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The molecular basis for metameric pattern in the *Drosophila* embryo

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Summary

The metameric organization of the *Drosophila* embryo is generated in the first 5 h after fertilization. An initially rather simple pattern provides the foundation for subsequent development and diversification of the segmented part of the body. Many of the genes that control the formation of this pattern have been identified and at least twenty have been cloned. By combining the techniques of genetics, molecular biology and experimental embryology, it is becoming possible to unravel the role played by each of these genes.

The repeating segment pattern is defined by the persistent expression of *engrailed* and of other genes of the 'segment polarity' class. The establishment of this pattern is directed by a transient molecular prepattern that is generated in the blastoderm by the activity of the 'pair-rule' genes.

Maternal determinants at the poles of the egg coordinate this prepattern and define the anteroposterior sequence of pattern elements. The primary effect of these determinants is not known, but genes required for their production have been identified and the product of one of these, *bicoid* is known to be localized at the anterior of the egg. One early consequence of their activity is to define domains along the A–P axis within which a series of 'cardinal' genes are transcribed.

The activity of the cardinal genes is required both to coordinate the process of segmentation and to define the early domains of homeotic gene expression. Further interactions between the homeotic genes and other classes of segmentation genes refine the initial establishment of segment identities.

Key words: *Drosophila melanogaster*, segmentation, homeotic genes, pattern, *in situ* hybridization, embryogenesis.

Introduction

In this review, I discuss the patterns of gene expression underlying the metameric organization of the early *Drosophila* embryo. I am concerned with two features of this embryonic pattern; the generation of repeating units along the anteroposterior axis and the regional specification of different identities within this array. These features first become apparent in the morphology of the animal 5 h or more after fertilization, but the underlying molecular patterns are generated earlier, during the formation of the blastoderm and gastrulation.

This early period of development culminates in the elaboration of the 'segmented germ band', a morphological stage that is strikingly conserved throughout the range of insects (Anderson, 1973). It is in the segmented germ band that the general and, presumably, most ancient features of the insect body plan are displayed most clearly, and that the homology of metameric units is most apparent. As we shall see below, this clarity extends to a molecular level. Genes controlling segmentation and segment identity establish a metameric pattern of activity in the early germ band embryo. Only vestiges of this relatively simple pattern can be discerned in the patterns of gene activity in later development.

The study of this process has been made posssible by the identification of mutations in approximately 50 genes (see Table 1). These disrupt the pattern of metameric units (the segmentation genes), the identity of segments (the homeotic genes), or both (e.g. mutations affecting the polarity of the whole embryo). Virtually all were first identified by the phenotypes of mutant alleles. Many of the homeotic mutations were identified fortuitously, by striking transformations of one segment into another (e.g. Bridges & Morgan, 1923; Lewis, 1963). The great majority of segmentation genes, however, have been identified by systematic, not to say Herculean,

Table 1. *Segmentation and homeotic genes in* Drosophila

Gene name (symbol, locus[a])	Maternal or zygotic expression	Classification by phenotype	References: Embryonic phenotype	References: Molecular cloning and structure	References: Pattern of expression	Comments
COORDINATE AND GAP GENES						
bicaudal (*bic*, II-67)	m	embryonic polarity reversal	2, 11, 99	—	—	Double-abdomen phenotype, but no duplication of pole cells. Variable penetrance.
Bicaudal-D (*Bic-D*, II-52.9)	m	,,	2, 12	—	—	Dominant 'gain of function' phenotype like *bicaudal*
Bicaudal-C (*Bic-C*, II-51)	m	,,	2, 12	—	—	Dominant haplo-insufficient phenotype like *bicaudal*
dicephalic (*dic*, III-46)	m	,,	13	—	—	Variably produces bicaudal and dicephalic phenotypes; alters location of nurse cells within follicle
**bicoid* (*bcd*, ANT-C)	m	anterior pattern defects	9	10	10	Null alleles delete head, gnathal and thoracic regions; telson but not abdomen or pole cells duplicated anteriorly. See text.
exuperantia (*exu*, II-93)	m	,,	6	—	—	Mutants disrupt anterior head structures; posterior midgut and proctodaeum duplicated anteriorly.
swallow (*sww*, I-14)	m	,,	2, 100	—	—	Phenotype similar to *exu*; also called *fs(1)1502* of Gans (14)
torso (*tor*, II-57)	m	anterior and posterior pattern defects	2, 6	—	—	Mutants disrupt anterior head structures; delete most posterior derivatives of the fate map (not pole cells)
trunk (*trk*, II-36)	m	,,	6	—	—	Similar to *torso*
fs(1)Nasrat (*fs(1)N*, I-0.0)	m	,,	98	—	—	Maternal phenotype similar to *torso*;
oskar (*osk*, III-48.5)	m	'posterior group' gap gene	7	—	—	Segment gap like *knirps*; pole cells not formed See text.
staufen (*stau*, II-83)	m	,,	6	—	—	Similar to *oskar*; also shows head defects and head fold shifted anteriorly.
tudor (*tud*, II-90)	m	,,	2, 6, 8	—	—	Similar to *oskar*; weak alleles affect only pole cells. No null alleles known.
valois (*vls*, II-53)	m	,,	6	—	—	Similar to *oskar*; cellularization often incomplete.
vasa (*vas*, II-51)	m	,,	6	—	—	Similar to *oskar*
**hunchback* (*hb*, III-48)	m, z	segment gap	1, 2, 4, 15, 34	17	16, 17	'Cardinal gene'; see Fig. 5; maternal expression partially rescues mutant phenotype, but is not required for normal segmentation; allelic with *Rg* (*pbx*)
**Kruppel* (*Kr*, II-107.6)	z	,,	1, 2, 3, 18, 19, 20	16, 21, 22	16, 23	'Cardinal gene'; see Fig. 5; mutants also eliminate Malpighian tubules
knirps (*kni*, III-47)	z	,,	1, 2, 4	—	—	'Cardinal gene'? see Fig. 5
giant (*gt*, I-1)	z	,,	5, 57, 96	—	—	Mutants disrupt head and A4–8
tailless (*tll*, III-102)	z	,,	4, 24	—	—	Zygotic mutant phenotype similar to maternal effect of *torso*, but limited to ectoderm.
unpaired (*upd*, I-59)	z	Segment gap/irregular defect	5, 57	—	—	Mutants disrupt principally T2 and A5
hopscotch (*l(1)hop*)	m, z	Segment gap/irregular defect	97	—	—	Maternal or zygotic function sufficient for normal segmentation Absence disrupts T2, T3, A4, A5, A8.

Table 1. *Continued*

Gene name (symbol, locus[a])	Maternal or zygotic expression	Classification by phenotype	References: Embryonic phenotype	References: Molecular cloning and structure	References: Pattern of expression	Comments
PAIR-RULE AND SEGMENT POLARITY GENES						
**even-skipped* (*eve*, II-55)	z	pair rule	1, 2, 3, 33	25, 27	25, 26, 27	Null mutants eliminate all segmental periodicity; gene contains homeobox. See Fig. 4.
**hairy* (*h*, III-27)	z	,,	1, 2, 4, 28	29, 30	31	Pattern deletions out of frame with, but overlap, those of *ftz*. See Fig. 4; function also required for adult bristle pattern
runt (*run*, I-65)	z	,,	1, 2, 5, 35, 36	—	—	Pattern deletions cover more than full metamere; mutant also shows segment polarity reversals
**fushi-tarazu* (*ftz*, ANT-C)	z	,,	2, 4, 37	38, 39, 42	40, 41, 45	Pattern deletions correspond to even numbered parasegments See Fig. 4. Gene contains *Antp*-like homeobox.
**paired* (*prd*, II-45)	z	,,	1, 2, 3, 33	43	43	Pattern deletions similar to *hairy*. Gene contains homeobox, and sequences homologous to *bicoid* and *gooseberry*. See Fig. 4.
odd-paired (*opa*, III-48)	z	,,	2, 4	—	—	Pattern deletion complementary to *paired*
odd-skipped (*odd*, II-08)	z	,,	1, 2, 3, 33	—	—	Pattern deletions cover less than full metamere, also shows segment polarity reversals
sloppy-paired (*slp*, II-08)	z	,,	2, 3	—	—	
**engrailed* (*en*, II-62)	z	pair rule/segment polarity	1, 2, 3, 46	46, 47, 48	45, 48, 49, 50, 51	Mutant shows variable pair-rule fusions and segment polarity reversals. Expression defines P compartments
**gooseberry* (*gsb*, II-104)	z	segment polarity	1, 2, 3, 55, 59	52	52	Posterior compartments replaced by anterior duplications. Phenotype defined only by deficiencies of at least two genes
**wingless* (*wg*, II-30)	z	,,	1, 2, 3, 53, 55	54	54	Null mutants abolish periodicity of cuticular pattern
armadillo (*arm*, I-1)	m, z	,,	2, 5, 55, 56, 57	—	—	Polarity phenotype similar to *gooseberry*, autonomous in clones. Lack of maternal function causes egg shape defect.
cubitus-interruptus (ci^D, IV-0)	z	,,	1, 2, 59	—	—	Polarity phenotype of null allele similar to *gooseberry*; Ci^D shows gain of function phenotype. Allelic with *l(4)13*
fused (*fu*, I-59.5)	m, z	,,	1, 2, 55, 57, 58	—	—	Polarity phenotype similar to *gooseberry*; maternal or zygotic expression sufficient for normal segmentation
hedgehog (*hh*, III-90)	z	,,	1, 2, 4, 59	—	—	Null mutant phenotype like *wingless*, but head normal.
naked (*nkd*, III-47)	z	,,	4	—	—	Eliminates denticle belts
patched (*ptc*, II-59)	z	,,	3	—	—	Null mutants show duplication of segment boundary region in reversed polarity.
dishevelled (*l(1)dsh*, I-34)	m, z	,,	55	—	—	Maternal or zygotic function sufficient for normal segmentation. Absence of function results in phenotype like *wingless*

Table 1. *Continued*

Gene name (symbol, locus[a])	Maternal or zygotic expression	Classification by phenotype affected segments (parasegments)	References: Embryonic phenotype	References: Molecular cloning and structure	References: Pattern of expression	Comments
HOMEOTIC GENES SHOWING REGION SPECIFIC EXPRESSION[b]						
proboscipedia (*pb*, ANT-C)	z	(adult transformation)	37	—	—	Mutants show no detectable embryonic phenotype
Deformed (*Dfd*, ANT-C)	z	Segment transformation Mandible/maxilla (PS 0/1)	60, 61	61, 62	63, 64, 65	
Sex combs reduced (*Scr*, ANT-C)	z	Segment transformation Labial/T1 (PS 2/3)	4, 37, 66	67	57, 65, 67, 68	
Antennapedia (*Antp*, ANT-C)	z	Segment transformation T1–T3 (PS 3–5)	4, 37, 66, 69	70–73	74–78	
Ultrabithorax (*Ubx*, BX-C)	z	Segment transformation T3–A7 (PS 5–13)	66, 79, 80	81–83	84–86	
Abdomunal-A (*abd-A*, BX-C)	z	Segment transformation A2–A8 (PS 7–14)	79, 87, 88, 89	90	68	Equivalent to *iab-2* or *iab-2*/*iab-4* region of BX-C
Abdominal-B (*Abd-B*, BX-C)	z	Segment transformation A5–A8/9 (PS 10–14)	79, 87, 88, 91	90	63, 68, 95	Equivalent to *iab-7* or *iab-5*/*iab-8* region of BX-C
caudal (*cad*, II-38E)	m, z	Loss/transformation A10/11 (PS 15) or telson	92	93, 94	92	Absence of zygotic function alone affects anal pads; absence of both maternal and zygotic function also variably disrupts segmentation
F90-2 (*Ant-C*)	z	Uncertain	—	95	95	Mutant phenotype not described, but probably corresponds to *labial* (101). Expressed anterior to *Dfd* and posterior to *Abd-B*

Genes have been grouped somewhat arbitrarily according to the classification of their phenotypes. Within each group, genes for which molecular information is available have been placed first. These are marked with an asterisk.

[a] Gene loci are shown by chromosome (Roman numeral) and genetic map position, except for genes in the ANT-C (III, 47.5), the BX-C (III, 58.8) or, in the case of *caudal*, where only chromosome hybridization data are available.

[b] Only the homeotic genes of the ANT-C and BX-C are listed, together with one other, *caudal*, for which molecular and mutant data are available. All of these genes contain a homeobox, and all are known to be expressed at restricted locations along the AP axis. (The inclusion of *proboscipedia* is based on the unpublished data of M. Pulz and T. Kaufman). Other genes mutate to homeotic phenotypes, but no others are clearly analogous to the 'segment selector' genes of the ANT-C and BX-C.

Key to references

(1) Nüsslein-Volhard & Wieschaus, 1980; (2) Nusslein-Volhard *et al.* 1982; (3) Nüsslein-Volhard *et al.* 1984; (4) Jurgens *et al.* 1984; (5) Wieschaus *et al* 1984*a*; (6) Schüpbach & Wieschaus, 1986; (7) Lehmann & Nüsslein-Volhard, 1986; (8) Boswell & Mahowald, 1985; (9) Frohnhöfer & Nüsslein-Volhard, 1986; (10) Frigerio *et al.* 1986; (11) Bull, 1966; (12) Mohler & Wieschaus, 1986; (13) Lohs-Schardin, 1982; (14) Gans *et al.* 1975; (15) Lehmann & Nüsslein-Volhard, 1987; (16) Jäckle *et al.* 1986; (17) Tautz *et al.* 1987; (18) Gloor, 1950; (19) Wieschaus *et al.* 1984*b*; (20) Seifert *et al.* 1986; (21) Preiss *et al.* 1985; (22) Rosenberg *et al.* 1986; (23) Knipple *et al.* 1985; (24) Strecker *et al.* 1986; (25) Macdonald *et al* 1986; (26) Harding *et al.* 1986; (27) Frasch *et al.* 1987; (28) Ingham *et al.* 1985*c*; (29) Holmgren, 1984; (30) Ish-Horowicz *et al.* 1985; (31) Ingham *et al.* 1985*a*; (33) Nüsslein-Volhard *et al.* 1985; (34) Bender *et al.* 1987; (35) Gergen & Wieschaus, 1985; (36) Gergen & Wieschaus, 1986*a*; (37) Wakimoto & Kaufman, 1981; (38) Laughan & Scott, 1984; (39) Kuroiwa *et al.* 1984; (40) Hafen *et al.* 1984*a*; (41) Carroll & Scott, 1985; (42) Weiner *et al.* 1984; (43) Kilcherr *et al.* 1986; (45) Weir & Kornberg, 1985; (46) Kornberg, 1981; (47) Poole *et al.* 1985; (48) Fjose *et al.* 1985; (49) Kornberg *et al.* 1985; (50) Ingham *et al.* 1985*b*; (51) Dinardo *et al.* 1985; (52) Bopp *et al.* 1986; (53) Baker, 1987*a*; (54) Baker, 1987*b*; (55) Perrimon & Mahowald, 1987; (56) Wieschaus & Riggleman, 1987; (57) Gergen & Wieschaus, 1986*b*; (58) Martinez-Arias, 1985; (59) Martinez-Arias & Ingham, 1985; (60) Kaufman, 1983; (61) Regulski *et al.* 1987; (62) Regulski *et al* 1985; (63) McGinnis *et al.* 1984; (64) Chadwick & McGinnis, 1987; (65) Martinez-Arias *et al.* 1987*a*; (66) Struhl, 1983; (67) Kuroiwa *et al.* 1985; (68) Harding *et al.* 1985; (69) Kaufman & Abbott, 1984; (70) Garber *et al.* 1983; (71) Scott *et al.* 1983; (72) Schneuwly *et al* 1986; (73) Laughon *et al.* 1986; (74) Hafen *et al.* 1983; (75) Levine *et al.* 1983; (76) Martinez-Arias, 1986; (78) Carroll *et al.* 1986*a*; (79) Lewis, 1978; (80) Lewis, 1981; (81) Bender *et al.* 1983; (82) Hogness *et al.* 1985; (83) Weinzierl *et al.* 1987; (84) Akam & Martinez-Arias, 1985; (85) White & Wilcox, 1985; (86) Beachy *et al.* 1985; (87) Sanchez-Herrero *et al.* 1985; (88) Tiong *et al.* 1985; (89) Morata *et al.* 1983; (90) Karch *et al.* 1985; (91) Casanova *et al.* 1986; (92) Macdonald & Struhl, 1986; (93) Mlodzik *et al.* 1985; (94) Mlodzik & Gehring, 1987; (95) Hoey *et al.* 1986; (96) Petschek *et al.* 1987; (97) Perrimon & Mahowald, 1987; (98) Degelman *et al.* 1986; (99) Nüsslein-Volhard, 1977; (100) Zalokar, M. *et al.* 1975; (101) Kaufman, T. C. (1983); R. Diederich and T. Kaufman, personal communication.

searches for mutations affecting the pattern of structures in the cuticle of dying embryos (Nüsslein-Volhard & Wieschaus, 1980; Wieschaus, Nüsslein-Volhard & Jürgens, 1984*a*; Jürgens, Wieschaus, Nüsslein-Volhard & Kluding, 1984; Nüsslein-Volhard, Wieschaus & Kluding, 1984*b*; Perrimon, Engstrom & Mahowald, 1984; Schüpbach & Wieschaus, 1986).

Mutations in the segmentation genes have a variety of effects, which fall into three broad classes (Nüsslein-Volhard & Wieschaus, 1980; Nüsslein-Volhard, Weischaus & Jürgens, 1982). Mutations in the 'gap genes' cause embryos to develop with large gaps in the array of segments. Mutations in the 'pair-rule' genes cause embryos to develop with only half the normal number of segments, and the characteristics of the remaining segments suggest that elements of each alternate segment have been deleted. Mutations in the 'segment polarity' genes affect the sequence of pattern elements within segments, resulting in extensive pattern deletions, duplications and reversals of polarity within each segment.

engrailed *as a marker for segmentation*

The segmentation gene *engrailed* has played a unique role in our understanding of metameric pattern in *Drosophila*. It was first identified by the effects on adult flies of what we now recognize to be a rather unusual mutant allele. This mutation allows adult survival, but in several segments it results in the partial replacement of posterior structures by anterior ones (Tokunaga, 1962; Garcia-Bellido & Santamaria, 1972). Morata & Lawrence (1975) showed by genetic analysis that *engrailed* was required to maintain the lineage boundary that separates, and defines, the anterior and posterior compartments within each segment primordium of the developing adult. Subsequently, more typical *engrailed* mutations were found to display an embryonic segmentation defect, resulting both in the fusion of segments and in alterations of segment polarity (Kornberg, 1981).

engrailed and many other of these genes have now been cloned. In some cases, antibodies are available to detect the proteins that they encode. Thus, it is possible to examine the process of pattern generation with molecular probes. The result has been a remarkable triumph for developmental genetics. Virtually all of the genes identified by mutant phenotypes in late embryos or adults have proved to be involved in the early processes of embryonic patterning; many appear to play key roles. Expression of the *engrailed* gene, in particular, has provided a molecular marker to visualize the metameric pattern of developing embryos as it is first established.

The metameric organization of the early germ band

I shall use the term germ band to refer to the whole region of the developing egg that will give rise to the embryo proper (Anderson, 1973). In *Drosophila*, cells fated to form the germ band cover most of the surface of the blastoderm and about two-thirds of these will give rise to the metameric or segmented part of the body (Poulson, 1950; Campos-Ortega & Hartenstein, 1985; Technau, 1987). The remaining cells generate the procephalon, or head, the endoderm and the amnioserosa, an extraembryonic membrane (Fig. 1).

The characteristic morphology of the early germ band is generated from the blastoderm by the processes of gastrulation, without any cell division (Fig. 2). At first, the region that will give rise to the segmented part of the body appears as a uniform double-layered structure, mesoderm within and ectoderm without. The first morphological sign of segmentation appears in the mesoderm, as a series of repeated thickenings. Shortly thereafter, grooves appear in the outer surface of the ectoderm (Poulson, 1950; Turner & Mahowald, 1977). These grooves do not demarcate the future segments, but parasegments (Martinez-Arias & Lawrence, 1985), metameric units which include cells in the posterior part of one segment and the anterior part of the next. 14 parasegments are clearly visible, though at least some regions of parasegments 0 and 15 are probably specified in the initial pattern. Cells in the first four parasegments rapidly rearrange to generate the mandibular, maxillary and labial lobes of the mouthparts. The following thoracic and abdominal parasegments remain very similar in structural organization until much later in embryogenesis (Turner & Mahowald, 1979; Petschek, Perrimon & Mahowald, 1987).

The metameric organization of the germ band is displayed most beautifully by the pattern of expression of the *engrailed* gene. *In situ* hybridization (Kornberg, Siden, O'Farrell & Simon, 1985) and antibody staining (DiNardo, Kuner, Theis & O'Farrell, 1985) reveal that the *engrailed* gene product is expressed in 15 evenly spaced rows of cells within the germ band. These rows, each only two or three cells wide, lie immediately posterior to the grooves that define each parasegment (Ingham, Martinez-Arias, Lawrence & Howard, 1985*b*), suggesting that the parasegments are identical to the lineage units defined by a P–A pair of compartments (Fig. 3).

Metamerism of the germ band is also apparent in the pattern of expression of homeotic genes and, here again, parasegments appear to be the metameric units defined by the earliest patterns of gene expression. At least seven homeotic selector genes, four in the Antennapedia complex (ANT-C; Kaufman, 1983)

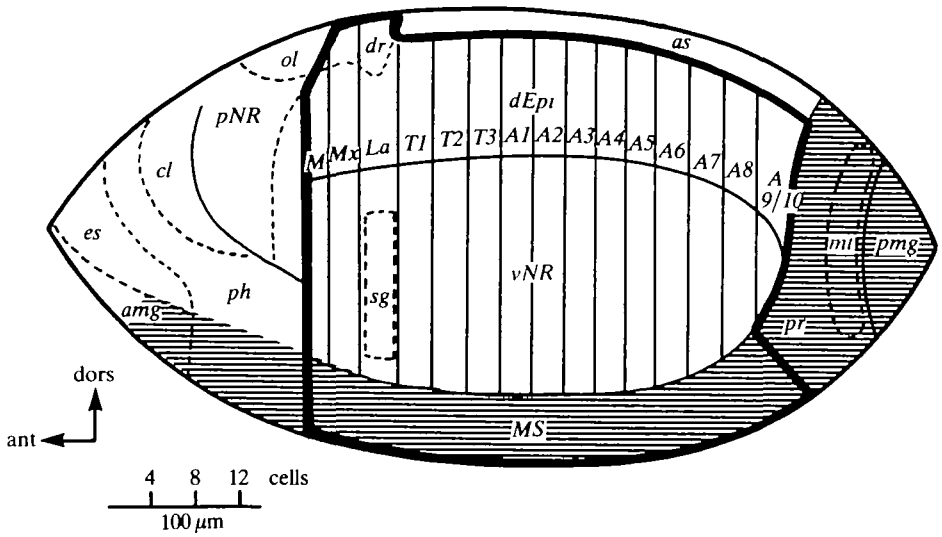


Fig. 1. A fate map of the *Drosophila* blastoderm (from Campos-Ortega & Hartenstein, 1985). The shape is a planimetric reconstruction of the blastoderm surface. All parts of the egg surface contribute to the embryo proper, except the narrow dorsal primordium for the amnioserosa (*as*). Hatched areas will invaginate at gastrulation. Cells that will generate metameric structures are enclosed by a thick line. Abbreviations: *amg*, anterior midgut; *as*, amnioserosa; *cl*, clypeolabrum; *dEpi*, dorsal epidermis; *dr*, dorsal ridge; *es*, oesophagus; *mt*, Malpighian tubules; *MS*, mesoderm; *ol*, optic lobe; *ph*, pharynx; *pmg*, posterior midgut; *pNR*, procephalic neurogenic region; *pr*, proctodeum; *sg*, salivary gland; *vNR*, ventral neurogenic region; *M*, mandibular segment; *Mx*, maxillary segment; *La*, labial segment; *T1–T3*, thoracic segments; *A1–A10*, abdominal segments; ant, anterior; dors, dorsal.

Fig. 2. Early development of *Drosophila melanogaster*. Diagrams on the left show the morphology of stages during early development. (Timings are given as hours at 25°C after fertilization.) Corresponding panels on the right illustrate patterns of gene activity established at each of these stages. (Numbers locate the anterior of parasegments.)

(A) Cleavage stage 7–8 of Foe & Alberts (1983). Nuclei are shown migrating to the periphery of the egg. Cytoplasmic transplantation experiments show that determinants are localized at the poles of the egg. Crosses – location of maternally derived transcripts from the *bicoid* gene; dots – location of polar granules and of posterior determinants dependent on the *oskar* group genes. (The polar granules subsequently segregate to the pole cells.) Maternal transcripts of the genes *hunchback* (pink) and *caudal* (not shown) are initially uniformly distributed, but graded distributions are established during early cleavage.

(B) Syncytial blastoderm (cleavage stage 12). Most nuclei reach the perimeter of the egg and become transcriptionally active. Pole cells have formed. Yolk segregates to the middle of the egg, leaving a peripheral layer of clear cytoplasm. Localized transcription of the cardinal genes *hunchback* and then *Krüppel* (brown) is established from the zygotic genome. The transcriptional patterns of these genes become more elaborate at later stages (not shown); both are subsequently expressed in an additional zone at the posterior of the cellular blastoderm.

(C) Cellular blastoderm (cleavage stage 14A/B; embryonic stage 5 of Campos-Ortega & Hartenstein, 1985). Cell membranes are extending down from the egg cortex, but cells are not yet closed. The activity of maternal determinants falls rapidly. Expression of the pair-rule genes, first apparent at about cleavage stage 13, resolves into a well-defined pattern of overlapping stripes showing double segment periodicity. (*fushi-tarazu* domains – yellow/green; *even-skipped* domains, purple/pink; each of these domains is shown as subdivided by the superimposed expression of one other gene (blue) but the actual sequence of cell states defined by all of the pair-rule genes is more complex. See Fig. 4.)

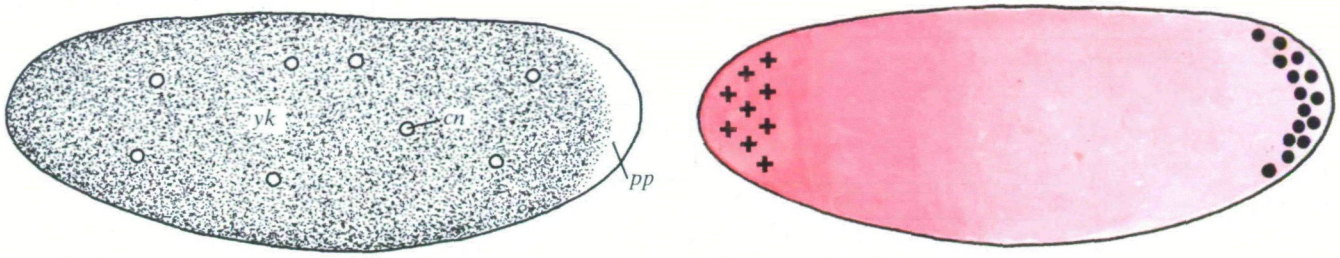
(D) Gastrulation (embryonic stage 7). Mesoderm invaginates ventrally; anterior and posterior midgut invaginations form the endoderm. Pole cells lie within the posterior midgut invagination and are carried dorsally and forward by the beginning of germ band elongation. The transient cephalic furrow demarcates an approximate boundary between head and body regions. Cells expressing high levels of *engrailed* protein (orange) begin to define the definitive metameric repeat. Expression of the pair-rule genes continues to evolve (not shown).

(E) After germ band extension (embryonic stage 10). The metameric region forms a uniform double-layered structure extending around the posterior pole of the egg and the most posterior segments are apposed to the head. Cell division is underway, most blastoderm cells undergoing two or three rounds of mitosis before differentiation. Cells expressing *engrailed* (orange) and *wingless* (green) define the definitive metameric pattern and flank presumptive parasegment borders. Products of the pair-rule genes are decaying rapidly.

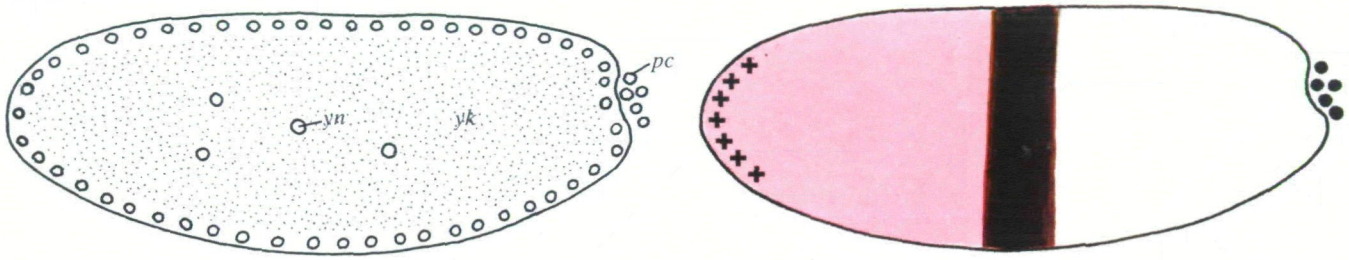
References for the patterns of gene expression are listed in Table 1. Abbreviations as Fig. 1 and *ect*, ectoderm; *yk*, yolk; *yn*, yolk nuclei; *pp*, polar plasm; *pc*, pole cells; *cf*, cephalic furrow; *st*, stomodeum.

Morphology *Gene activity*

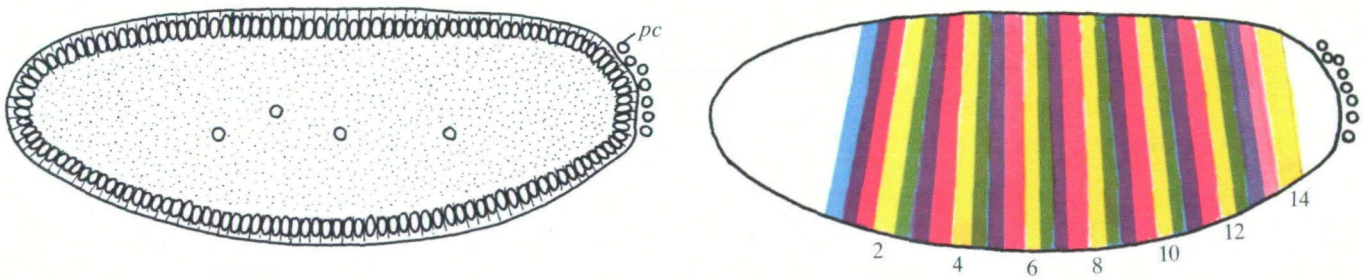
A. Nuclear migration (1·25 h, ~128 nuclei)



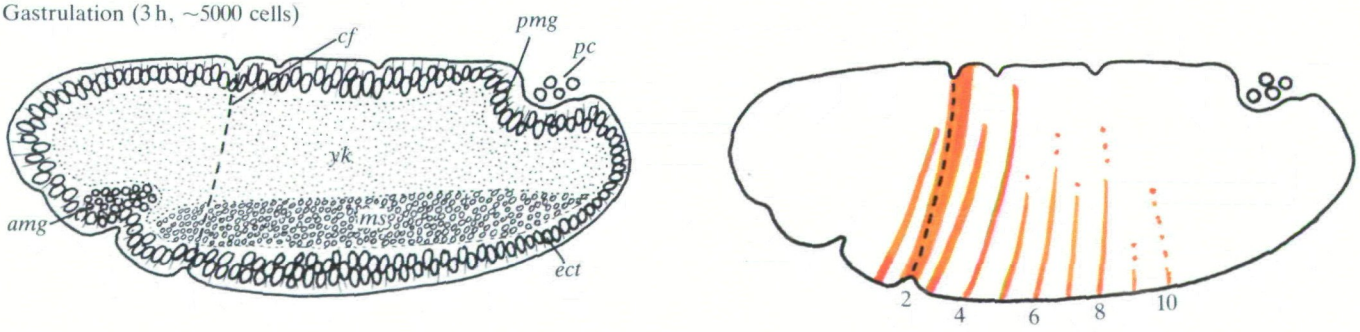
B. Syncytial blastoderm (2 h, ~1500 nuclei)



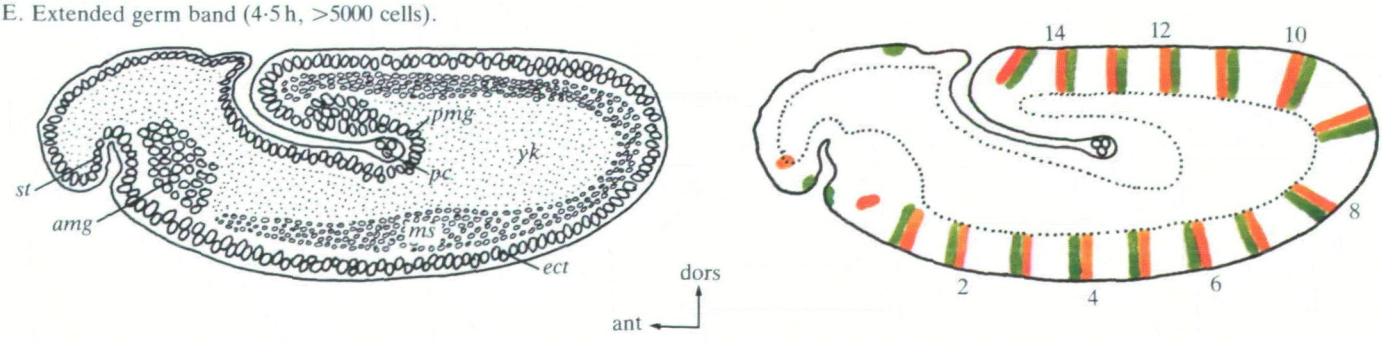
C. Cellular blastoderm (2·5 h, ~5000 cells)



D. Gastrulation (3 h, ~5000 cells)



E. Extended germ band (4·5 h, >5000 cells).



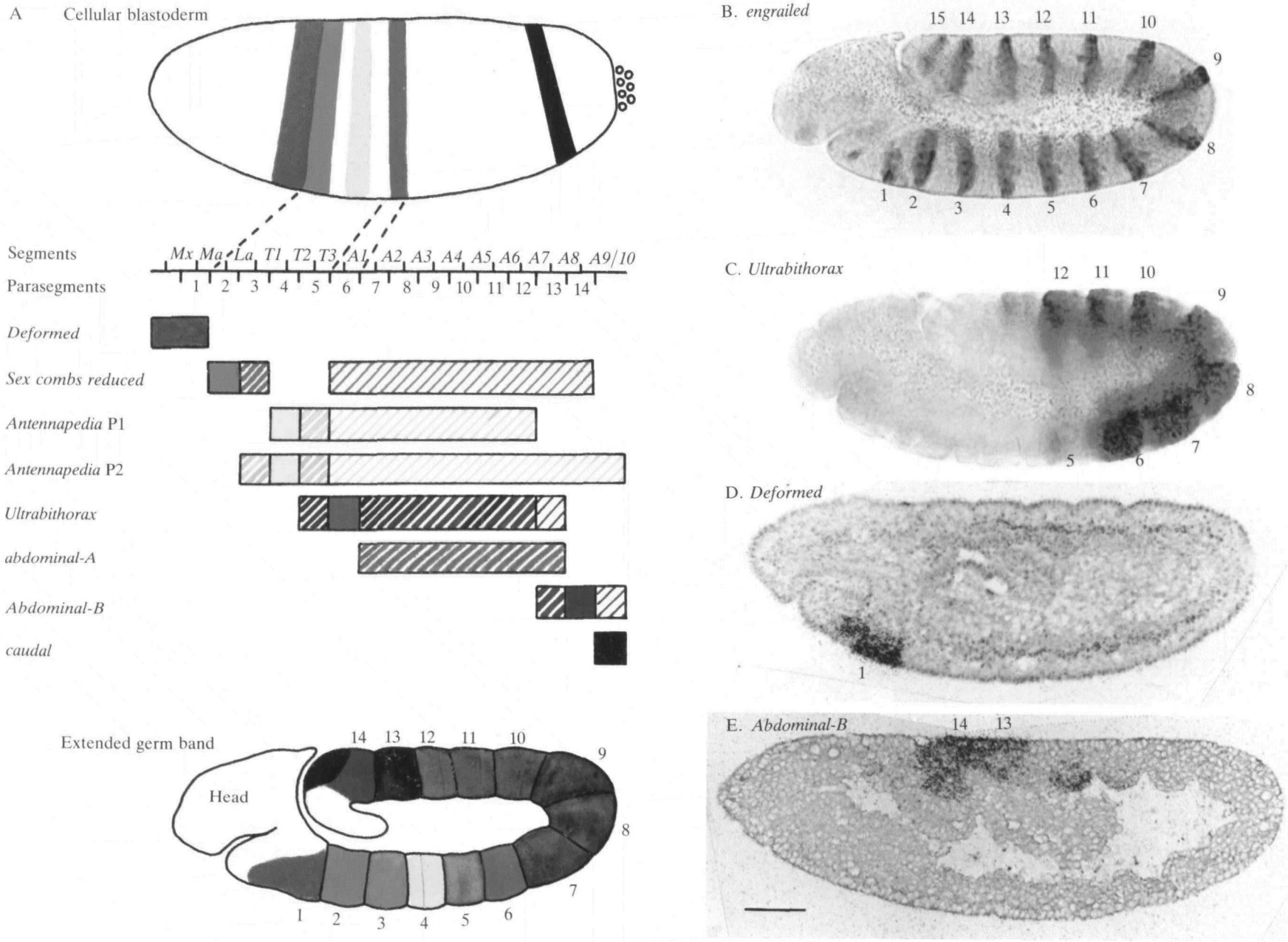

A
Cellular blastoderm
Segments
Mx Ma La T1 T2 T3 A1 A2 A3 A4 A5 A6 A7 A8 A9/10
Parasegments
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Deformed
Sex combs reduced
Antennapedia P1
Antennapedia P2
Ultrabithorax
abdominal-A
Abdominal-B
caudal
Extended germ band
Head
14 13 12 11 10
9
8
7
1 2 3 4 5 6
B. engrailed
15 14 13 12 11 10
9
8
1 2 3 4 5 6 7
C. Ultrabithorax
12 11 10
9
8
5 6 7
D. Deformed
1
E. Abdominal-B
14 13

Fig. 3. (A) Expression of homeotic genes in the early *Drosophila* embryo. Domains of expression for each homeotic gene are indicated schematically by the array of horizontal bars below the parasegment/segment ruler. Colour has been used to delimit regions of the stage-10 embryo within which transcripts of each gene can be detected. At this stage, shortly after germ band extension and before formation of the gnathal lobes, the domains of expression for most, if not all, of these genes appear to respect parasegment boundaries. Blocks of solid colour show parasegments where a single homeotic gene is expressed at high levels in most or all cells of the ectoderm. Elsewhere, two or more homeotic genes are expressed and contribute to the final morphology. In these regions, broad stripes of colour have been used when the activity of a gene is known to be important for normal development; narrow stripes for regions where transcripts have been detected, but where mutations have no apparent effect on embryonic development. Domains of expression in the mesoderm sometimes differ from those shown here. Transcription from the two *Antennapedia* promoters (P1 and P2) is depicted by separate bars.

Changes in the deployment of these genes with time are emphasized by depicting homeotic gene expression in the cellular blastoderm (above) and in the extended germ band (stage 10, below). Cellular blastoderm: *Deformed* (grey) and *Caudal* (black) are expressed in sharply defined zones during cellularization of the blastoderm. These delimit the metameric region of the embryo. *Sex combs reduced*, *Antennapedia* and *Ultrabithorax* are all transcribed at low level in broad, overlapping domains within this metameric region, but transcripts accumulate to high levels only in narrow stripes, each about four cells wide. These probably coincide with the primordia for parasegments 2 (*Scr*), 4 (*Antp* P2 promoter) and 6 (*Ubx*). The *Antp* P1 promoter is active at blastoderm in a wider region (parasegments 4–6) but transcripts from this 100 kb transcription unit are not completed until well after gastrulation. *abdominal-A* is not transcribed at high levels until after gastrulation; the early transcription of *Abdominal-B* is not yet well defined.

Extended germ band: overlapping domains of homeotic gene expression give most parasegments of the extended germ band a unique identity, shown here by the mixing of colours. Thus parasegment 3 (orange) expresses both *Scr* (magenta) and *Antp* (yellow); parasegment 5 (green) expresses both *Antp* and *Ubx* (blue). *abd-A* is exceptional in that it always acts either in combination with *Ubx* (parasegments 7–12) or with *Abd-B* (parasegment 13). (In this diagram, the domain of expression shown for each gene is limited to that in which its expression is known to affect segment development.)

In several cases, the domain within which each gene is transcribed and/or required changes during later development. Most notably, *abd-B* is expressed and required in parasegments 10–12 during later stages of embryogenesis and for adult development, but it is not detectably expressed in these regions in the early extended germ band. Hence the early development of parasegments 7–12 appears to involve qualitatively similar selector gene activity.

(B) Extended germ band embryo (late stage 10) stained for *engrailed* protein with the antibody of DiNardo *et al.* (1985). Stripes of expression define the metameric repeat. *engrailed* stripes are numbered to correspond with parasegments, though the parasegmental grooves are not yet visible. Patches of expression are just becoming apparent in the head. (Whole mount of demembranated embryo prepared as described by White & Lehmann, 1986; antibody visualized by peroxidase staining with Vectastain ABC reagent.)

(C) A similar preparation of a slightly older embryo stained for *Ubx* protein with the antibody of White & Wilcox (1985). Parasegmental grooves are clearly visible. Parasegments 6–12 contain high levels of *Ubx* protein, parasegments 5 and 13 lower levels. Modulation of the level of *Ubx* protein within each parasegment is already clearly visible. The P compartments, corresponding to the *engrailed* stripes in B above, contain much lower levels of the *Ubx* protein than the A compartments.

(D) Sagittal section of a stage-11 embryo hybridized with a probe for transcripts of the *Dfd* gene. Autoradiography reveals *Dfd* transcripts in parasegment 1 and in neural tissue lying immediately anterior to this.

(E) Section of a similarly staged embryo hybridized with a probe for transcripts of the *Abdominal B* gene. The transcriptional organization of *Abdominal B* is not yet clear, but this probe (overlying and extending 3′ from the homeobox) hybridizes to most or all *Abd-B* transcripts. In the ectoderm, transcripts are detected only in, and posterior to, parasegment 13. By this stage, they are also conspicuous in the mesoderm of parasegment 12, but they are restricted to the more posterior segments of younger embryos.

Data for the patterns of gene expression are taken from the references in Table 1 and also from the unpublished results of A. Martinez-Arias (*Antp*) A. Rowe (*abd-A*) and E. Sanchez-Herrero (*Abd-B*). A. Martinez-Arias kindly provided the preparations for panels B, C and D; E. Sanchez-Herrero that for panel E. (The different appearance of embryos D and E reflects differences in fixation and staining procedures.) Bar, 50 μm.

and three in the bithorax complex (BX-C; Lewis, 1978) are expressed in specific regions of the germ band (Fig. 3; see also Harding, Wedeen, McGinnis & Levine, 1985). Ubiquitous and high level expression of *Sex combs reduced* (*Scr*) defines parasegment 2 (Kuroiwa, Kloter, Baumgartner & Gehring, 1985; Martinez-Arias, Ingham, Scott & Akam, 1987*a*), of *Antennapedia* (*Antp*), parasegment 4 (Carroll *et al.* 1986*a*; Martinez-Arias, 1986;) and of *Ultrabithorax* (*Ubx*), parasegment 6 (Akam & Martinez-Arias, 1985; White & Wilcox, 1985). *Deformed* (*Dfd*) is expressed in the region immediately anterior to parasegment 2, including parasegment 1 but including also more anterior structures (Chadwick & McGinnis, 1987; Martinez-Arias *et al.* 1987*a*). Other parasegments are characterized by the expression of combinations of homeotic genes.

As the germ band develops, this metameric pattern of homeotic gene expression is rapidly obscured. Cell movements disrupt the initial patterns (DiNardo *et al.* 1985). Within cells of a single parasegment, the expression of each homeotic gene is modulated according to position and cell type and, in some structures, homeotic genes are activated in domains that are not obviously related to the metameric specification of the animal (White & Wilcox, 1985; Akam & Martinez-Arias, 1985; Carroll *et al.* 1986*a*; Martinez-Arias, 1986). It is probably an oversimplification to suggest that the homeotic selector genes simply define the identity of metameres at any stage in development, but their observed patterns of expression fit this model most closely in the early germ band stages.

A scheme for pattern formation along the A–P axis

A picture is emerging of the processes that generate the metameric pattern in the germ band (Fig. 2). There is no evidence for any periodic pattern preformed in the egg at the time of fertilization. The only localized maternal determinants are probably at the poles of the egg (Sander, 1984; Frohnhöfer, Lehmann & Nüsslein-Volhard, 1986; Frohnhöfer & Nüsslein-Volhard, 1986; Lehmann & Nüsslein-Volhard, 1986). During the early stages of cleavage, these determinants coordinate pattern and polarity throughout the embryo. The earliest signs of this activity are the generation of a gradient in the distribution of at least one maternally encoded product (Mlodzik, Fjose & Gehring, 1985; Macdonald & Struhl, 1986) and the localized activation of a small set of genes, (the segment gap genes) at defined positions along the A–P axis of the egg (Knipple *et al.* 1985; Tautz *et al.* 1987). Cross regulatory interactions between these genes sharpen the boundaries between the regions within which each is active (Jäckle *et al.* 1986) and so a relatively precise set of spatial domains are established along the axis of the egg. I shall refer to these as the cardinal domains, a term suggested by Meinhardt (1986; see below).

At almost the same time, another set of genes (the pair-rule genes) are activated within the segmented body region. Initially the transcription of these genes is either uniform, or simply localized, but complex periodic patterns rapidly evolve (Hafen, Kuroiwa & Gehring, 1984*a*; Ingham, Howard & Ish-Horowicz, 1985*a*; Carroll & Scott, 1985; Harding *et al.* 1986; Macdonald, Ingham & Struhl, 1986; Kilcherr *et al.* 1986). These patterns characteristically exhibit a stage showing a double segment periodicity. Local order in this evolving pattern probably depends on some reaction diffusion mechanism, but long-range order requires an interaction between the products of the genes that define cardinal domains and the pair-rule genes or their products (Carroll & Scott, 1986; Carroll, Winslow, Schüpbach & Scott, 1986*b*; Ingham, Ish-Horowicz & Howard, 1986).

The pattern generated by the gap and pair-rule genes is transient and can appropriately be described as a prepattern (Stern, 1968). It is elaborated during cellularization of the blastoderm and decays rapidly during gastrulation and formation of the germ band. It serves, however, to establish a series of qualitatively different cell states (the 'singularities' of Stern) that define the patterns of expression of *engrailed* (Howard & Ingham, 1986) and of other genes that mediate the definitive segment pattern.

Recent results suggest that the same cardinal domains serve to define the initial regions within which each homeotic gene is potentially active (White & Lehmann, 1986; Martinez-Arias and M. Akam, unpublished results). Subsequently, the evolving patterns of homeotic gene expression reflect their interactions with both the pair-rule generated segmental prepattern (Ingham & Martinez-Arias, 1986; Duncan, 1986), with each other (Hafen, Levine & Gehring, 1984*b*; Struhl & White, 1985), and with *engrailed* and other persistent components of the segmental pattern (Martinez-Arias & White, 1987).

In the following sections I elaborate on various aspects of this scheme. I progress backwards in developmental time, because the analysis of mutations affecting each developmental stage relies on the interpretation of altered patterns appearing later in development.

The appearance of the definitive segment pattern

The expression of the *engrailed* gene provides a molecular assay for the developing segment pattern.

By the time the germ band is fully extended, the *engrailed* stripes are remarkably similar throughout the segmented region of the body (Fig. 3), but they do not all appear simultaneously. The first cells to express high levels of *engrailed* protein do so just as gastrulation is starting (DiNardo *et al.* 1985). These cells lie ventrally, in the region of the future maxillary segment. They will come to form the *engrailed* stripe of parasegment 2. In the next 30 min, the pattern spreads dorsally and posteriorly. A similar time course for the evolution of pattern has been observed for all of the genes that stripe in the blastoderm, and probably indicates that equivalent molecular decisions are taken at slightly different times in different regions of the embryo. As we shall see below, events leading to the activation of *engrailed* in odd- and in even-numbered parasegments are not strictly equivalent, but depend on different elements of the prepattern. Probably for this reason, even-numbered stripes appear before the odd ones, with both sets showing the same progressive spread (Weir & Kornberg, 1985).

After the germ band has extended, *engrailed* protein begins to accumulate in discrete regions of the head and in parts of the hindgut. The *engrailed* gene may therefore play a role in the development of pattern in these parts of the body, but obvious signs of metamerism are now obscured, even at the molecular level. The regions of *engrailed* expression are patches, not stripes, even when they are first apparent; they appear later than in the metameric region and they appear in regions where the underlying molecular prepattern is quite different from that in the metameric region (DiNardo *et al.* 1985).

It is clear that genes other than *engrailed* are necessary from the time of gastrulation onwards to establish or maintain normal pattern within each segment. The most obvious candidates are the known segment polarity genes (Table 1). Only two of these genes have yet been isolated; *wingless* (Baker, 1987*a*,*b*) and *gooseberry* (Bopp *et al.* 1986; Coté *et al.* 1987). Both of these show a pattern of expression comparable with that of *engrailed*. *Wingless* is expressed from gastrulation until late in embryogenesis. In the early germ band embryo, *wingless* transcripts accumulate in a narrow stripe of cells at the posterior margin of each parasegment (Fig. 2). Thus the *wingless* and *engrailed* stripes presumably abut and define the cells either side of each parasegment boundary (Baker, 1987*a*,*b*). The putative *gooseberry* transcripts are also expressed in narrow stripes from gastrulation onwards, but the registration of these with the *engrailed* stripes has not been determined. It remains to be seen how many different regions within each segment are similarly defined in the early germ band by the activity of specific genes.

The prepattern underlying segmentation

Detailed observations of the way in which the *engrailed* stripes first appear suggest that their location must be controlled by a prepattern. Along each of the presumptive stripes, which initially will be only one or at most two cells across, individual cells accumulate relatively high levels of *engrailed* protein. Neighbouring cells along the stripe may transiently show very different levels of *engrailed* protein and cells between stripes show none at all. This suggests that each cell turns the *engrailed* gene on independently and that the precise timing of this event is a stochastic response to an underlying prepattern (DiNardo *et al.* 1985).

The products of the pair-rule genes are directly implicated in the generation of this prepattern. Mutation in any one of them alters the pattern of *engrailed* expression in the extended germ band, (Howard & Ingham, 1986; Martinez-Arias & White, 1987; S. DiNardo and P. H. O'Farrell, personal communication; see Fig. 4) implying that all of them disrupt the initial metameric organization of the embryo.

Many of the pair-rule genes have now been cloned and the patterns of expression of four have been described (see Table 1). In all of these cases, the genes are first transcribed in the syncytial blastoderm, at or very soon after the time that transcription of the zygotes own genes is first activated (Edgar & Schubiger, 1986) and about 40 min before the *engrailed* pattern first appears. A striped pattern of pair-rule transcript distribution evolves while the cellular blastoderm is forming. Characteristically, the pattern at its climax in the cellular blastoderm is one of seven annuli or stripes of transcript accumulation encircling the embryo. These are uniform in size, except at the ends, and spaced at two-segment intervals throughout the segmented body region (Figs 2, 4).

At gastrulation, the cells of the blastoderm are finally closed off from the yolk syncytium (Rickoll, 1976). Within these cells, the distributions of the pair-rule gene products define a series of different cell states in a repeating pattern along the A–P axis. Most components of this pattern have a two-segment periodicity. However, during and after gastrulation the patterns of pair-rule gene expression continue to evolve. In some cases, a pattern with single-segment periodicity appears, either by the splitting of existing stripes (*paired*, Kilcherr *et al.* 1986) or by the intercalation of additional ones (*even-skipped*, *eve*, MacDonald *et al.* 1986). Finally, by the time the germ band is fully extended, transcripts of the pair-rule genes are virtually undetectable in the metameric region. At least in the case of *fushi-tarazu* (*ftz*, read as

futz) and *eve*, the protein products are rapidly disappearing (Carroll & Scott, 1985; Frasch *et al.* 1987). Some of the pair-rule genes are expressed again as the nervous system develops, but this later expression shows no trace of a pair-rule repeat (DiNardo *et al.* 1985), and is under quite different regulation from the early expression in the blastoderm (Hiromi, Kuroiwa & Gehring, 1985).

It is not yet clear how the transient blastoderm prepattern regulates the activity of *engrailed*. Eliminating the product of any pair-rule gene alters the pattern of *engrailed* expression, but these effects may be direct or indirect, for the pair-rule genes themselves interact (see below). In some cases, the effects of pair-rule mutations on *engrailed* expression are conceptually simple, suggesting a fairly direct interaction. For example, in mutant embryos that lack a functional *ftz* gene, those stripes of *engrailed* expression that normally overlie a *ftz* stripe are absent, but the alternate stripes persist. In *paired* mutants, only these alternate stripes persist. In other cases, however, more complicated pattern alterations result (Fig. 4). It seems likely that both hierarchical (Ingham & Howard, 1985; Ingham & Martinez-Arias, 1987) and combinatorial (O'Farrell & Scott, 1986; Gergen, Coulter & Wieschaus, 1986) interactions define cell states that activate the *engrailed* gene.

Generation of the blastoderm prepattern

When the pair-rule genes are first transcribed, their expression shows no trace of periodic patterning. *hairy* is expressed uniformly throughout virtually the whole egg (Ingham, Howard & Ish-Horowicz, 1985*a*); *ftz* and *eve* throughout the segmented body region only, and *paired* within a localized region around the cephalic furrow (the centre from which the segmental pattern spreads). This suggests that the periodicity of the segment pattern is generated after the genes are initially activated and raises the possibility that the system of pair-rule genes and their products constitute a system that generates pattern *de novo*, using a mechanism analogous to that proposed by Turing (1952), in which reaction and diffusion coefficients define many properties of the pattern (see Meinhardt, 1982). In such a system, it would be the accumulating products of the pair-rule genes themselves that regulate the activity of other members of the set.

In the case of one of the pair-rule genes, *ftz*, we know that regulation must take place at the level of transcription or RNA processing; protein degradation and transport can at most play a secondary role, for when the *ftz* promoter and untranslated leader sequences are fused to heterologous protein-coding sequences (e.g. bacterial β-galactosidase) the resulting RNA and protein distributions are indistinguishable from those of the *ftz* products themselves (Hiromi *et al.* 1985).

One prerequisite for a reaction–diffusion model is that the products of the pair-rule genes should have a life time that is short in comparison to the timescale of pattern evolution. This appears to be the case. In the presence of α-amanitin, *ftz* RNA in the blastoderm has a half-life of about 10 min (Edgar, Weir, Schubiger & Kornberg, 1986). Moreover, in the syncytial blastoderm, the proteins that regulate *ftz* transcription also have a very short lifetime; injection of cycloheximide into eggs just as the *ftz* stripes are forming results in the re-establishment of uniform transcription. However, when the stripes are well established in the cellular blastoderm, the transcription pattern becomes stable to cycloheximide injection (Edgar *et al.* 1986).

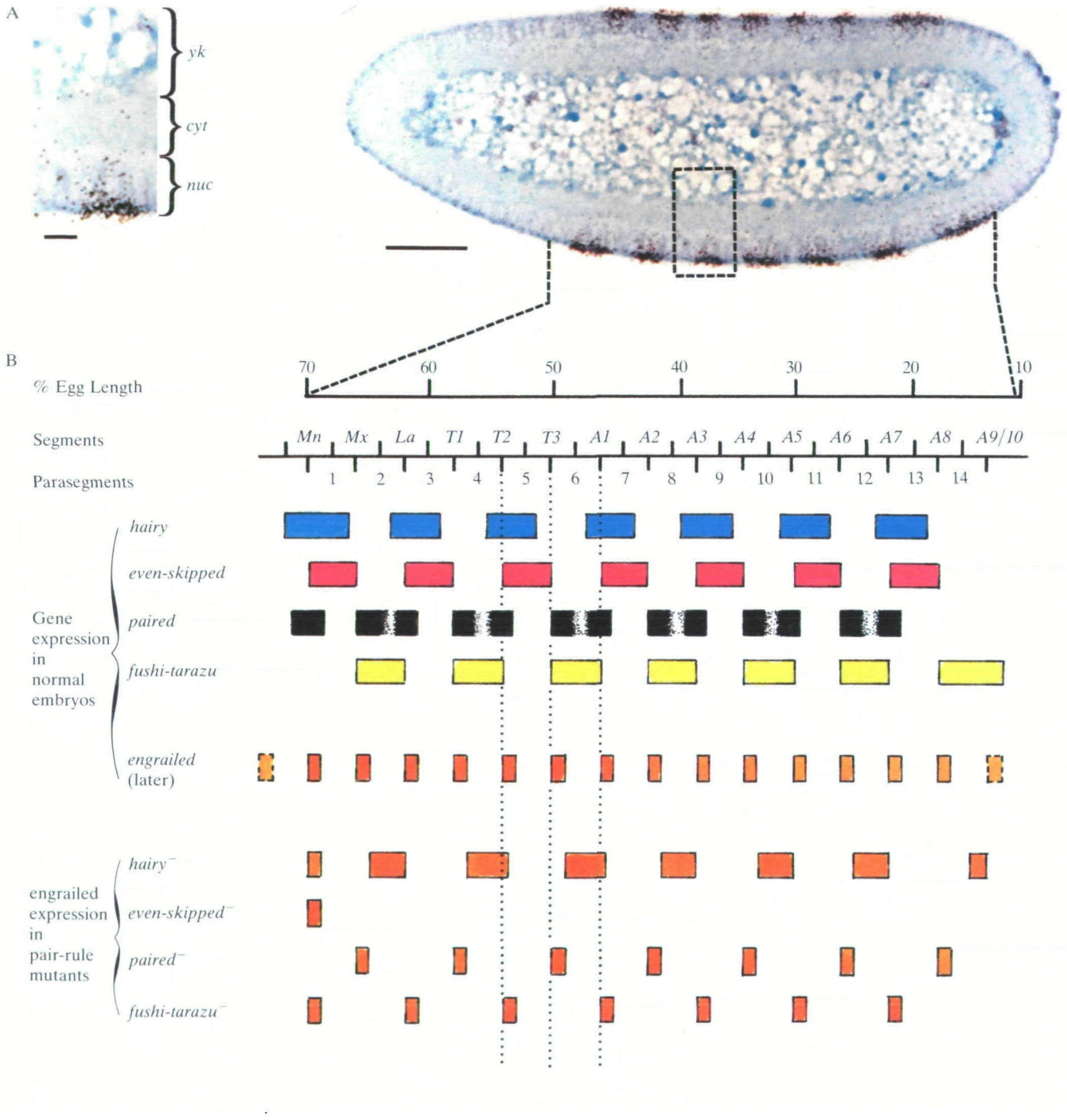
Fig. 4. Expression of segmentation genes in the *Drosophila* blastoderm.

(A) Embryo at cleavage stage 14A/B hybridized with a ^{3}H-labelled probe for transcripts of the pair-rule gene *fushi-tarazu*. The autoradiograph maps the distribution of *ftz* transcripts within the embryo. Compare Fig. 2C. The enlarged panel at left shows the location of nuclei (nuc), lying peripheral to the bulk of the cytoplasm (cyt). Hybridization lies over the cortex of the egg, not over the deeper cytoplasm. Bar, 50 μm; for enlargement, 10 μm.

(B) Approximate registration of pair-rule stripes, *engrailed* expression and metameric units. The patterns of expression are shown for four of the pair-rule genes at about the same stage as the embryo shown above. The later patterns of *engrailed* expression have been projected onto the same diagram, even though at this mid-blastoderm stage hybridization reveals only a single well-defined *engrailed* stripe (stripe 2).

The bands of *engrailed* expression define P compartments and so lie at the anterior margin of each parasegment. Stripes of *even-skipped* and *fushi-tarazu* expression are each approximately four cells wide at mid blastoderm, and appear to lie out of phase with each other. Double-labelling experiments in later embryos suggest that the anterior margins of both *ftz* and *eve* stripes coincide precisely with the *engrailed* stripes, and hence define parasegment boundaries (Lawrence, Johnston, Macdonald & Struhl, 1987). *Hairy* stripes are about the same width, but are displaced slightly with respect to parasegments and overlap those of *ftz*. *Paired* stripes are broader than a single metameric repeat, but the seven stripes split into fourteen before gastrulation.

The bottom part of the figure shows the effect that null mutations in each of the same four pair-rule genes have on the subsequent expression of *engrailed*. In *paired* and *ftz* mutant embryos, it appears that alternate engrailed stripes have simply been deleted. (Autoradiograph provided by P. Ingham; see Ingham *et al.* 1985*a* for further details. Other data taken from references in Table 1.)


A
yk
cyt
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B
% Egg Length
70
60
50
40
30
20
10
Segments
Mn
Mx
La
T1
T2
T3
A1
A2
A3
A4
A5
A6
A7
A8
A9/10
Parasegments
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Gene expression in normal embryos
hairy
even-skipped
paired
fushi-tarazu
engrailed (later)
engrailed expression in pair-rule mutants
hairy⁻
even-skipped⁻
paired⁻
fushi-tarazu⁻

A second expectation of any dynamic model is that the pattern of any one component will depend on the activities of other elements of the system. Such interactions have been observed by monitoring the distribution of transcripts or proteins from one pair-rule gene in embryos that lack the products of another. These experiments reveal a hierarchy among the pair-rule genes. For example, the pattern of *ftz* stripes is abnormal in embryos that lack the product of the *hairy* gene, but the *hairy* pattern is normal in *ftz* mutant embryos (Howard & Ingham, 1986). Of the set of pair-rule genes, only *hairy*, *runt* and *eve* are necessary for the normal pattern of *ftz* expression (Carroll & Scott, 1986); the *ftz* protein itself is not required to establish the normal pattern of *ftz* transcription. Thus *ftz* cannot form an essential part of the mechanism that generates the periodic pattern but *hairy*, *runt* and *eve* may play such a role. *ftz* must play a secondary role in stabilizing the pattern or mediating its effects on subsequent development (see below).

The molecular details of these interactions are not known. Since most of the process takes place in a syncytium, the communication that defines the spatial elements of the pattern must be between genes in adjacent nuclei, rather than between cells. Remarkably, the *ftz* pattern is unaffected when the size and spacing of nuclei in the blastoderm are altered (Sullivan, 1987). In a classical reaction–diffusion model, spatial communication would be mediated by freely diffusible factors. In the context of the syncytial blastoderm, it is likely that the cytoskeletal architecture will play an important role (see Foe & Alberts, 1983). Indeed, it has been noted that the transcripts of several of the pair-rule genes accumulate in the cytoplasm immediately adjacent to the egg cortex (Fig. 4; Ingham *et al.* 1985*a*; Weir & Kornberg, 1985; Macdonald *et al.* 1986), in contrast to other RNA species, which at the same time are uniformly distributed throughout the peripheral cytoplasm of the syncytial blastoderm (MEA, unpublished results). Specific attachment to the cytoskeleton perhaps serves to restrict the diffusion of these RNAs before cellularization, allowing the synthesis of their protein products to remain tightly localized.

The terminal stripes of the blastoderm prepattern have unique characteristics, as we would expect for any pattern generated by the dynamic interactions of its components. The most posterior *ftz* stripe, for example, is approximately twice as wide as the others, presumably because cells on its posterior flank are not subject to the same set of interactions as those in the more anterior stripes (Figs 2, 4). If *ftz* and other components of the pair-rule pattern define the structure of metameric units, we would not expect the units defined at the boundaries of the metameric region (parasegments, or pseudoparasegments, 0 and 15) to be equivalent, or in morphological parlance truly 'homologous', to those generated internally. We might, however, expect the internal elements of the pattern to be strictly homologous, both in terms of final structure and in the sense that their generation would depend on the same gene products. This is not the case. Additional gene products are required in specific regions of the egg to allow normal generation of the pair-rule pattern. These include the products of the segment gap genes.

Segment gap genes and the initial subdivision of the egg

The genes that mutate to segment gap phenotypes fall into two broad classes; five that are required principally after fertilization, during development of the zygote, and at least eight others that function largely or exclusively during oogenesis (Table 1). These classes may themselves embrace genes with very varied roles in development, but at least three of the zygotically acting gap genes, *hunchback*, *Krüppel* and *knirps*, appear to play analogous roles, each in a different region of the embryo. It is useful to refer to these three as cardinal genes (Meinhardt, 1986, see below).

Mutations in each of these three genes delete overlapping regions of the segment pattern (Fig. 5; Nüsslein-Volhard & Wieschaus, 1980; Nüsslein-Volhard *et al.* 1982). These pattern deletions result from a primary effect on segment specification. For example, in the cuticle of embryos that lack the *Krüppel* gene product, the head and gnathal segments are normal, but the last gnathal segment is appended directly to posterior abdominal segments. Three thoracic and five abdominal segments are missing, and replaced by one abdominal segment in reversed polarity (Wieschaus *et al.* 1984*b*). The localized effect of the *Krüppel* mutation is already apparent in blastoderm stage embryos. Cells form normally within the primordia for the deleted segments, but within this region the pattern of *ftz* and *hairy* transcription never resolves into stripes and *engrailed* expression is never established (Ingham *et al.* 1986; Carroll & Scott, 1986).

The genes *Krüppel* and *hunchback* have been cloned (Preiss *et al.* 1985; Tautz *et al.* 1987). Both are transcribed in the early syncytial blastoderm, shortly before the pair-rule genes are active. *hunchback* is initially transcribed throughout the anterior half of the egg, but by early stage 14 (when the pair-rule stripes are beginning to appear), *hunchback* and *Krüppel* are transcribed only within sharply defined A–P zones of the egg, located within but smaller than the region within which each gene affects segment

pattern (Tautz *et al.* 1987; Knipple *et al.* 1985). Thus, although the segment deletion zones of *hunchback* and *Krüppel* overlap, the blastoderm transcription domains appear to abut. Expression of one of these two genes probably represses the other, for in *hunchback* mutants the transcription of *Krüppel* extends anteriorly, and in *Krüppel* mutants the transcription of *hunchback* extends posteriorly (Fig. 5; from Jäckle *et al.* 1986). Meinhardt (1986) predicted such a relationship for the genes defining each of a set of adjacent cardinal domains. If such a relationship does exist, then the expression of these cardinal genes can be seen to define the first precise spatial subdivisions along the A–P axis of the egg. These cardinal domains probably play a major role not only in the control of metamerization, but also, by interacting with homeotic genes, in the control of regional identity (see below).

Some interaction between the pair-rule system and the cardinal domains is evident not only from the gaps in the segment pattern, but also from alterations in the spacing of the remaining pair-rule stripes in gap mutant embryos (Carroll & Scott, 1986; Carroll *et al.* 1986; Mahoney & Lengyel, 1987). Both of these observations indicate that the pattern-generating properties of the pair-rule system are dependant on,

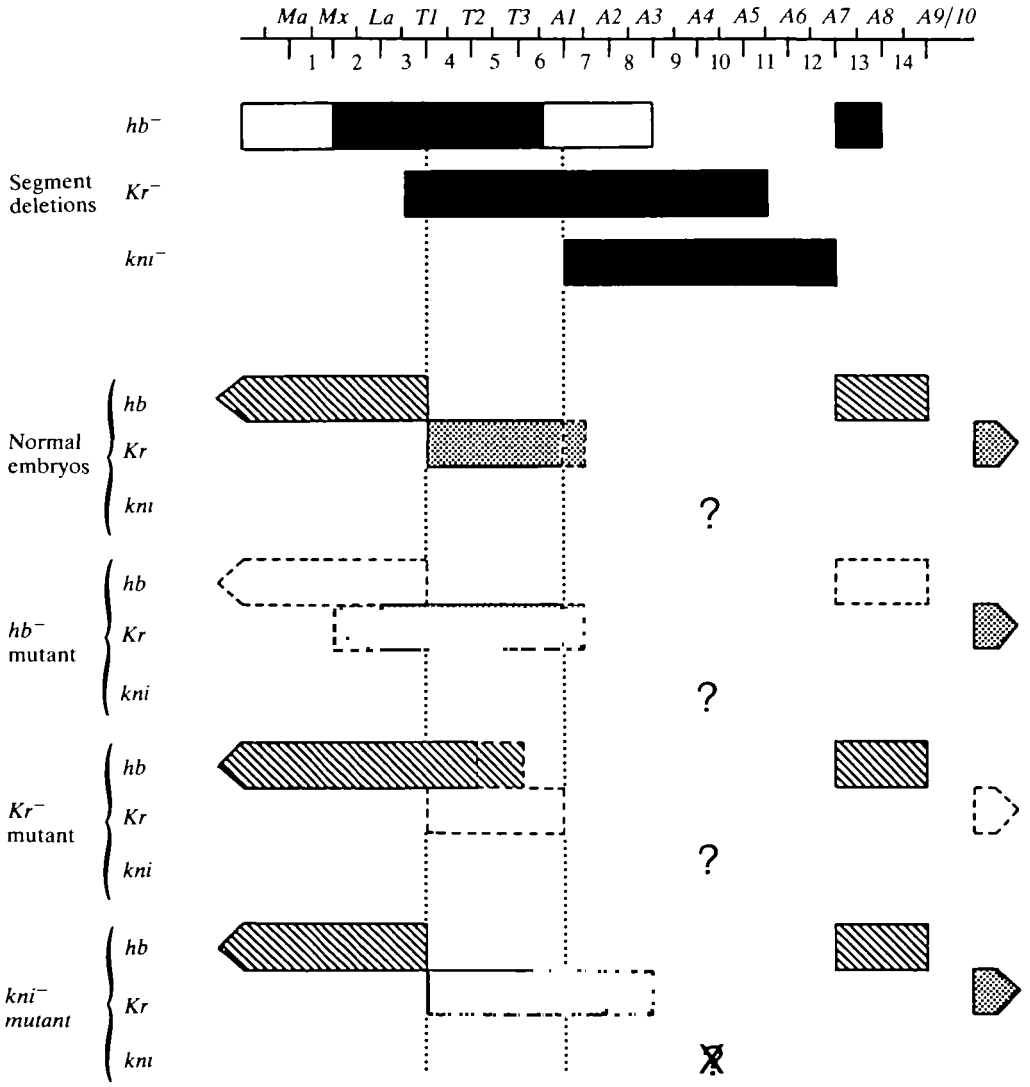

Fig. 5. Phenotypes and transcriptional domains of the cardinal genes.

The extent of segment deletions are shown for null mutations at each of the three genes *hunchback*, *Kruppel* and *knirps*. Solid bars show the deletions due to loss of zygotic gene function. The longer open bar shows the greater deletion resulting from the loss of both maternal and zygotic activity of the *hunchback* gene.

Domains of expression for the two cloned genes, *hunchback* and *Krüppel*, are shown in the wild type and in each of the three mutant classes. Both the early, anterior transcription zone (compare Fig. 2B) and the slightly later posterior domains are shown. Note that both anterior and posterior transcription zones correlate with regions of pattern deletion, but only the anterior zones are affected in the mutant genotypes. Dotted lines show the uncertainty in the location of boundaries. (Redrawn from Jäckle *et al.* 1986.)

and can be modified by, a set of different proteins in different regions of the egg. The extent of autonomous pattern propagation by the pair-rule system alone, for example by a reaction–diffusion mechanism, must be strictly limited.

Essentially nothing is known about this interaction. Both the *Krüppel* and the *hunchback* proteins have homology to transcription factor IIIa, in the putative nucleic acid binding domain of the metal fingers (Rosenberg *et al.* 1986; Tautz *et al.* 1987), but this alone should probably not be taken as evidence that either acts as a transcription factor. There are further homologies between the two proteins, however, which suggest that their equivalent developmental roles are mediated by similar molecular activities (Tautz *et al.* 1987).

The nature and role of maternal information

The activity of the gap and segmentation genes after fertilization elaborates the segment pattern, but it is the structure of the egg established during oogenesis that must establish the fixed relationship between the axes of the egg and the blastoderm fate map. Screens for maternal effect mutations affecting embryonic pattern have identified components of two mechanisms, one of which determines the dorsal–ventral polarity of the egg (for reviews see Anderson & Nüsslein-Volhard, 1984; Anderson, 1987) and a second defining the A–P axis.

The phenotypes of these mutations suggest that at least three aspects of the A–P pattern are specified independently. One group of maternally acting genes, typified by *oskar*, are necessary for the formation of certain posterior structures, including the primordial germ cells and the whole abdominal region of the embryo (Boswell & Mahowald, 1985; Lehmann & Nüsslein-Volhard, 1986; Schüpbach & Wieschaus, 1986). Other maternal genes, best exemplified by *bicoid*, are necessary only for the formation of anterior structures (Frohnhöfer & Nüsslein-Volhard, 1986). A third group, typified by *torso*, are necessary for the formation of structures at both ends of the embryo (Schüpbach & Wieschaus, 1986). At the posterior end, mutations of the *oskar* and *torso* groups affect different sets of structures (*torso* affects the 'tail' (Jürgens, 1987) but not most of the abdomen). The effects of the *torso* and *oskar* group genes are independent and additive in double mutant combinations, suggesting that they interfere with two independent processes.

The results of cytoplasmic transplantation experiments strongly suggest that *bicoid*, and genes of the *oskar* group, are directly involved in the synthesis of specific anterior and posterior determinants which are localized before fertilization. This predicted localization has been visualized for transcripts of the *bicoid* gene by *in situ* hybridization (Frigerio *et al.* 1986).

In the case of *oskar*, and possibly also *bicoid*, these determinants have two activities. One is to specify directly the fate of those cells to which they are segregated (e.g. for *oskar*, the germ cells). The second activity is to generate an influence or gradient which affects the organization of the blastoderm fate map in a large region of the embryo. *bicoid* mutations, for example, eliminate head structures, but also shift the location of the cephalic furrow, which separates head from gnathal structures (Frohnhöfer & Nüsslein-Volhard, 1986; Lehmann & Nüsslein-Volhard, 1986).

It is certainly possible that other, as yet unidentified, maternal determinants specify those features of the blastoderm fate map that lie anterior or posterior to the metameric region. These regions give rise to no cuticular derivatives in the embryo and so such mutations would be difficult to detect. It is probably significant, however, that none of the maternal effect mutations identified to date result in interstitial gaps in the segment pattern. Experimental manipulation, of *Drosophila* and of many other insect eggs, suggests that only the anterior and posterior extremes of the A–P pattern are determined at the time of fertilization (Sander, 1976). All elements of the metameric pattern other than those at the extreme poles are sensitive to manipulations that, like ligation or ultraviolet irradiation, alter the influence of these polar determinants (Schubiger, Moseley & Wood, 1977; Schubiger & Newman, 1982). This is in marked contrast to the situation at cellular blastoderm, by which time the egg behaves as a mosaic within which most pattern elements are determined (Simcox & Sang, 1983).

Mutations in *bicoid*, and in the *oskar* class of genes, result in gaps in the segment pattern that are similar to those generated by mutations in some of the zygotically acting gap genes. For example, the effects of *oskar* mutations on the segment pattern are similar to those of mutations in the cardinal gene *knirps*. This suggests that one role of the *oskar* and *bicoid* determinants is to specify the initial domains within which each of the cardinal genes is activated – and hence specify the sequence of cardinal domains.

We do not know how these determinants act. One suggestion as to how they might act comes from studies of two other maternal transcripts, those from the genes *hunchback* and *caudal*. Both the cardinal gene *hunchback* and the homeotic gene *caudal* are expressed zygotically in specific regions of the egg, but both are also expressed during oogenesis, so that at fertilization the egg contains maternally derived transcripts which are uniformly distributed along the

A–P axis (Mlodzik *et al.* 1985; Mlodzik & Gehring, 1987; Tautz *et al.* 1987). During early cleavage stages, before syncytial blastoderm formation and before zygotic transcription of the cardinal genes, these maternal transcripts become differentially distributed, probably by selective degradation. In the case of *caudal*, the protein translated from the maternal transcripts also shows a graded distribution, accumulating in the posterior part of the egg. This protein gradient is apparently established before any asymmetric distribution of RNA and so may depend on the selective translation of the RNA (Macdonald & Struhl, 1986; Mlodzik & Gehring, 1987). Whatever the mechanism, this early sign of asymmetric activity throughout the length of the egg is presumably initiated by the localized polar determinants, for it occurs in unfertilized eggs (Macdonald & Struhl, 1986), in the absence of nuclear replication and zygotic gene activity.

Intriguing as these observations are, it is unlikely that the maternal transcripts of either *caudal* or *hunchback* play a critical role in initiating the zygotic pattern of gene activity. Oocytes lacking either of these gene products can develop normally, provided that they are fertilized by sperm bearing an active copy of the gene.

Another class of maternal effect mutations result not in gaps in the segment pattern, but in polarity reversals affecting the whole anterior (*bicaudal*) or posterior (*dicephalic*) region of the egg (Bull, 1966; Nüsslein-Volhard, 1977; Lohs-Schardin, 1982; Mohler & Wieschaus, 1986). It is likely that these mutations are altering the maternal localization of determinants, rather than eliminating them.

The specification of regional identity

Establishing the pattern of homeotic gene expression

Models for segmentation and for the control of segment identity have been developed largely in isolation, but the two processes must be intimately linked (Akam, 1985; Meinhardt, 1986). States of homeotic gene expression change abruptly at parasegment boundaries, and experimental manipulation generally perturbs segmentation and segment identity coordinately. Normal segmentation can be established in the absence of any segment diversity when, for example, an embryo lacks much of the Antennapedia and bithorax complexes, so it must presumably be the homeotic genes that take their cues from the segmentation mechanism.

It is now clear that segmentation genes at several levels in the regulatory hierarchy are intimately involved in establishing the spatial activity of homeotic genes. Mutations in the maternal and zygotic gap genes and in some of the pair-rule genes affect the very earliest patterns of homeotic gene expression.

Mutations in *hunchback* and *oskar* have particularly dramatic, and largely reciprocal effects, on the early expression of *Antp* and *Ubx*. *Hunchback* activity appears to repress *Ubx* in the head and thorax (White & Lehmann, 1986), but to be necessary for the activation of *Antp* (A. Martinez-Arias, personal communication). The posterior determinants dependant on *oskar* are necessary for the initial activation of *Ubx* in the abdomen, and also for the repression of *Antp* in the same region (Martinez-Arias and MEA, unpublished). These interactions affect protein distributions as soon as they can be determined in the extended germ band, but they can be visualized as alterations in the pattern of transcript distribution as early as the blastoderm..

Within its wide domain of activity, a homeotic gene may play very different roles in adjacent segments. *Ubx*, for example, serves quite different functions (Lewis, 1978; Casanova, Sanchez-Herrero & Morata, 1985) and is expressed in strikingly different patterns in parasegments 5 and 6 (Akam & Martinez-Arias, 1985; Beachy, Helfand & Hogness, 1985; White & Wilcox, 1985). This differential regulation in adjacent parasegments depends on the activity of some but not all of the pair-rule genes.

Mutations in one pair-rule mutation, *oddpaired* (*opa*), fuse parasegments 5 and 6, but have little effect on the expression of *Ubx* (Ingham & Martinez-Arias, 1986). Other pair-rule genes, however, clearly perturb both segmentation and homeotic gene expression, and one in particular, *ftz*, appears to play a major role in coupling the spatial regulation of homeotic genes to the evolving segment pattern (Duncan, 1986; Ingham & Martinez-Arias, 1986). In normal embryos, the domains of *ftz* expression coincide with even-numbered parasegments (see Fig. 4). In the blastoderm of *ftz* mutant embryos, the early peaks of *Scr*, *Antp* and *Ubx* expression are abolished. These peaks normally lie within and probably define the primordia for parasegments 2, 4 and 6. Later in development, the uniform and high levels of homeotic gene expression that normally characterize these even-numbered parasegments are not observed. Instead, the double-segment units generated in *ftz* mutant embryos behave as single entities with respect to the expression of these homeotic genes, and most closely resemble odd-numbered parasegments in their levels and patterns of homeotic gene expression. This behaviour is in marked contrast with the bipartite pattern of homeotic gene expression observed in the morphologically similar units of the *opa* embryo (Ingham & Martinez-Arias, 1986).

A direct interaction between the *ftz* protein and genes of the Antennapedia and bithorax complexes

seems particularly likely. *ftz* is the only segmentation gene to contain a 'class 1' homeobox (i.e. one with a sequence that is very closely related to those of other genes in the Antennapedia and bithorax complexes (McGinnis *et al.* 1984; Scott & Weiner, 1984)). In later development, the products of the Antennapedia and bithorax complex genes regulate one-anothers' transcription (see below); at early stages, the *ftz* protein may play a similar role.

Elaborating the pattern of homeotic gene expression

Once the germ band is defined, a new set of controls are established to maintain and further elaborate the pattern of homeotic gene expression. This must be the case, for the gap and pair-rule genes that establish the early pattern are expressed only transiently, and at least in the case of *ftz*, their protein products decay rapidly (Carroll & Scott, 1985).

These later controls involve at least three factors. One set of products is required to maintain the repression of many homeotic genes in those segments where each is not normally active. These are the products of the genes *Polycomb* (Lewis, 1978), *extra sex combs* (*esc*, Struhl, 1981, 1983) and others with similar phenotypes (Duncan and Lewis, 1982; Ingham, 1984; Jürgens, 1985; Dura, Brock & Santamaria, 1985). Mutations in all of these genes allow inappropriate expression of both ANT-C and BX-C genes in all body segments and even in the head. This phenotype originally suggested that their products might be involved in the initial positional activation of the homeotic genes, perhaps serving to define a gradient (see Ingham, 1985). This cannot be the case, at least for *Polycomb* or *esc*, as mutations in these genes have no effect on homeotic gene expression until after the germ band is established (Struhl & Akam, 1985; Wedeen, Harding & Levine, 1986).

A second, and independent, set of controls involve interactions between the homeotic genes themselves. Among the genes *Antp*, *Ubx*, *abdominal-A* (*abd-A*) and *Abdominal-B* (*Abd-B*), each of the more posteriorly expressed genes represses the expression of other genes initially active within its own domain (Hafen *et al.* 1984*b*; Harding *et al.* 1985; Struhl & White, 1985). This effect is seen most clearly in the nervous system during later stages of embryogenesis, but even here it is not complete. *Ubx*, for example, is expressed in most or all neural cells of parasegment 6 throughout embryogenesis and, in the absence of the *abd-A* and *Abd-B* genes, it is similarly expressed in parasegments 7 through 13, where it was initially active. In normal development, however, the activity of *abd-A* and *Abd-B* in parasegments 7 to 13 limits *Ubx* expression to only a defined subset of neural cells in each segment (Struhl & White, 1985).

This cell-specific pattern of expression within individual metameres implies that *Ubx*, and presumably other homeotic genes, are also regulated by a third class of factors – those which identify positions or cell types in each metamere. The differential expression of homeotic genes within regions of a single parasegment is seen in many tissues and becomes more elaborate from early germ band stages onwards (White & Wilcox, 1985). For *Ubx*, it clearly depends on genes that define the anteroposterior organization of the parasegment, including *engrailed* (Martinez-Arias & White, 1987) and *wingless* (A. Martinez-Arias & N. Baker, personal communication). It probably also depends on genes that define the differences between cell types, but these have yet to be identified.

An evolutionary perspective

Cells fated to give rise to the segmented region of the *Drosophila* embryo occupy a large fraction of the blastoderm (Fig. 1) and the metameric pattern is essentially defined by the time of gastrulation (see Technau, 1987, for review). In this respect, *Drosophila* and other long-germ insects are exceptional within the annelid–arthropod lineage. In less-specialized insects, the abdominal segments are generated after gastrulation, or even postembryonically, by mitotic division within a growth zone. (Anderson, 1972; Jura, 1972). The full pattern of the germ band is not determined in the blastoderm. In the annelid- and myriapod-like forms believed to be ancestral to the insects, sequential growth of the metameric region of the body is generally observed and this frequently occurs postembryonically (Anderson, 1973). Indeed, the archetypal pattern of annelid development exemplified by primitive polychaetes is characterized by quite the opposite strategy of development. Here, the primary pattern-forming processes of embryogenesis are concerned principally with the generation of those parts of the body that are not metamerically segmented. Of the 64 cells generated by the first six divisions of the stereotyped spiral cleavage pattern, it is typically only the 4d cell and four progeny of the 2d cell that give rise to the entire metameric region of the body. These form stem cells or teloblasts, which form a growth zone within the trochophore larva, from which the segmented body develops. The remaining cells of the blastula are otherwise specified to form pre-oral structures including the brain, the gut and specialized larval cells (Wilson, 1892; Anderson, 1973).

Since the pattern of spiral cleavage is essentially indistinguishable in annelids and molluscs, and appears in slightly modified form in flatworms (Macbride, 1914), we must suspect that it is an ancient

developmental mechanism, and not a secondary adaptation to generate specialized larval structures. So, if we accept a common origin for metamerization in annelids and arthropods, then the mechanism of segmentation in *Drosophila* must have evolved from a budding process that originally occurred later in development. It is perhaps significant in this regard that localized maternal determinants, which appear to play such a major role in the specification of embryonic lineages in the spirally cleaving embryos, probably have no direct role in the specification of individual segments or of regional differences within the metameric region of the insect embryo.

At present, we do not know what elements of the segmentation mechanism have been conserved between annelids and insects. We might expect that genes like *engrailed*, directly involved in maintaining the segment pattern throughout development, would play equivalent roles in all those organisms that share with *Drosophila* a common origin of metamerism. On the other hand, the transient pair-rule prepattern may be an invention of the insects that relates specifically to the subdivision of an extended metameric region in the blastoderm. This need not be the case, however. Interacting systems that generate extended patterns in space can, with little change in regulatory networks, generate patterns that have both temporal and spatial components (Meinhardt, 1982).

The evolving role of homeotic genes

It is generally assumed that homeotic genes serve to define segment identities. This seems to be an appropriate description of their role in most segments of the *Drosophila* adult, all of which are unique (Lewis, 1978). It is probably not, however, appropriate to think in this way about homeotic genes in primitive members of the annelid–arthropod lineage. In many annelids, segment number is not defined and many segments have no unique identities.

I find it more likely that homeotic genes of the Antennapedia–Bithorax class originally served to distinguish between the presumptive metameric region and other parts of the embryo. We have little idea what the role of such an ancestral *Antennapedia*-like gene might have been within these cells, though it could perhaps have been related to the requirement for continued postembryonic growth of the segmented region. Subsequently, as segment diversity arose, these same genes must have been utilized to define different parts of the metameric region (see Martinez-Arias, 1987), but it is hard to imagine that precise boundaries between uniquely defined segments arose immediately.

In the early embryo of even such an advanced arthropod as *Drosophila*, the expression of homeotic genes suggests that all parasegments are not qualitatively distinguished. The regions of the mouthparts and the 'tail' (Jürgens, 1987) seem to be 'hard-wired', in that the *Deformed* and *caudal* genes are initially activated in precisely the correct regions of the blastoderm (see Fig. 3), but definition of the thoracic and abdominal segments has to evolve, by interactions and modulations of the set of genes expressed in parasegments 3–13. Parasegments 3, 4, 5 and 6 rapidly come to have unique identities, but parasegments 7 to 12 are remarkably similar throughout much of embryogenesis. The catalogue of structures in their musculature and peripheral nervous system is identical (Ghysen *et al.* 1986; Campos-Ortega & Hartenstein, 1985; Hooper, 1986) and in the early germ band they show the same qualitative patterns of homeotic gene expression (see Fig. 3).

With this perspective, it is interesting to revive the views of a classical arthropod morphologist. Snodgrass (1935) selected two features to characterize the arthropods; the possession of jointed limbs (a criterion familiar to all elementary students of zoology) and the subdivision of the body into regions or 'tagmata', each composed of structurally and hence developmentally similar segments. The particular array of tagmata serves to define the major classes of arthropods – insects having 3 gnathal, 3 thoracic and 8–11 abdominal segments; arachnids and crustacea possessing different tagmatal organizations.

Any model that seeks to explain segment diversity within the arthropods must account for both the similarity of segments within a tagma and the differences between them. It may be that early in the evolution of the arthropods, a single homeotic gene defined, not segment identity, but the developmental characteristics of segments throughout each tagma. Expression of the *Antennapedia* gene would define all thoracic parasegments, and expression of a prototypical bithorax complex gene would define abdominal parasegments. This correlation between tagmatic identity and homeotic gene expression is no longer clear in the adult roles of homeotic genes in the Diptera, but it is strongly suggested by their patterns of expression in the early germ band.

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