent	1101→1100	1	*c*
		leg 3 → leg 1		0101→1100	2	*c*
Polycomb	Pc	antenna → leg 2	1001→1101	1001→1101	1	*c*
lethal(4)29	l(4)29	leg 2 → leg 1	convergent	1101→1100	1	*a*
		leg → leg 1		0101→1100	2	*c*

A set of homoeotic mutants causing the same transformation is represented by one member: [1] Antennapedia, Antennapedix, aristapedia, aristatarsia; [2] Opthalmoptera, opthalmoptera, eyes-reduced; [3] tetraltera, Metaplasia, Haltere mimic; [4] extrasexcombs, Extrasexcomb, reduplicated sex comb, sparse arista. (From Kauffman *et al.* (1978).) References: (*a*) Gehring & Nöthiger (1973); (*b*) Shearn *et al.* (1971); (*c*) Ouweneel (1976); (*d*) Lewis (1978); (*e*) Gehring (1966); (*f*) Stepshin & Ginter (1972); (*g*) Postlethwait *et al.* (1972).

early embryogenesis, the egg is sequentially subdivided into finer subdomains by a series of compartmental events. Each compartmental event reflects the assumption of the two isolated polyclones (Crick & Lawrence 1975) of two alternative developmental commitments. A terminal compartment carries a commitment encoded by a unique combination of the alternative commitments taken during its formation. Each commitment, once taken, is maintained thereafter by the continued activity of a master gene system in one of two alternative states of activity. Transdetermination and at least some homoeotic mutants act by altering the states of activity of the master gene systems carrying the committed states. Despite the attractiveness of the combinatorial model, none of these proposals is at present fully substantiated.

Sequential subdivision of the early embryo

Current evidence fairly convincingly suggests that the early embryo is sequentially subdivided. Work in our laboratory, described below, indicates that before blastoderm formation, an initial clonal restriction in the first abdominal segment divides the egg into anterior and posterior compartments (Kauffman & von Allmen 1981). Early lethal mutants recently described by Nüsslein-Volhard & Wieschaus (1980) and Sander *et al.* (1980) form embryos with half the number of segments; each is an apparent fusion of, or failure to separate, two adjacent segments. These mutants strongly suggest that a paired segment stage may be an obligatory intermediate in the formation of the fully segmented embryo. Analysis of clonal restrictions among clones induced at or shortly after the cellular blastoderm stage (Wieschaus & Gehring 1975; Steiner 1976; Lawrence & Morata 1977; Lawrence *et al.* 1978) reveals that at the blastoderm stage, all longitudinal segments are clonally isolated. By about one division after blastoderm, anterior and posterior compartments within the thoracic and leg discs are clonally isolated. By two divisions after blastoderm, dorsal thoracic anlagen are clonally isolated from their corresponding leg disc anlagen. Therefore, after blastoderm, clonal restrictions arise sequentially within segments.

To test whether the clonal restrictions present at the cellular blastoderm arise sequentially, we performed three experiments. We induced clones among 2100 flies in 30 min age windows from 0 to 6 h after oviposition and analysed over 3000 clones to test whether they straddled among longitudinal segments, and, if so, whether they exhibited any longitudinal restrictions before blastoderm; we analysed gynandromorphs to test whether early compartmental events might distort the fate map; and we analysed time-lapse videotapes to see whether organized cleavage cytoplasmic movements might account for preblastoderm clonal restrictions. Analysis of the frequencies of mitotic recombinant clones per fly in irradiated versus control flies substantiated our success in inducing blastoderm clones. By performing Student t tests for the significance of coincidence of clones in all possible pairs of segments, we found that among flies irradiated from 0 h onwards, clones straddled all segments in the anterior half-egg from the head to the first abdominal segment. In the posterior half egg, clones straddled all segments from the metathorax to the 7th abdominal segment (figure 5*a*). Clones did not cross between these anterior and posterior blocks of segments, which overlap in both including the metathorax and first abdominal segments. By about 2–3 h after oviposition, clones in the posterior block ceased straddling beyond the second abdominal segment, yielding a restriction between the first and second abdominal segments (figure 5*b*, *c*).

The apparent preblastoderm clonal restriction between the first and second abdominal

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segments might be exected to be visualized on gynandromorphic fate maps. To test this, we analysed 165 *ysn/Inw*^{vc} (Lindsley & Grell 1967) gynandromorphs, from which we obtained a typical fate map, and looked in detail at male–female mosaics in the abdominal tergites. We found that the frequency of mosaics in the first abdominal segment was more than twice that in other abdominal tergites (table 3). We further distinguished two classes among these

TABLE 3. MOSAICS WITHIN ABDOMINAL SEGMENTS

abdominal segment...	1	2	3	4	5	6
mosaic frequency	0.26	0.11	0.11	0.08	0.11	0.08
A/P mosaic	41	8	6	5	2	2
patchy mosaic	43	27	30	21	33	22
sturt	19.2	6.6	6.4	4.8	5.7	4.0

A/P mosaics, number of instances in left or right half-segment of a mosaic in which the anterior bristles are all of one genotype, while posterior bristles are the other. Patchy mosaic, number of instances of scattered, patchy mosaics. Sturt, sturt distance from anterior to posterior margin of each left or right half-abdominal segment.

mosaics: patchy mosaics in which male and female cells were scattered in a salt–pepper distribution, and anterior–posterior mosaics, in which the anterior margin of a tergite was entirely of one sex, the posterior margin entirely the other. The frequency of such anterior–posterior mosaics was very much higher in the first abdominal tergite (table 3) than in other tergites.

(*a*)	Hd	Pro	Ms	Mt	1	2	3	4	5	6
Pro (iii)	**8.37**									
(ii)	2.85									
(i)	17									
Ms	**3.78**	**7.18**								
	9.41	12.08								
	21	37								
Mt	**3.73**	**4.54**	**3.18**							
	1.88	2.63	7.99							
	7	10	17							
1	*1.34*	*2.16*	*2.23*	3.75						
	5.77	7.91	24.88	5.34						
	9	14	36	14						
2	−0.57	0.32	0.58	**3.67**	**2.72**					
	6.5	9.01	22.92	5.99	18.30					
	5	10	31	15	30					
3	1.73	**3.80**	0.21	*2.39*	**2.55**	**2.83**				
	8.07	11.25	34.76	7.46	22.83	25.67				
	13	24	36	14	35	40				
4	−0.55	0.69	1.88	*2.34*	*2.02*	**3.23**	**4.60**			
	9.72	13.47	41.81	8.98	27.43	31.00	38.44			
	8	16	45	16	38	49	67			
5	1.13	−0.47	*2.27*	**2.94**	*2.19*	**3.15**	**3.56**	**4.01**		
	10.36	14.81	45.67	9.80	30.00	33.71	19.69	50.48		
	14	13	61	19	42	52	65	79		
6	−0.50	1.52	1.66	0.84	1.37	**5.25**	**4.98**	**3.37**	**6.59**	
	7.37	10.16	31.64	6.80	10.31	25.53	29.13	35.28	38.23	
	6	15	41	9	27	49	56	55	79	
7	−0.82	−0.87	−0.33	**2.59**	**3.45**	**3.15**	**2.90**	**3.86**	**5.46**	**4.45**
	3.56	4.92	15.31	10.04	11.37	19.09	17.05	3.29	10.51	12.96
	2	3	14	8	21	22	25	33	42	29

FIGURE 5. Continued opposite.

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The result of both the high frequency of mosaics and the high frequency of anterior–posterior mosaics in the first abdominal segment was that the sturt distance from the anterior to posterior margin within the first abdominal segment in our data was 19, twice to three times the sturt distance within other abdominal tergites (table 3), and comparable with that *between* major thoracic segments in our data.

The simplest consistent interpretation of our data is that an early preblastoderm clonal restriction divides the egg into anterior and posterior domains in the middle of the first abdominal tergite. On this interpretation, the anterior half of the first abdominal tergite is thoracic in origin. This interpretation is supported by the recent discovery that first abdominal-like 'adventitial bristles' reliably map to the posterior margin of the metathoracic disc (Adler 1978).

Time-lapse videotapes of early cleavage reveal that the cytoplasm typically undergoes two types of rhythmic, reversible streaming, probably in association with cleavage divisions: (1) a whole egg flow in which cortical cytoplasm displaces in the posterior direction while the yolk displaces in the anterior direction; (2) two half-egg 'cells' of streaming, which meet at mid-egg, in which the cortical cytoplasm simultaneously displaces from mid-egg toward both poles while yolk displaces from both poles toward the mid-egg, followed by movement in the reverse directions. Figure 6 shows the frequencies with which these types of bilaterally symmetric

(*b*)

	Hd	Pro	Ms	Mt	1	2	3	4	5	6
Pro (iii)	**7.51**									
(ii)	2.39									
(i)	14									
Ms	**2.95**	**5.93**								
	7.13	9.11								
	15	27								
Mt	**3.24**	**4.49**	*2.49*							
	1.71	2.26	6.60							
	6	9	13							
1	1.47	*2.34*	1.51	**3.54**						
	4.79	6.18	18.53	4.49						
	8	12	25	12						
2	−0.40	0.37	0.23	*2.17*	1.57					
	5.42	7.02	20.96	5.10	14.10					
	3	8	22	10	20					
3	19.1	*2.43*	−0.01	*2.13*	1.70	**3.91**				
	6.22	8.09	24.05	5.85	16.16	18.09				
	11	15	24	11	23	35				
4	−1.76	−0.04	1.16	1.28	*2.45*	**3.40**	**2.90**			
	7.98	10.27	30.83	7.50	20.81	23.51	26.93			
	3	9	37	11	32	40	42			
5	1.11	−1.40	0.93	0.91	*2.01*	**2.78**	**4.06**	**2.50**		
	7.87	10.27	30.83	7.50	20.81	23.51	26.93	43.50		
	11	9	36	10	30	37	48	60		
6	−0.81	1.19	1.02	0.15	0.59	**4.12**	**4.39**	**4.83**	**5.52**	
	5.99	7.69	23.08	5.63	15.67	17.69	20.20	30.39	32.50	
	4	11	28	6	18	35	40	57	64	
7	−0.49	−0.34	−1.19	*2.04*	**3.17**	1.61	*2.08*	**3.22**	**4.61**	**4.53**
	2.83	3.66	10.95	2.66	7.38	8.35	9.56	14.66	15.71	10.99
	2	3	7	6	16	13	16	27	34	26

FIGURE 5. Continued overleaf.

(*c*)

	Hd	Pro	Ms	Mt	1	2	3	4	5	6
Pro (iii)	**6.87**									
(ii)	1.79									
(i)	11									
Ms	*2.16*	**5.21**								
	5.12	7.61								
	10	22								
Mt	*2.40*	**3.65**	**2.79**							
	1.28	1.93	4.57							
	4	7	12							
1	*2.38*	*2.45*	1.45	**3.63**						
	3.54	5.34	15.33	3.81						
	8	11	21	11						
2	−1.44	0.51	0.11	**2.86**	1.18					
	3.82	5.76	16.52	4.16	12.78					
	1	7	17	10	17					
3	0.76	*2.08*	0.01	1.95	1.90	**3.42**				
	4.40	6.64	18.94	4.79	13.11	14.15				
	6	12	19	9	20	27				
4	−1.57	−0.58	0.78	1.28	*2.25*	**3.48**	**2.93**			
	5.78	8.73	25.09	7.50	17.51	18.90	21.44			
	2	7	29	11	27	34	35			
5	*2.18*	−0.57	0.80	0.91	1.08	**2.82**	**2.84**	0.61		
	5.71	8.69	11.99	7.50	17.48	18.80	22.50	28.74		
	11	7	29	10	22	31	36	32		
6	−1.13	0.94	1.17	0.15	0.59	**3.39**	**3.69**	**3.71**	**3·53**	
	4.36	6.58	18.91	5.63	15.67	14.22	16.16	21.70	21.60	
	2	9	24	6	18	27	31	39	38	
7	−0.80	−0.71	−1.45	*2.04*	**3.17**	1.46	**2.78**	*2.48*	**2.81**	**2.75**
	2.18	3.30	9.45	2.66	7.38	7.10	8.09	10.80	10.78	8.15
	1	2	5	6	16	11	16	19	20	16

FIGURE 5. All possible pairwise Student *t* test correlations between longitudinal segments in *Drosophila*, asking, for each bilateral half-egg, whether somatic clones tend to be present at above-chance levels in the two segments compared. Each set of three numbers gives (i) the number of coincidences observed, (ii) the number expected, and (iii) the significance (t = obs. − exp.)/s.d.) of the deviation of expected from observed. Values of t greater than 2.5, corresponding to a probability of less than 0.02 of arising by chance, are printed in bold type. Values of t greater than or equal to 2, but less than 2.5, corresponding to probabilities less than 0.05, are printed in italic type. Horizontal and vertical lines emphasize that clones in anterior and posterior half-egg straddle each half-egg, but do not often cross into the other half-egg. (*a*) All clones seen in all irradiated flies (N = 2076); (*b*) all clones seen in flies irradiated after 2 h after oviposition (N = 1217); (*c*) all clones seen in flies irradiated after 3 h after oviposition (N = 933). About 70 % of all eggs were precellular when irradiated in (*a*), about 50 % in (*b*) and about 35 % in (*c*). Examination of clone straddling patterns in age windows from 0 to 3 h, in which all eggs were precellular, and in which the minimum number of bristles per clone analysed varied from 1 to 4, consistently shows failure to clones to straddle from the anterior to posterior half-egg.

movement were observed in *Drosophila melanogaster* eggs. Similar results have been reported in *D. montana* (Kinsey 1967). In both cases, the bidirectional cells appear to meet at a mid-egg position roughly between 50 % and 60 % e.l.

Nuclei can be observed to translate over about 10 % e.l. in either anterior or posterior 'cell', but do not appear to exchange between these 'cells'. It seems plausible that this coordinated streaming plays a role in mediating the straddling of clones throughout the anterior or posterior half-eggs, and their confinement to either half-egg. The value 50 % e.l. corresponds to the first abdominal segment. The variability in position where streaming 'cells' meet may suffice

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to explain overlapping anterior and posterior blocks of segments (figure 5*a*), but appears to preclude their role in more precise location of early compartmental events.

In summary, the hypothesis that the early *Drosophila* embryo is sequentially subdivided is currently supported by evidence that the preblastoderm egg is initially divided into anterior and posterior half-egg compartments, that a fused two-segment stage may be an obligatory stage in the establishment of the fully segmented embryo, and by data indicating that intra-segmental compartmental boundaries arise successively in the early post-cellular blastoderm embryo.

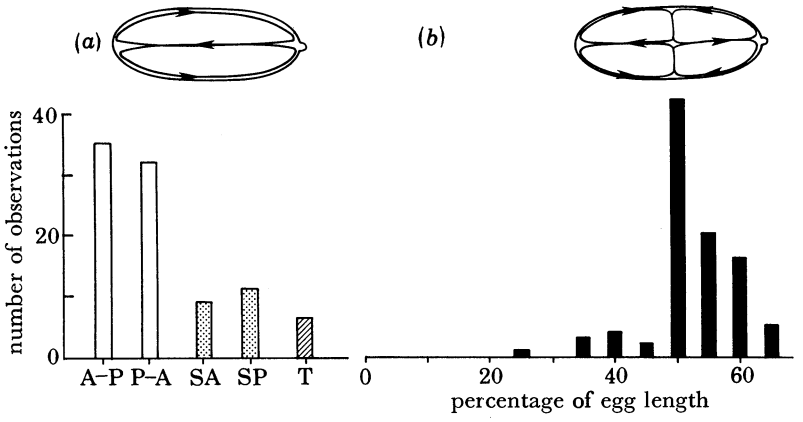

FIGURE 6. Patterns of rhythmic motion seen in cleavage-stage eggs: (*a*) whole egg flow; (*b*) two half-egg 'cells' meeting at mid-egg. Flow in each direction reverses, on average about 10 min later. A–P, P–A, whole egg cortical flow from anterior to posterior, or posterior to anterior. SA, SP, single directional cortical flows confined to anterior or posterior half-egg. T, triple patterns with three longitudinal 'cells' along egg. (*b*) Position mid-egg where anterior and posterior half-egg 'cells' meet, measured from posterior pole.

DO COMPARTMENTAL BOUNDARIES REFLECT DEVELOPMENTAL COMMITMENTS?

The central tenet of the compartmental hypothesis is that compartmental events reflect alternative commitments by the isolated polyclones (Crick & Lawrence 1975; Garcia-Bellido 1975). This core postulate is not yet established. Clonal isolation of two cell or nuclear populations, whether in the preblastoderm egg, in the blastoderm or in later imaginal disc development, is logically incapable of demonstrating that the isolated groups of cells carry different commitments. The most convincing evidence available has been the analysis of the *engrailed* mutant (Morata & Lawrence 1975, 1978), which in favourable cases transforms the entire posterior compartments of wing, leg, and head discs into mirror-image anterior compartments. This has suggested that en^+ is active only in the posterior compartments of these discs and maintains their posterior commitment. The *bithorax* (*bx*) and *postbithorax* (*pbx*) mutants transform the anterior and posterior compartments of the metathoracic disc to corresponding mesothoracic compartments (Lewis 1964, 1978; Morata & Garcia-Bellido 1976), and have been given similar interpretations (Garcia-Bellido 1975).

These data are important, but the conclusions are not firm. They rest on indirect interpretations from homoeotic mutants to the function of their wild-type alleles. Specifically, the assumption is made that en^+, bx^+ and pbx^+ are 'selector' genes affecting, or part of, the maintained determined states of their compartments (Garcia-Bellido 1975). Evidence described below suggests that *bx* does not affect determination itself, while *pbx* does (Adler 1978). No evidence

with respect to *engrailed* is available. The indirectness of interpretation is brought out by Russell's (1978) recent three-variable spherical coordinate model of positional information in imaginal discs, in which *engrailed* is interpreted as a loss of one coordinate, revealing an underlying twofold symmetry in the remaining two-variable system. In this view, the *engrailed* phenotype is a failure of positional information, not a failure of a bistable selector gene switch.

Direct demonstration that a compartmental boundary reflects a development commitment requires transplantation of cells or nuclei across the location of the compartmental boundary before its formation and transplantation after its formation. Before boundary formation, transplanted material should carry no heritable commitment to one or other compartmental fate, and should integrate into and form host site structures. After formation of the compartmental boundary, transplanted materials should carry heritable commitments to the fates of their compartment of origin. Using this paradigm, we have begun to test whether the first compartmental boundary, which divides the egg into anterior and posterior compartments, actually reflects a developmental commitment.

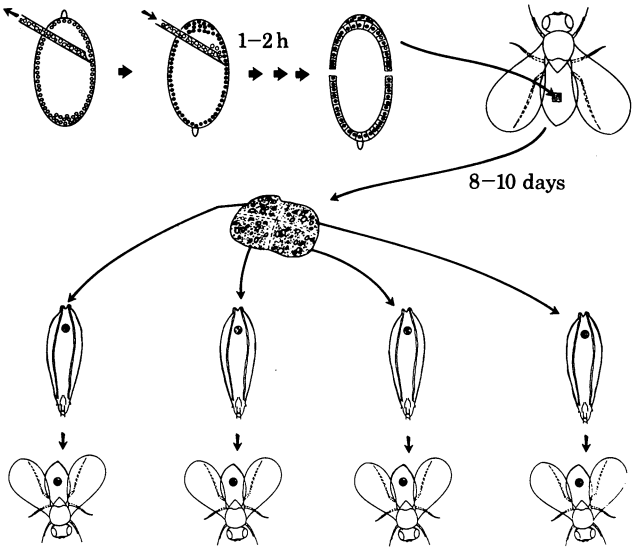

FIGURE 7. Schematic diagram of heterotopic transplantation experiment (see text for details).

Figure 7 summarizes our procedure, which utilized the transplantation of genetically marked donor nuclei from the anterior flank of a donor syncytial blastoderm to the posterior flank of a syncytial blastoderm host, or transplantations from the posterior flank of a donor to the anterior flank of a host. After cellularization of the host, the egg was cut in half, and the half containing donor nuclei injected for culture into adult abdomen. A week later, larval implants were removed, injected into host larvae, and recovered from emerged adults. Our results (Kauffman 1980) demonstrate that anterior syncytial blastoderm nuclei transplanted to the posterior flanks of host eggs uniformly yield anterior head and thoracic adult structures. Posterior nuclei injected into anterior flanks formed posterior abdominal and genital structures in all but one instance. This dominant donor autonomy, seen in 14 out of 15 donor implants, suggests that preblastoderm nuclei have adopted at least anterior or posterior somatic commitments that are stable during exposure to heterologous cytoplasm during the 45–100 min between the injection of donor nuclei and cellularization, and are heritable for the remainder of development after cellularization. Current experiments with [^{3}H]thymidine-labelled donor

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nuclei suggest that nuclei transplanted before the 13th cleavage division into 11th or 12th division hosts undergo two or more divisions before host cellularization. Nuclei transplanted after the 13th division into 12th division or earlier hosts appear to undergo at least one mitotic division. These results tentatively suggest that the preblastoderm nuclear commitments to anterior or posterior fates may also be heritable over nuclear division cycles *before* cellularization.

Since nuclear transplantations from the anterior to the posterior half-egg carried out in early cleavage reveals no donor autonomy (Okada *et al.* 1974; Zalokar 1971), and gynandromorphs demonstrate that the first cleavage division products are totipotent (Hotta & Benzer 1972; Janning 1978), the available data demonstrate that transplantation across the location of the first mid-egg clonal restriction before its formation allows donor material to form host site structures, while transplantation across the boundary after its formation reveals donor autonomy.

At the syncytial blastoderm stage, nuclei might carry commitments to single segments. In contrast, if an initial anterior–posterior clonal restriction creates two half-egg compartments, then at that stage the 'anterior' compartment should be a single committed domain (figure 4*a*). Anterior nuclei transplanted within the anterior compartment from head to thorax, or from thorax to head sites, should show no autonomy and should form host site, not donor site, structures. We are currently testing this prediction. Among eight donor implants derived from head–thorax transplantation, five formed thoracic tissue, and three formed head tissue. This frequency of non-autonomy among head–thorax transplants is significantly greater than the non–autonomy observed in anterior–posterior or posterior–anterior transplants at the same stages, 1 of 15. Transplantations from thorax to head regions are in progress. If the results show that transplanted thoracic nuclei also form head structures at high frequency, the data will suggest that in the syncytial stage egg, the anterior half-egg may in fact be a single anterior compartment. If so, quite good direct evidence for the existance of initial anterior and posterior commitments in the *Drosophila* embryo will be available.

In summary, no conclusive evidence yet available rigorously demonstrates that compartmental boundaries actually reflect assumption of alternative heritable somatic commitments.

Are disc or compartment commitments combinatorial?

Evidence for this hypothesis remains entirely indirect. The capacity of a combinatorial model to account for one step and sequences of transdetermination, for coordinated effects of homoeotic mutants, and for mutants affecting complementary subsets of discs, have been described. All are indirect support for the combinatorial hypothesis. For example, evidence adduced in favour of the combinatorial hypothesis includes the additivity of homoeotic mutants (Garcia-Bellido 1975). The inference is plausible but indirect. The fact that mutant A transforms tissue 1 to tissue 2, and mutant B transforms tissue 2 to tissue 3, while the joint mutants A,B transform tissue 1 to tissue 3, shows transitivity. The minimal conclusion, without further work, is that each mutant transforms its target tissue to a true version of the transformed tissue. It does not yet follow that each tissue's committed state comprises combined states of activity or inactivity of the wild-type homoeotic alleles. Direct evidence for the combinatorial hypothesis may be achievable by nuclear transplantation experiments in preblastoderm eggs, or other approaches.

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Are commitments, once taken, maintained by continued gene activity?

This postulate of the compartmental, combinatorial code hypothesis remains largely unsubstantiated. It should first be noted that this assumption is not a logical requirement of these models. Commitments might be encoded in alternative combinations of cascading gene activities.

Although there is relatively little evidence supporting the hypothesis that genes act continuosly throughout development to maintain disc determination, the combination of several experiments now suggests that genes in the *bithorax* complex may in fact act continuously to maintain mesothoracic and metathoracic commitments. Lewis (1978) has described deletions in the *bithorax* locus that act in embryonic development. Somatic recombinant clones yielding homozygous clones of the recessive homoeotic mutant *bx* in a heterozygous *bx*/+ background, reveal that such *bx*/*bx* clones in the anterior metathorax exhibit the *bithorax* transformation if generated before mid third instar (Morata & Garcia-Bellido (1976). Since *bx* is known to be a hypomorph in which the homoeotic transformation is due to loss of wild-type function (Lewis 1978), this experiment demonstrates that the latest, but perhaps only, time that the wild-type allele is needed to prevent the homoeotic transformation is during early to mid third instar. We recently discovered that the heterozygote *Cbx*/*TM2 Ubx*^{130} is temperature sensitive at all stages of embryonic and larval development. At 29 °C, flies exhibit a strong *Cbx* phenotype; shift or pulses to 17 °C at any stage increase the *Ubx* phenotype significantly (Kauffman 1981 *a*). Together with the other results noted, these suggest that the *Cbx Ubx* system may act continuously from embryonic to late larval life to maintain mesothoracic and metathoracic properties in these two tissues.

The hypothesis that master gene systems act continuously to maintain alternative commitments would seem to predict that many temperature–sensitive homoeotic mutants with broad spectrum times of activity might be uncovered. In fact, most temperature-sensitive homoeotic alleles have quite restricted times of action (Grigliatti & Suzuki 1971; Stepshin & Ginter 1972; Postlethwait 1974). This may reflect the fact that such mutants impinge on underlying gene systems maintaining alternative commitments without being parts of those systems, or that the hypothesis of stable gene activities maintaining commitments is largely incorrect.

Do homoeotic mutants alter determined states?

Homoeotic mutants are widely regarded as 'switch genes'. Yet it is not clear whether homoeotics often alter determination states themselves. Homoeotic mutants might alter the initial positional information triggering heritable developmental commitments, might alter such heritable commitments once taken, or might alter the execution of those commitments during metamorphosis or earlier. Two experimental approaches have addressed the latter distinction. Adler (1978) supposed that if a homoeotic mutant alters a disc to another disc's determined state, then the kinds of regeneration obtained from the transformed tissue should be identical to that obtained when the true disc is tested for regeneration. He tested whether posterior halves of the metathoracic disc, transformed by *postbithorax* (*pbx*) to posterior wing, regenerated anterior wing or anterior metathorax. He found that such fragments regenerated anterior wing. In contrast, anterior metathorax fragments transformed to anterior wing by *bx*

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regenerated posterior metathorax, not posterior wing. He concluded that *pbx* transforms the determined state of posterior haltere to posterior wing, while *bx* only transforms the execution of the anterior haltere to anterior wing.

The second paradigm to test whether a homoeotic mutant alters determination itself was employed in our laboratory. *Nasobemia* (*Ns*) is a dominant homoeotic transforming antenna to mesothoracic leg. In *Nasobemia* flies, the mesothoracic leg is normal; hence the determined state in its mesothoracic leg disc should be normally heritable. If *Ns* truly transforms antenna cells to the leg disc determined state, it should be possible to remove *Ns* from a clone after *Ns* has acted, and the Ns^+/Ns^+ clone should heritably express the leg state. This was tested in *Ns/bld Sb* flies. *Ns* is known to be temperature sensitive between 48 and 65 h (Stepshin & Ginter 1972). Our results (Kauffman & Ling 1980) showed that if *Ns* is removed by somatic recombination before 48 h the *bld Sb* Ns^+/*bld Sb* Ns^+ clones exhibit a low level of leg transformation. In contrast, after 65 h, the *bld Sb* Ns^+/*bld Sb* Ns^+ clones continued to express maximal leg transformation over the remainder of development, and up to eight or nine cell divisions. The possibility that persistence of the *Ns* phenotype was due to *perdurance* (Garcia-Bellido 1972, 1975) of *Ns* product in Ns^+ cells is highly unlikely on two grounds, lack of temperature sensitivity after 65 h, and persistence of maximal leg expression regardless of Ns^+ clone sizes, ranging up to 500 cells. Adler's (1978) results and ours, combined, suggest that some homoeotic mutants do affect determination itself, while others probably affect its execution. Careful distinction between these classes is warranted when interpreting the actions of homoeotics, and making inferences to their wild-type alleles. For example, in a combinatorial model, the execution of disc-specific genes requires 'reading' and responding to the combined states of several master genes. In a combinatorial model the class of homoeotics altering execution would not necessarily be expected to affect sets of tissues coordinately.

To summarize this appraisal, data related to the sequential compartment, combinatorial code hypothesis remain provocative but inconclusive.

Models of positional information suggested by sequential compartmentalization

The locations of compartmental boundaries arising successively on the wing disc are shown in figure 3. Since massive sorting-out of distinct cell types is excluded during imaginal disc development, the locations in which successive clone restriction lines arise pose the problem of how their locations, numbers, and, on the wing disc, their obvious twofold symmetries are to be explained. Elsewhere in this volume, Murray describes the capacity of chemical systems undergoing reaction and diffusion to generate a rich variety of different spatially inhomogeneous chemical patterns, which might account for the patch and band marking patterns seen on a variety of animals. Several years ago, my colleagues and I (Kauffman *et al.* 1978) proposed essentially the same model to account for the particular sequence, number and symmetries of compartmental boundaries in the wing disc.

Like Murray, we proposed a reaction–diffusion system composed of two substances, *X* and Y, undergoing chemical synthesis or destruction and diffusion in a bounded wing disc-shaped spatial domain with no flux boundary conditions. As summarized in figure 8, such systems of partial differential equations are conveniently explored by studying the stability of their

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spatially homogeneous steady states to spatially distributed perturbations in the chemical variables. With appropriate values of the linearized reaction constants and the diffusion constants (table 4), such chemical systems have been known since Turing's work (1952) to be capable of amplifying perturbations with a specific range of peak to peak spatial wavelengths. Spatial wavelength perturbations of shorter or longer wavelength decay to the spatially homogeneous steady state. Imposition of specific boundary conditions, such as no flux of X or Y across the boundary, implies that only a discrete family of differently shaped chemical concentration patterns, eigenfunction solutions to the diffusion Laplacian operator for that specifically shaped domain, can 'fit' onto the domain. At any given tissue size and shape, the establishment of a chemical pattern requires jointly that a chemical eigenfunction pattern fit onto the domain, and that its wavelength be one of those that is amplified by the chemical reaction–diffusion system. If the spatial domain is so small that the longest wavelength eigenfunction pattern that might fit onto the domain is less that the minimal excitable wavelength, the spatially homogeneous solution is stable and no pattern can form (Gmitro & Scriven 1966).

TABLE 4. OUTLINE OF ANALYSIS OF GENERAL NONLINEAR REACTION–DIFFUSION SYSTEM: SUCCESSIVE EIGENFUNCTION MODEL OF COMPARTMENTALIZATION

Nonlinear reaction–diffusion system:

$$\partial x/\partial t = F(x,y) + D_x\nabla^2 x;$$
$$\partial y/\partial t = G(x,y) + D_y\nabla^2 y.$$

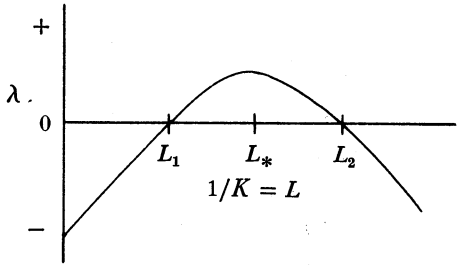
Linearize about spatially homogeneous steady state and perform stability analysis:

$$\begin{vmatrix} A_{11} - K^2D_x - \lambda & A_{12} \\ A_{21} & A_{22} - K^2D_y - \lambda \end{vmatrix} = 0.$$

Under conditions:

(i) $A_{11} + A_{22} > 0$;
(ii) $A_{11}A_{22} - A_{12}A_{21} > 0$;
(iii) $(A_{11} - A_{22})^2 > -4A_{12}A_{21}$;
(iv) $D_xA_{22} + D_yA_{11} > 0$;
(v) $\{\sqrt{(D_x/D_y)}A_{22} - \sqrt{(D_y/D_x)}A_{11}\}^2 > -4A_{12}A_{21}$.

The reaction–diffusion system has spatial patterns with a 'natural' wavelength L_*.



An inherent property of this class of models, is that as the spatial domain containing such a reaction–diffusion system enlarges and perhaps changes shape, a succession of differently shaped eigenfunction solutions can arise and decay (Herschkowitz-Kaufman 1975; Kauffman 1977; Kauffman *et al.* 1978; Nicolis *et al.* 1978; Erneux & Hiernaux 1980). Therefore, this class of models naturally predicts the sequential formation of differently shaped gradients of the same substances, as a tissue's size and shape, or other parameters, change.

Figure 8 shows the first five eigenfunction patterns for a wing disc shape (Kernevez 1980;

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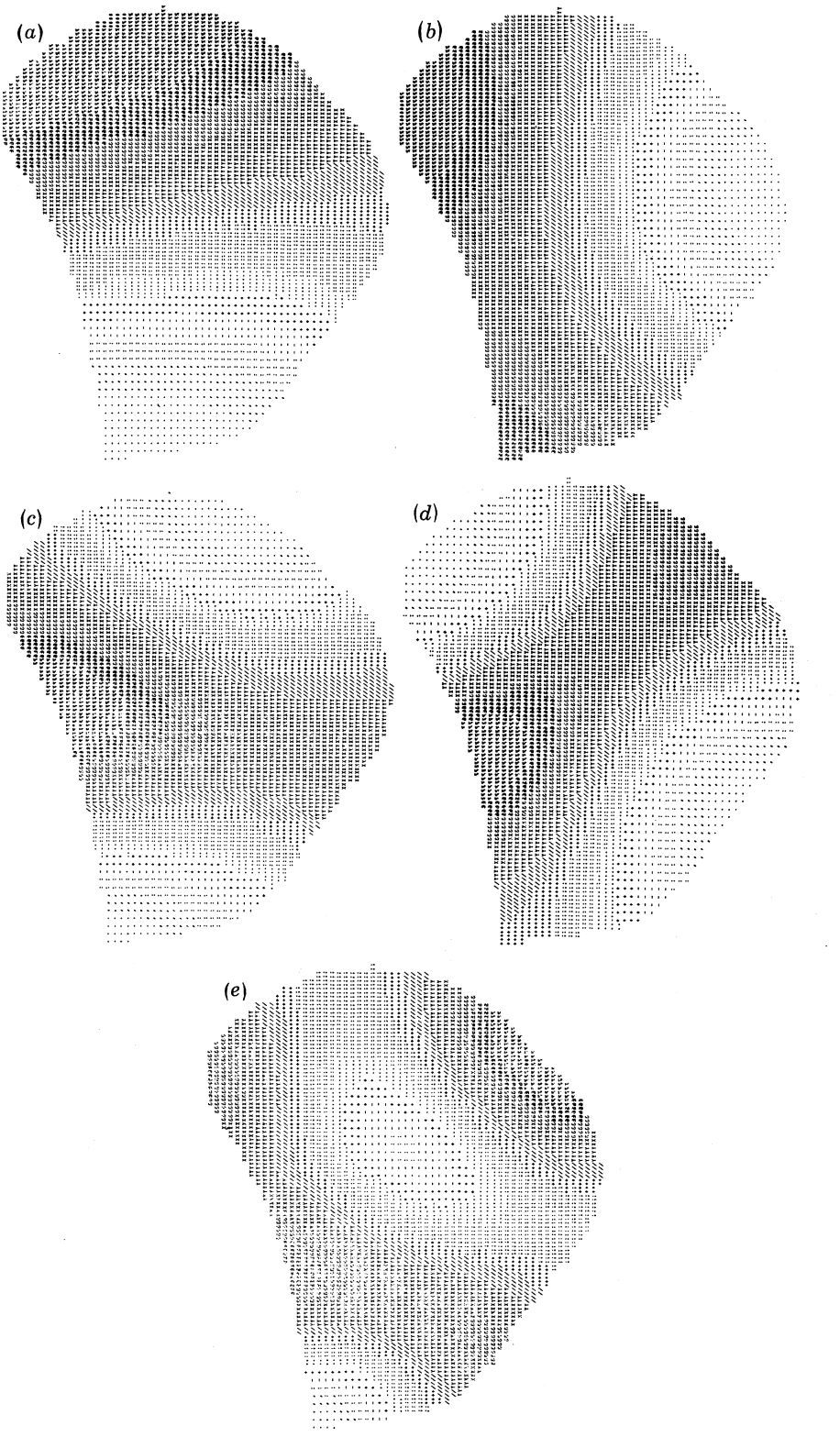

FIGURE 8. First five eigenfunction patterns computed for the actual wing disc shape as it increases in size (Kernevez 1980). Concentration profiles are shown in a density 'grey' code. Mid-density corresponds to the steady-state value of the system and constitutes a threshold. Cells experiencing concentrations above or below threshold adopt alternative heritable commitments that record the position of threshold compartmental boundary line after each chemical pattern disappears and is replaced by a subsequent pattern.

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Bunow *et al.* 1980). If one assumes that the steady-state level corresponds to a threshold in which cells experiencing above threshold concentrations adopt one commitment, those below it adopt an alternative commitment, maintained thereafter by master gene systems, then the sequence of 'nodal' steady-state lines of successive chemical patterns predicts a sequence of compartmental boundaries. The similarity between those predicted on the wing disc and those observed is strong (Kauffman *et al.* 1978). Despite its imperfections (Bunow *et al.* 1980), this model remains the only current serious attempt to explain the particular geometries of wing disc compartmentalization. In contrast, the assumption that position may be specific by monotonic gradients in a disc or embryonic tissue makes no specific predictions about the positions, sequences or symmetries of compartmental boundaries.

Application of the sequential chemical pattern model to the egg

The same class of models can be studied on the maturing egg. Because the egg does not grow in size, to obtain a succession of chemical patterns it is necessary to assume that some other parameter, such as diffusion constants, varies. The subsequent feature of this class of models is

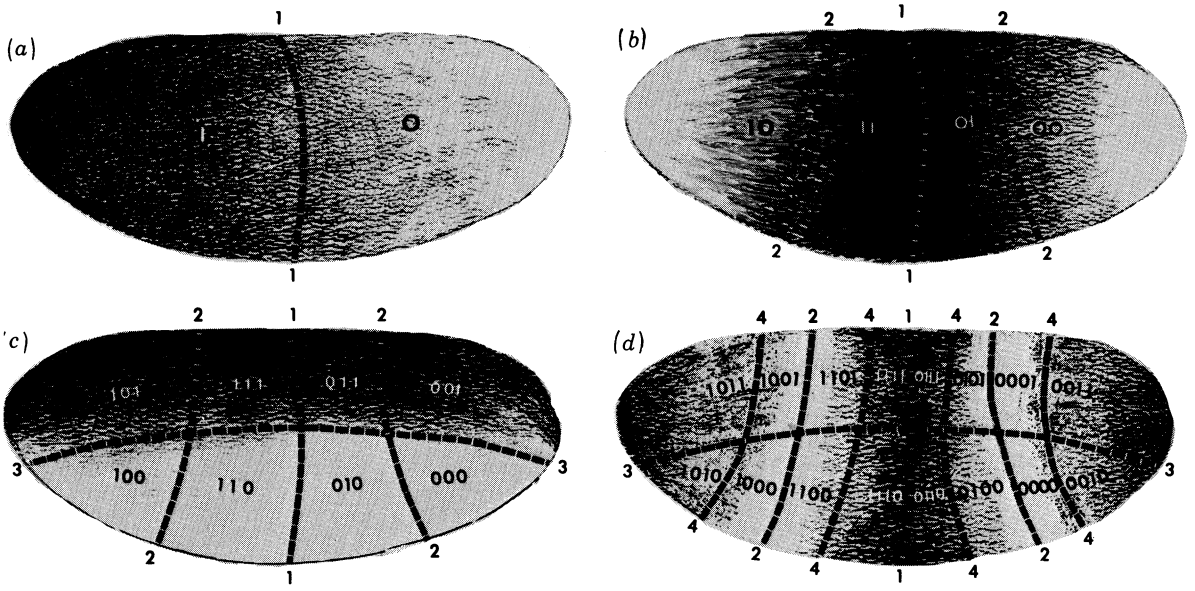

Figure 9. Approximate first four eigenfunction patterns on an egg shape. The first (*a*) is monotonic anterior to posterior. The second (*b*) is U-shaped, with a maximum in the mid-region of the egg; the third (*c*) has a maximum dorsally and minimum ventrally. The fourth creates a pattern with three longitudinal peaks and two troughs along the egg.

that they form a succession of different patterns on the egg. The first pattern is necessarily monotonic in the long axis of the egg (figure 9*a*). A mid-egg nodal line will yield a first mid-egg compartmental boundary. The second pattern is robust in generating a non-monotonic gradient with a maximum in the mid region of the egg and minima at both ends (or the inverse of this pattern) (figure 9*b*). Two nodal lines create 'end' and 'middle' compartments. It is worth stressing that this second pattern has the property of making the two ends of the egg carry the same 'end' commitment. This non-monotonicity therefore is critical to building up a combinatorial code in which distant regions can still have neighbouring development programmes. The third and fourth patterns (figure 10*c*, *d*) create further 'dorsal' and 'ventral', and 'even'

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and 'odd' commitments. This model was used above to generate the combinatorial code assigned to discs, which accounts well for transdetermination and many homoeotic mutants.

A property of this sequential chemical model that I now wish to note is that successive commitments carry a record of the mirror symmetries in the sequence of gradients that arose and triggered formation of compartmental boundaries. For example, the two 'end' commitments, compared with the 'middle' commitment, record the U shape of the second pattern. An implication is that mutants that disenable a specific commitment, say 'middle', might reveal the record of those gradient symmetries.

Mutants and procedures yielding symmetrical patterns

Several mutants, or experimental procedures, hint tantalizingly at such a record of sequential symmetries in pattern formation. In *Smittia*, irradiation of the anterior pole of the egg before blastoderm results in formation of a mirror-symmetrical double-abdomen embryo (Kalthoff 1979). Kalthoff has found that variations in the parameters that alter the frequency of double abdomen do not alter the location of mirror symmetry in the anterior abdomen, and that embryos are sometimes obtained in which one bilateral half is normal, the other double abdomen. Based on this, he has suggested that in *Smittia*, a bistable 'anterior'–'posterior' switch operates in a system with a U-shaped gradient, monotonic in each half-egg, supplying positional information to guide metamerization. Ultraviolet irradiation would disenable the anterior state and generate a double-abdomen phenotype. With our data in *Drosophila* for a first anterior–posterior compartmental boundary in the anterior abdomen and anterior or posterior somatic nuclear autonomy in transplantations across this boundary, Kalthoff's data are consistent with a first anterior or posterior binary decision in insect development. Examination of figures 4*a* and 9*d* shows that disenabling the first 'anterior' commitment so that the tissues fall to a 'posterior' state also yields a mirror-symmetrical double-abdomen phenotype in the sequential chemical pattern model, owing to recording of the history of symmetries in successive patterns in the resulting combinatorial code.

Conversion of the 'middle' commitment in figures 4*d* and 9*d* to a ground 'end' state would yield an embryo that had no thoracic segments or proximal abdominal segments, but might have mirror-symmetrical posterior abdominal segments in the posterior half-egg, and mirror-symmetrical head segments in anterior egg. It is possible, but not clear, that *Krüpple* mutants might correspond to such a phenotype (Nüsslein-Volhard & Wieschaus 1980). These lethals lack thoracic and proximal abdomen segments. The anteriormost observed abdominal segment, the 6th, is repeated with mirror symmetry. In some cases, the 6th, 7th and 8th abdominal segments are present in mirror symmetry in the posterior half embryo. The head region is grossly abnormal, and not yet closely scored for mirror symmetries (E. Wieschaus, personal communication 1980).

In their collection of embryonic lethals, Nüsslein-Volhard & Wieschaus (1980) describe several other mutants with mirror-symmetrical patterns. *Runt* appears to have six two-segment domains, each with a mirror-image duplication of the anterior part of one band. Several mutants have mirror-symmetrical patterns within each segment, the posterior region being replaced by mirror-symmetrical anterior region patterns. Finally, *engrailed* in adults shows a similar intra-disc mirror symmetry, as described earlier.

It is tempting to suppose that these mutants, which reveal mirror-symmetrical patterns on

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length scales ranging from half-egg to within segments, may reflect a developmental history in which successively shorter wavelength patterns form on the egg or early embryo, and supply positional information to trigger developmental commitments.

Weaknesses of the sequential chemical pattern model

The sequential eigenfunction chemical pattern model faces certain difficulties. One is the stability of patterns to shape changes in the egg. Although the first several patterns are quite insensitive, later chemical patterns, with shorter wavelengths, are deformed by modest changes in the shape of the egg (Bunow *et al.* 1980). This sensitivity derives from the assumption that all eigenfunction patterns reflect the egg's shape as a whole. One direction to modify sequential pattern models is to suppose only the first one or few robust chemical patterns divide the egg into a few functionally isolated subdomains, each further subdivided by the first few chemical patterns to arise within it. Monotonic gradient models face analogous difficulties in setting many serial threshold levels, and the sensitivity of threshold contours on the two-dimensional surface of the egg to changes in its size and shape.

A monotonic gradient model explains the gap phenomenon economically by supposing that ligation distorts the monotonic gradient such that it is steep across the ligated area, owing to low diffusion, and shallow in the pole areas. The several serial threshold values present in the steep region of the gradient are lost or not expressed, in the ligated area; hence segments flanking the ligation are lost. A sequential chemical pattern model replaces the serial threshold simultaneously present in the monotonic gradient with a succession of thresholds formed by the successive chemical patterns. As in the monotonic gradient model, ligation distorts each pattern so that it is steep (of shorter wavelength) across the ligated area. This displaces threshold values into the ligated area, with a similar loss of segments flanking or near the ligation. Thus sequential models do not appear to be ruled out by the gap phenomenon, but they do not provide the simplest explanation for it.

The models discussed have assumed that segment specification is identical to the process of segmentation itself. Lewis's (1978) demonstration that deletion of the entire *bithorax* region yielded larvae with serial mesothoracic segments, shows that metamerization does not require correct assignment of segment character. How these two are normally kept in register is an open question (Sander 1980).

Two-dimensional positional information: a tentative unifying hypothesis

The relation of transdetermination, homoeotic mutants, and, in particular, sequential compartmentalization in *Drosophila* to phenomena revealed in epimorphic pattern regulation is unclear. Since Wolpert's (1969, 1971) formulation of the problem of pattern formation in terms of positional information, serious effort has been devoted to discovery of the appropriate coordinate systems to carry positional information. Three alternative models have been proposed: polar coordinate (French *et al.* 1976), spherical coordinate (Russell 1978) and Cartesian coordinate (Cummins & Prothero 1978; Winfree 1980; Kauffman 1978; Kauffman & Ling 1981) models.

Debate about alternative coordinates is not trivial. Different coordinate systems require

the assumption of different special processes beyond intercalary smoothing of positional discontinuities to account for the data. In particular the polar model requires special rules of distal regeneration (Harrison 1918, 1921; Dent 1954; Butler 1955; Rose 1962; Bryant & Iten 1976; French *et al.* 1976; Schubiger & Schubiger 1978) by a truncated limb, or proximal leg disc annulus, since simple intercalation by juxtaposed proximal radial values will not 'fill in' missing distal values. In the past several years, several workers have independently realized that the older concept of monotonic transverse gradients forming a Cartesian model can account for all the data initially explained by the polar model (Cummins & Prothero 1978; Winfree 1980; Kauffman & Ling 1981). Furthermore, such models account for distal regeneration (Schubiger & Schubiger 1978) by simple intercalary smoothing of positional discontinuities in a proximal imaginal disc annulus, or truncated limb.

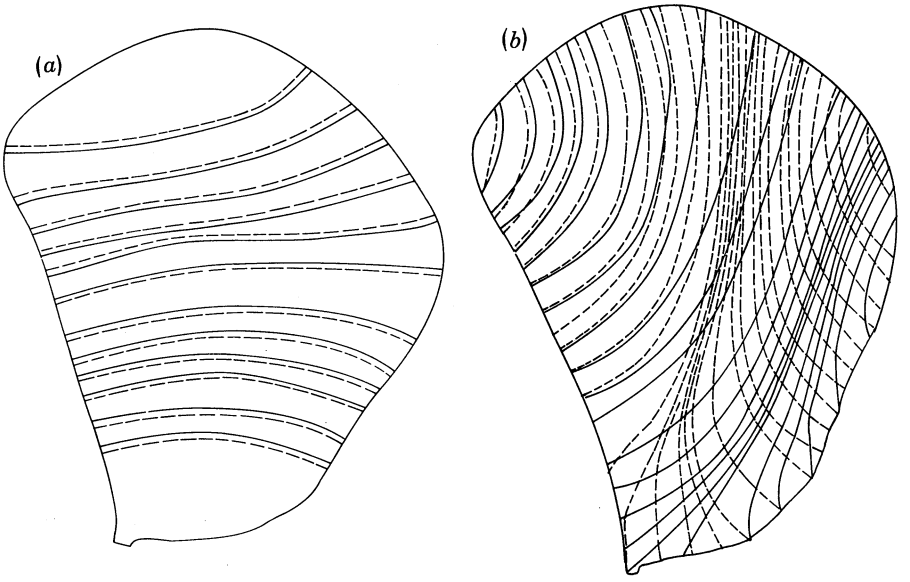

FIGURE 10. Patterns of X and Y chemical concentrations on an enlarging wing disc. Solid lines are lines of equal concentration of X, dotted lines equal concentrations of lines of Y. The first pattern to emerge is monotonic in the long axis of the disc, X and Y have parallel concentration profiles and parallel lines of constant concentration, and hence supply only one axis and one dimension of positional information. As the disc enlarges, monotonic gradients in both long and short axes can amplify, the superimposed mixed eigenfunction patterns yield non-parallel lines of X and Y constant concentration, and hence a second positional axis and two dimensions of positional information.

A feature of reaction diffusion models is that they can form transverse gradients in a particularly inevitable way (Kauffman 1981*b*). Such a chemical system will amplify any eigenfunction pattern that both satisfies the boundary conditions on the domain and is an amplifiable wavelength. For any chemical system that amplifies a finite range of wavelengths, if the range of amplified wavelengths is narrow, then on an enlarging spatial domain the first several eigenfunction patterns will arise and decay in succession. However, when the physical domain is large enough, the system will amplify two successive chemical patterns simultaneously, since both will fit onto the domain. When this occurs, the underlying chemicals, X and Y, will form non-identical gradient shapes whose lines of constant chemical concentrations run transverse to one another, and generate domains in which two dimensions of positional information are specified.

Figure 10 shows the succession of chemical patterns that arise on an enlarging wing disc in a

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particular nonlinear model in which the range of spatial wavelengths amplified was wide, rather than narrow. The wider range allows monotonic amplifiable patterns to fit onto a growing disc in both the long and short axes. When the disc reaches a size allowing the first simplest monotonic pattern to form in the long axis, that single pattern yields parallel concentration profiles of both X and Y. As the disc enlarges and allows chemical patterns to amplify in both the long and short axes, the modified eigenfunction patterns that emerge cause the two monotonic gradients of X and Y to rotate with respect to one another, generating transverse gradients. Reference to the non-parallel lines of X and Y constant concentrations can therefore provide two-dimensional positional information in the tissue.

These results suggest a unifying hypothesis aiming to tie together the concepts of compartments, codes and two-dimensional positional information. The properties of reaction–diffusion systems on growing domains that become large enough predict that a succession of monotonic and non-monotonic patterns arise. The first monotonic pattern yields one axis of positional information. After tissue growth or other parameters change, allowing more than one eigenfunction pattern to be simultaneously amplified, non-parallel gradients arise, and supply a second axis and two-dimensional information. Thus positional axes should form sequentially (Harrison 1918, 1921), the first in the long axis of the embryo or field. However, eventual non-monotonicity in the gradients will render the entire positional field multivalued, with a patchwork of neighbouring domains with mirror symmetries between them. If each domain carried a different commitment from its neighbours, and corresponded to a secondary field, cells in each area would consult their local-two-dimensional monotonic map of positional information and interpret it according to their determined state. In particular, if a combinatorial encoding of a sequence of chemical patterns generated distinct terminal compartments or secondary fields, each with its own monotonic cross-gradients, the code would carry the determined state of each domain, the cross-gradient its internal positional information. In this view, secondary fields (compartmentalization?) became a necessary part of the process of maintaining unique positional information as underlying gradients pass from monotonic to highly non-monotonic and multivalued. Alternatively, Slack (1980) has suggested that simple monotonic gradients may underlie a combinatorially coded set of compartments through gradient thresholds. In this view, compartments are not required to maintain unique positional information. Discrimination among these alternative models requires testing whether neighbouring compartments in one disc have mirror-symmetrical positional fields. Some evidence favours this. The observation that even small homozygous *engrailed* clones in the posterior margin of *en*/+ wings form anterior wing margin structures suggests that the underlying positional information in anterior and posterior wing compartments is mirror-symmetric (Morata & Lawrence 1978). Whether mirror symmetries in positional fields are generally found in adjacent compartments, and, if so, the implications for intradisc pattern regulation, remain to be tested.

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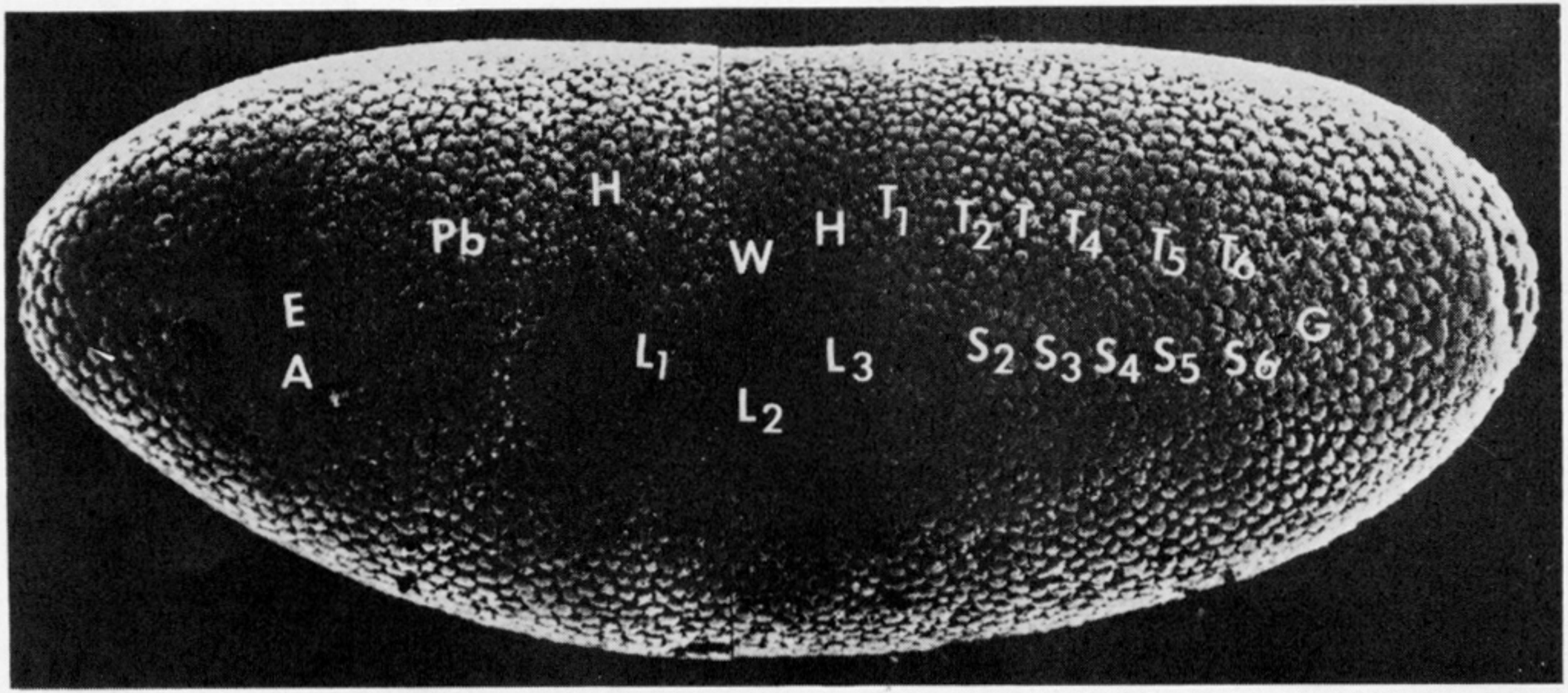

FIGURE 1. Fate map of blastoderm projected onto scanning electron micrograph (magn. ×600) of a *Drosophila* egg just after completion of blastoderm and onset of cephalic furrow formation. Pole cells visible at posterior pole. A, antenna; E, eye, Pb, proboscis; H, humerus; W, wing–thorax; H, haltere; L1–L3, prothoracic, mesothoracic and metathoracic legs; T1–T6, first to sixth abdominal tergites; S2–S6, second to sixth abdominal sternites; G, genital.

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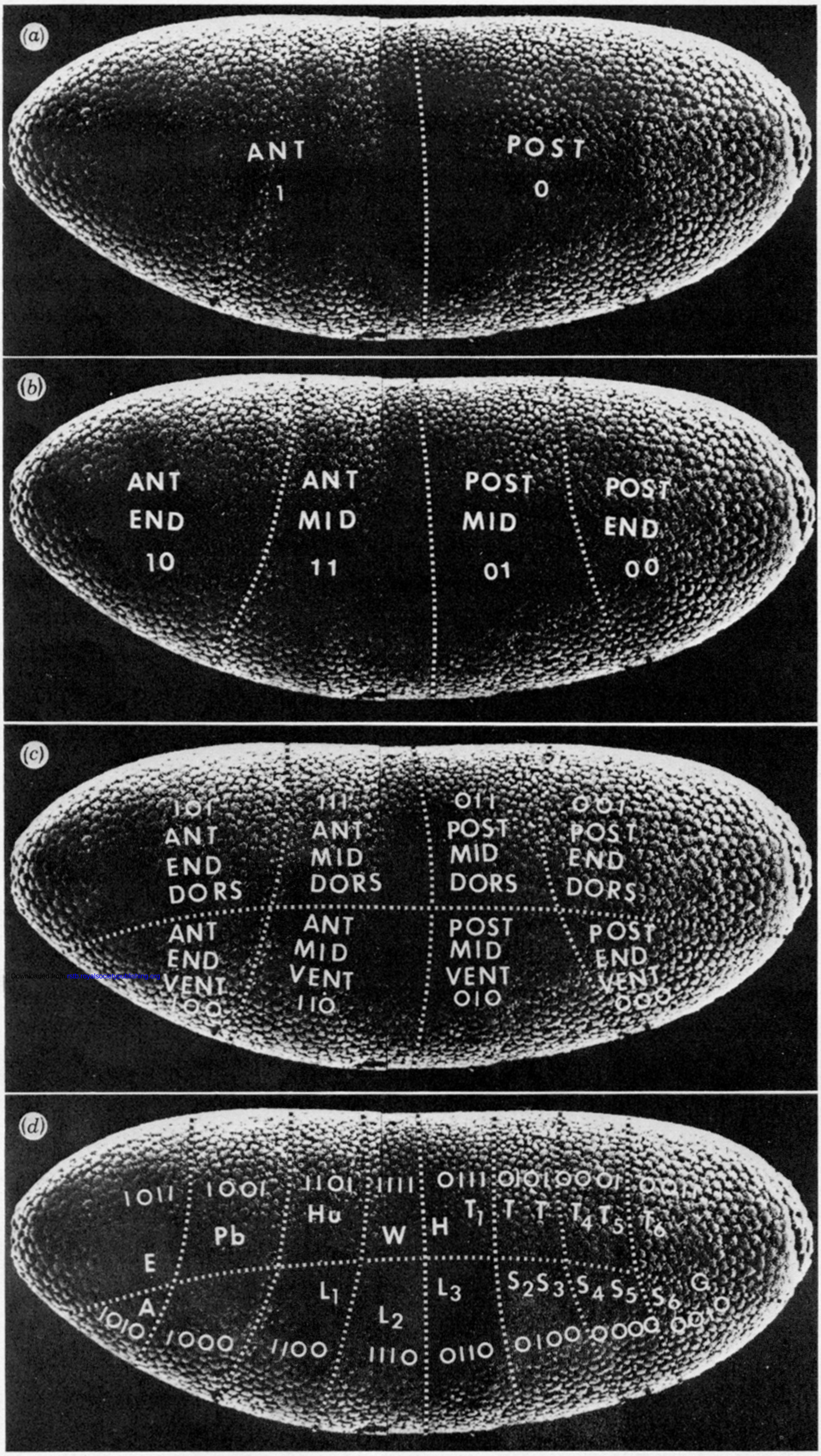
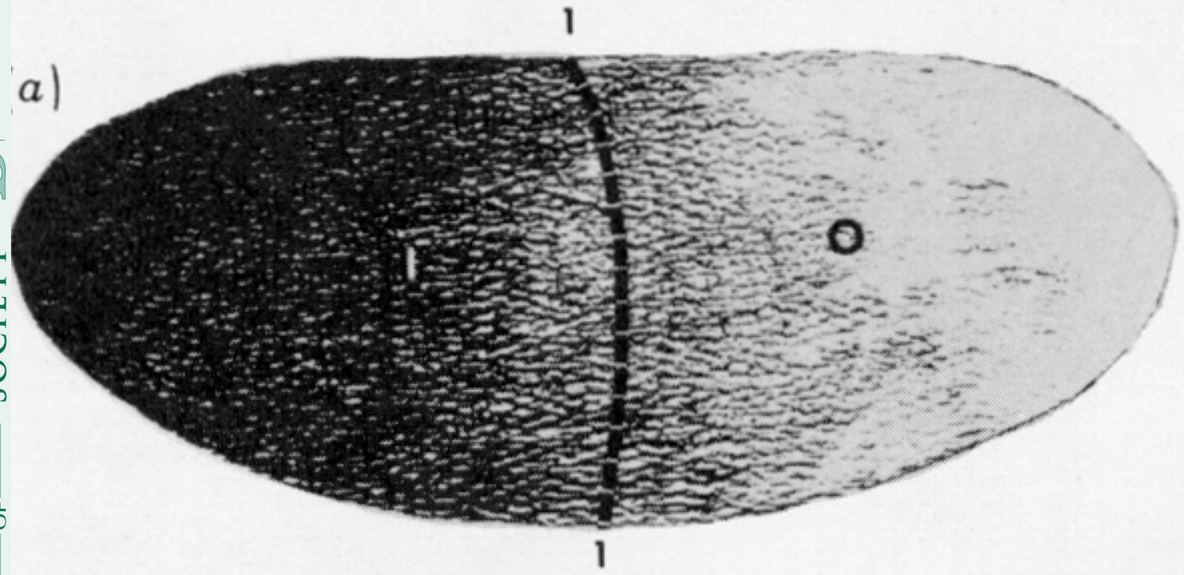
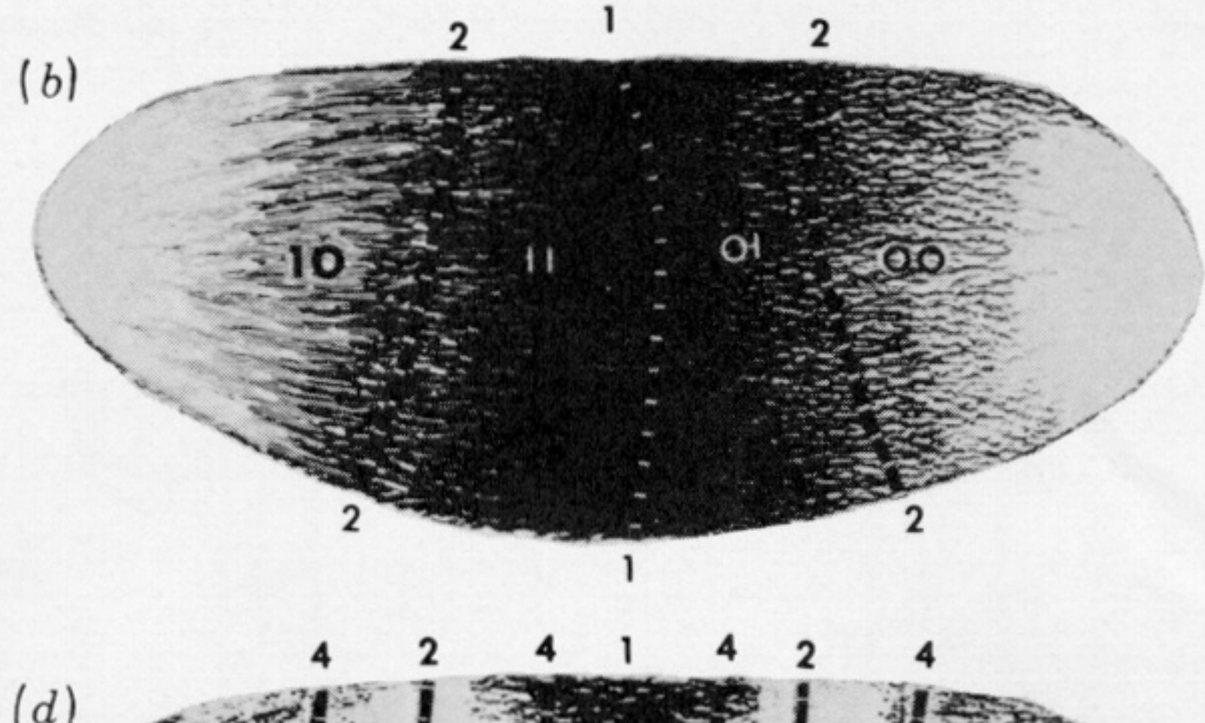
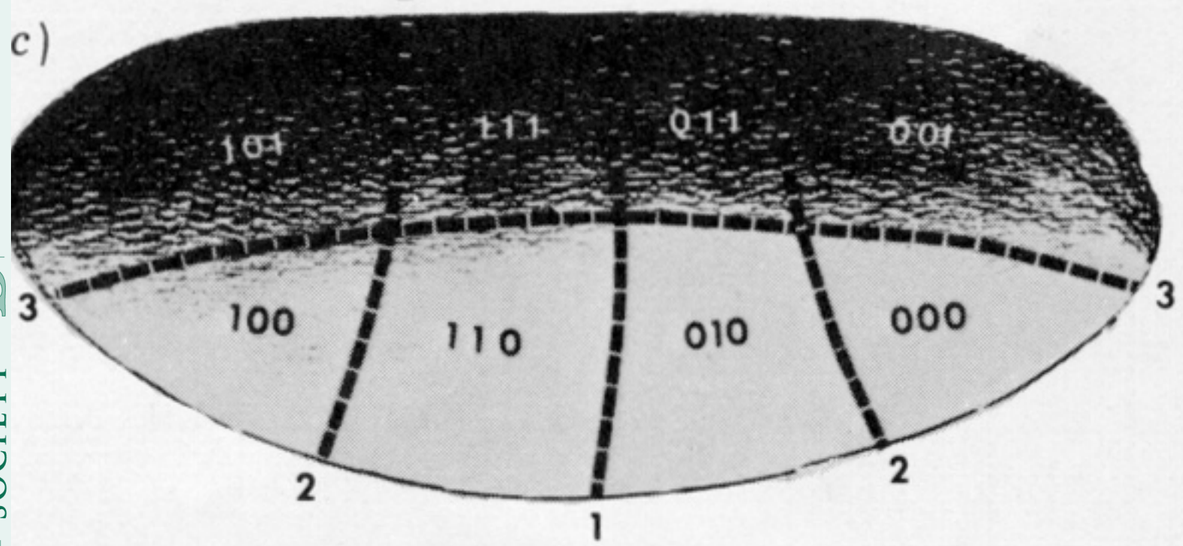


FIGURE 4. Four hypothetical compartmental events successively dividing the egg. (*a*) The first divides the egg into anterior–posterior half-egg domains; (*b*) a second event forms a pair of compartmental boundaries defining end and middle compartments; (*c*) the third event creates a dorsal–ventral boundary; (*d*) the fourth event creates four compartmental boundaries defining even and odd compartments. Each terminal compartment carries a record of the alternative commitments taken during its formation, e.g. 'anterior', 'middle', 'odd', 'dorsal' (1111) = mesothorax. The combinations of alternative commitments constitute an epigenetic code.







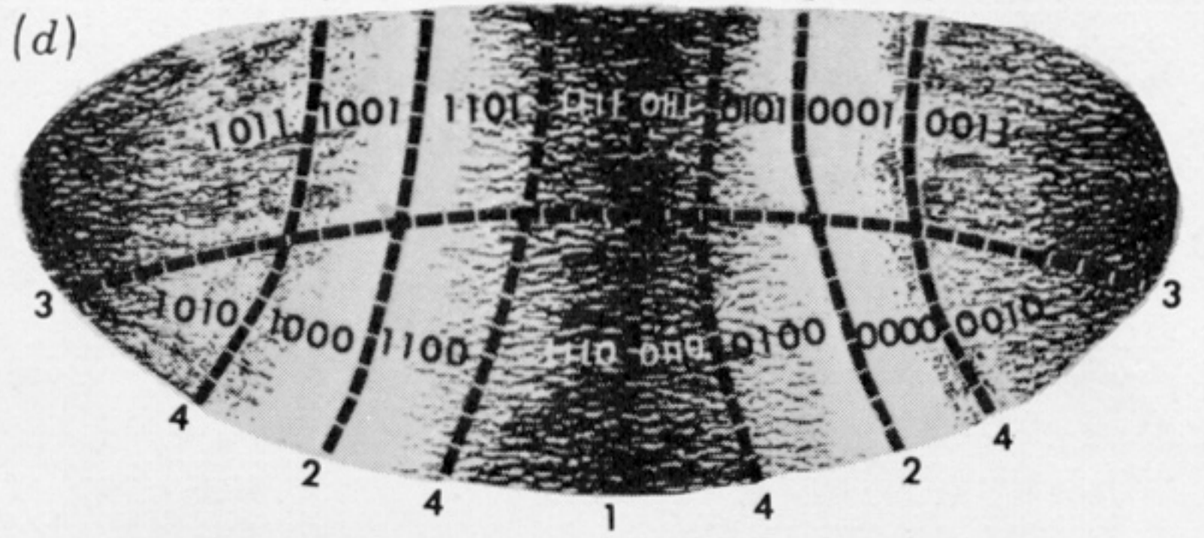


FIGURE 9. Approximate first four eigenfunction patterns on an egg shape. The first (*a*) is monotonic anterior to posterior. The second (*b*) is U-shaped, with a maximum in the mid-region of the egg; the third (*c*) has a maximum dorsally and minimum ventrally. The fourth creates a pattern with three longitudinal peaks and two troughs along the egg.