Segmentation of the *Drosophila* embryo

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Segmentation in *Drosophila* is a sequential process directed by at least 30 genes that encode various types of proteins, including: many transcription factors; a putative RNA-binding protein; a membrane-associated receptor kinase; several intracellular protein kinases; a number of secreted signaling molecules; and others of unknown function. Although the detailed molecular reactions used to generate the metameric subdivisions of the embryo are not yet understood, a general outline of the processes involved has been described. The manner in which spatial relations in the developing embryo are established can now be described.

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Introduction

Segments are among the earliest morphological manifestations of regional specialization in the *Drosophila* embryo. They are thought to represent equivalent developmental units of the organism, and to provide a framework for the diversification of body parts during subsequent differentiation. As a paradigm for studying how spatial information is encoded and transmitted, the processes that lead to the pattern of segments in the *Drosophila* embryo have been the subject of intense investigation during the past decade. From these studies has come the understanding that information defining position along the embryo's anterior/posterior (A/P) and dorsal/ventral (D/V) axes is encoded in the form of protein gradients.

The elegant studies which provide the experimental basis for this conclusion suggest that small differences in the concentration of protein morphogens direct the selection of different developmental pathways. The implications of this conclusion are both profound and far-reaching. Numerous reviews summarizing the results of these studies have appeared recently [1,2]. In addition, the genesis of the cephalic segments [3], the response of the gap genes to maternal regulatory signals [4], the interactions between the products of gap genes and the promoters of the pair-rule genes [5], and the cellular interactions which define the parasegmental subdivisions [6,7] have also been reviewed recently. It is beyond the scope of this discussion to explore in detail the many issues addressed in these previous reviews. Instead, we provide a general overview, while emphasizing those aspects relevant to understanding how the genes and gene products involved encode positional information along the A/P axis.

Formation of the anterior/posterior axis

The *Drosophila* egg is asymmetrically shaped, with anterior and posterior poles and dorsal and ventral surfaces that are clearly distinct. Although development reproducibly positions the embryo within this structured egg, remarkably few of the materials that make up the egg are sequestered in restricted locations. Rather, most of the RNA and protein in the egg appear to be distributed uniformly within either the egg membranes or cytoplasm, and there is little to suggest the presence of a blueprint for subsequent development. Indeed, the complex patterns produced in the first hours of development rely on few localized factors. Three separate and largely independent systems are involved in generating the A/P positional axis (Table 1). These have been called the anterior, posterior, and terminal systems. The terminal system has no known pre-localized germline components, and the other systems have few. Yet, within 2-3 h of fertilization, precise patterns are generated that can uniquely identify single rows of cells among the more than 4000 cells that make up the embryo.

Among the exceptional molecules in the maternal dowry that are localized during oogenesis are two that direct the anterior and posterior systems. In the anterior system, *bicoid* (*bcd*) mRNA is tightly sequestered at the anterior tip during oogenesis [8,9]. Sequences in the 3′ untranslated (3′ UTR) region of the *bcd* mRNA have been implicated in its anterior localization [10], a process requiring the participation of the products of three other genes, *exuperentia*, *swallow*, and *staufen*. Produced only after fertilization, the diffusion of bcd away from the anterior pole generates a concentration gradient during the precellular stages of embryogenesis [11]. The bcd pro-

Abbreviations

A/P—anterior/posterior; ***bcd***—*bicoid*; **D/V**—dorsal/ventral; ***eve***—*even-skipped*; ***ftz***—*fushi tarazu*; **3′ UTR**—3′ untranslated region.

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Table 1. Segmentation in *Drosophila* is directed by at least 30 genes.

Gene	Relevant sequence motif	Reference
Maternal: *bicoid, nanos*		
bicoid	Homeodomain	[9]
cappuccino		
exuperantia		
mago nashi		
nanos	No recognizable motif	[85]
oskar	No recognizable motif	[86,87]
pumilio	No recognizable motif	[88]
spire		
staufen	No recognizable motif	[89]
swallow	RNA-binding motif	[90]
tudor	No recognizable motif	[91]
valois		
vasa	Homology e1f4A	[92,93]
Gap		
buttonhead		
empty spiracles	Homeodomain	[14]
giant	Leucine zipper	[94]
huckebein	DNA-binding motif	[36]
hunchback	Zinc finger	[19]
orthodenticle	Homeodomain	[95]
knirps	Zinc finger	[96]
Krüppel	Zinc finger	[97]
spält	Zinc finger	[98]
tailless	Zinc finger	[99]
trunk		
Terminal system		
corkscrew	Tyrosine phosphatase	[25•]
Dsor1	MAP kinase	[27]
forkhead	forkhead domain	[100]
fs(1)Nasrat		
fs(1)pole hole		
huckebein	DNA-binding motif	[36]
l(1)pole hole	raf; Ser/Thr protein kinase	[101]
ras1	ras	[102]
son of sevenless	ras activator	[103]
tailless	Zinc finger	[99]
torso	Receptor tyrosine kinase	[29,104]
Pair rule		
even-skipped	Homeodomain	[105,106]
fushi turazu	Homeodomain	[107–109]
hairy	Helix-loop-helix	[110]
odd-paired	Zinc finger	(J Mullen, S DiNardo, personal communication)
odd-skipped	Zinc finger	[111]
paired	Homeodomain/paired domain	[112]
runt	No recognizable motif	[113]
Segment polarity		
armadillo	Plakoglobin homolog	[61]
arrow		
costal-2		
cubitus interruptus-Dominant	Zinc finger	[65]
dishevelled		
engrailed	Homeodomain	[53]
fused	Ser/Thr protein kinase	[57]
gooseberry	Homeodomain/paired domain	[55]
hedgehog	Membrane or secreted	[64,70,114,115]
lines		
naked		
patched	Membrane	[59,60]
porcupine		
shaggy (zeste-white 3)	Ser/Thr protein kinase	[61]
sloppy paired	forkhead domain	[56]
smoothened		
wingless	Secreted protein	[82,116,117]

Gaps in the Table indicate genes not yet cloned.

tein is a transcriptional regulator that is thought to act as a morphogen by eliciting distinct transcriptional responses from its several targets at different concentrations of protein.

The instructional capacity of the bcd gradient has been demonstrated in two ways: by showing that altering the maternal gene dosage of bcd alters the A/P proportionality of the embryo [11,12••], and by assaying expression from synthetic target genes to show how the broad monotonic gradient of bcd protein might be transduced into smaller domains of expression of its targets [12••,13]. These latter studies characterized putative target sequences in the *hunchback* promoter, and it bears stating that the relevance to *hunchback* expression of binding sites with a range of affinities for bcd protein is not understood. That is, it is not known what roles these different binding sites have in *hunchback* regulation. Furthermore, the implicit assumption that such binding sites exemplify the regulation of other target genes is only that. At this point, we can only assume that target genes activated in the more anterior regions have low-affinity binding sites for bcd, whereas target genes activated in the more posterior regions have sites that bind bcd with higher affinity. Binding sites that might mediate activation by bcd have been identified in the regulatory regions of several other putative targets of bcd regulation (e.g. *buttonhead*, *orthodenticle*, *empty spiracles*, *giant*, and *even-skipped* (*eve*); [3,14–21]). However, it has yet to be shown directly that different threshold concentrations of bcd determine where the domains of expression of these or other target genes are placed.

In the posterior system, *nanos* RNA is tightly sequestered at the posterior pole. Sequences in the 3′ UTR of the *nanos* mRNA have been implicated in its posterior localization, a process that requires the function of at least seven genes (*cappuccino*, *oskar*, *spire*, *staufen*, *tudor*, *valois*, and *vasa*). The nanos protein is produced only after fertilization and is found distributed in a gradient that peaks at the posterior pole [22]. The protein is thought to have a novel activity, targeting specific mRNAs for translational repression and/or degradation [23]. Sequence elements that are nanos-responsive have been characterized in the 3′ UTRs of *bcd* and *hunchback* mRNAs, and nanos-dependent repression of these transcripts is permissive for abdominal development. One presumes that the gradient of nanos protein is important for shaping the distribution of *hunchback* transcripts, such that the concentration of nanos protein provides an instructional measure of distance from the posterior pole by proportionally reducing the concentration of *hunchback* RNA. However, evidence to indicate that the form of the nanos gradient controls where abdominal segments develop has yet to be reported.

Despite the tight association of *bcd* and *nanos* RNAs with the anterior and posterior poles of the embryo, respectively, *bcd* and *nanos* functions are not primarily responsible for the development of the most anterior and posterior regions of the embryo. Rather, a separate terminal system is required for the anterior head (the acron) and for the tail (the telson). In contrast to the anterior and posterior systems, the terminal system has no determinants known to be localized in the embryo. The terminal pathways are thought to be initiated by a signaling pathway that is controlled by a tyrosine receptor kinase, torso. This protein is synthesized after fertilization, and distributed evenly within the plasma membrane of the embryo, but is thought to be activated in a spatially localized manner by cues emanating from one or several of the immediately adjacent somatic cells [24]. Genes products that have been implicated in the signal transduction pathway include l(1)pole hole (a serine-threonine kinase homolog), corkscrew (a tyrosine phosphatase homolog), ras1, son of sevenless (a positive regulator of ras1), and Dsor1 (a MAP kinase homolog) [25•,26•,27]. Ongoing studies promise to identify additional components of the torso signal transduction pathway [28], and, ultimately, to reveal how activation of torso leads to position-dependent responses of target genes in the terminal regions. It is unclear at present whether the distribution of activated torso at the poles is graded, and whether such a graded distribution is instructive. Such a model is formally possible [1,29], whereby this graded distribution elicits different concentration-dependent responses from the signal transduction pathway. Another possibility is that, "activated torso might act more like a switch, triggering terminal development after a threshold level of torso activity is achieved" [30]. Triggering such a switch might involve the synthesis or activation of an as yet unidentified transcription factor, whose distribution is subject to diffusion, and is therefore graded and instructional.

In summary, the A/P axis is established during the first several hours after fertilization of the *Drosophila* embryo by three independent systems, two of which rely on prior localization of determinants during oogenesis (bcd for the anterior system and nanos for the posterior system), and one of which relies upon localized activation of a signal transduction pathway (the terminal system). These systems operate during the period of rapid nuclear divisions, prior to cellularization of the embryo. In the anterior and posterior systems, positional information is thought to be encoded in the form of gradients of protein concentration, and to be elaborated by response elements in target nucleic acids that differ subtly in their relative affinities. The mechanism through which activation of the torso receptor leads to position-dependent gene activation at the embryo poles is less well understood.

Elaborating the pattern in the syncytial blastoderm

The broad concentration gradients of bcd and nanos protein that form along the length of the embryo activate a cascade of cross-regulating genes during the syncytial blastoderm stages. All of these genes encode transcription factors whose targets include other members of the cascade. Based upon their phenotypes and times of activation, these zygotically-expressed segmentation

genes can be classified into three classes — the gap, pair-rule, and segment polarity genes. The central gap genes, which are expressed in the middle of the embryo, include *hunchback, Krüppel, knirps*, and *giant*. They are expressed exclusively in restricted domains that will ultimately give rise to groups of contiguous segments along the A/P axis. Recent analysis suggests that the deployment of gap genes is accomplished in two steps [12••]. First, *hunchback* expression is activated anteriorly by bcd and translation of the *hunchback* transcripts is blocked posteriorly by nanos. Second, the resulting gradient of hunchback protein activates the *Krüppel, knirps*, and *giant* gap genes in a series of broad domains, presumably by providing a series of concentration thresholds that independently regulate each gene. The Krüppel, knirps, and giant proteins are themselves thought to form short-range morphogenetic gradients that help to refine their respective expression domains [31–34]. Such mutual interactions are thought to involve competition by activators and repressors for overlapping sites in the regulatory regions of the respective genes [35].

At the poles of the embryo in the zones where torso receptor is activated, two terminal gap genes, *tailless* and *huckebein*, are expressed. Although the spatial control of *tailless* and *huckebein* is not understood, mutual interactions between these genes, which both encode transcription factors, are not involved [36]. Nevertheless, tailless protein is distributed in a gradient that overlaps anteriorly with the *hunchback* domain and posteriorly with the *knirps* domain. Expansion of these domains toward the anterior and posterior poles in *tailless* and *huckebein* mutant embryos, respectively, suggests that one of the functions of the terminal gap genes is to repress *hunchback* and *knirps* [36]. A role for bcd in the development of the acron is suggested by the mis-expression of *tailless* and associated developmental defects in *bcd* mutant embryos [37].

In summary, a set of six transcription factors are synthesized in restricted domains along the A/P embryonic axis in response to maternal signals (Fig. 1). In the central part of the embryo, these domains are defined in part by broad gradients of bcd and nanos protein. Subsequent input from cross-regulatory interactions among the gap gene products helps to refine the relative placement of these domains along the A/P axis.

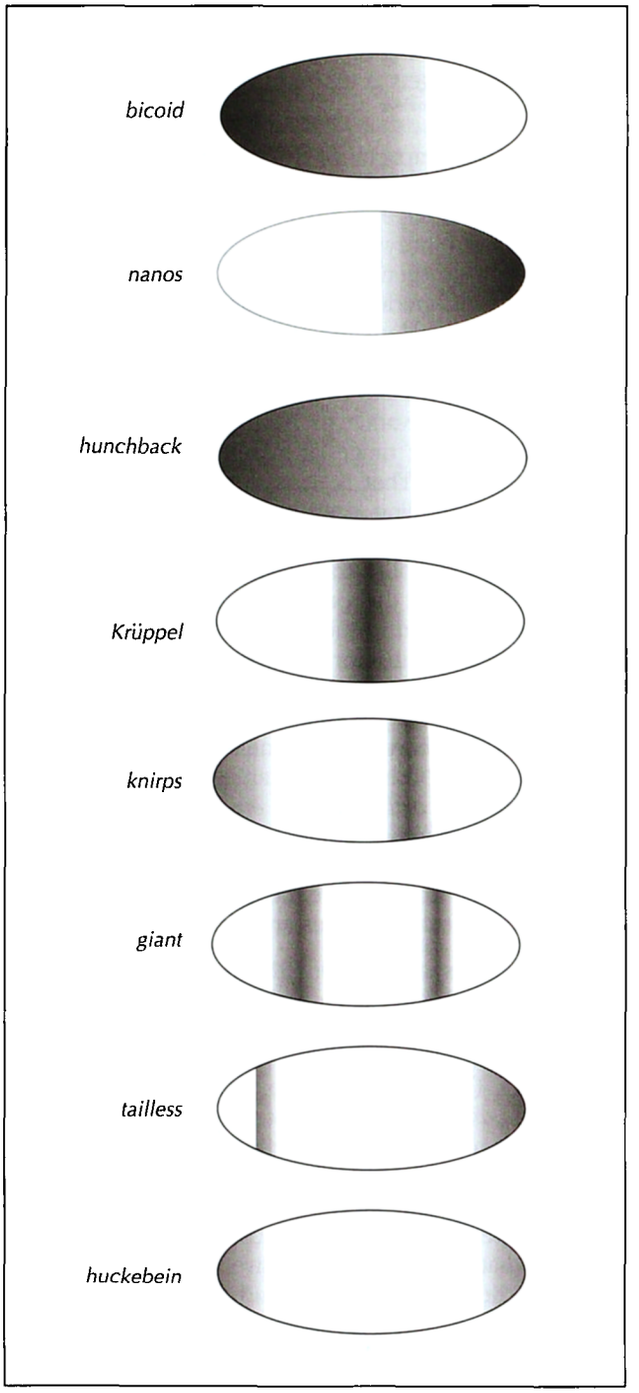

Fig. 1. Schematic representation of the expression of the *bicoid, nanos,* and the gap genes *hunchback, Krüppel, knirps, giant, tailless* and *huckebein* at the syncytial blastoderm stage. the six gap genes encoding transcription factors are expressed in defined domains along the A/P embryonic axis (indicated by differential standing). In the central region of the embryo, the domains of expression are partly determined by the bicoid and nanos protein gradients. Subsequent cross-regulatory interactions among the gap gene proteins further define the placement of the domains along the A/P axis.

Refining the pattern in the cellular blastoderm

The broad bands of gap gene expression along the A/P axis lead to a more refined and higher resolution pattern of pair-rule and segment polarity gene expression during nuclear cycle 14. For the pair-rule genes, expression patterns describe seven transverse stripes that correspond to two segment intervals along the body axis; their expression patterns bring the first signs of metamerization. Since the expression pattern of each pair-rule gene has a similar periodicity but different registration, unique combinations of pair-rule gene products are expressed in each cell.

Two types of mechanisms are thought to be involved in activating the striped domains of pair-rule gene expression. For the so called primary pair-rule genes, *hairy* and *eve*, different stripes are independently regulated by separate sequence elements, so called 'stripe response el-

ements' [38–40]. These sequence elements are thought to be regulated by overlapping subsets of gap genes that either activate (in the regions of expression) or repress (in the interstripe regions). The best studied example is the element responsible for expression of eve stripe 2. In this 480 bp element, closely linked binding sites for hunchback, Krüppel, giant, and bcd proteins have been mapped [41•]. Activation is thought to be mediated by the combined action of both hunchback and bcd. The limits of expression are thought to be defined by the repressive effects of giant anteriorly and by Krüppel posteriorly [42]. Presumably, the other eve stripes respond to other combinations of gap and maternal regulators.

Studies of the epistatic relationships among the pair-rule genes indicate that expression patterns of the so called secondary pair-rule genes (*fushi tarazu (ftz), paired, odd-paired*, and *odd-skipped*) depend upon the prior expression of *eve*, *runt* and *hairy*. Among these secondary pair-rule genes, regulation of *ftz* is best understood. In contrast to the organization and regulation of *eve* and *hairy*, control of all of the seven *ftz* stripes is mediated through a single regulatory element. The expression of *ftz* is initially activated in a broad domain extending from 10–70% of the egg length, and repression in the interstripe regions generates the striped pattern [43]. A 669 bp 'zebra element' in the *ftz* regulatory region has been shown to contain binding sites for a multitude of activating and repressing transcription factors [44–46]. The positive regulatory sites can mediate expression throughout most of the germ band, while the negative regulatory sites transform a continuous pattern of gene expression into discrete stripes.

The *eve* and *ftz* expression domains change shape continuously during the course of cellularization and gastrulation. Initially, the stripes are broad, with diffuse and overlapping borders, and the distribution of protein within each stripe is bell-shaped. These stripes subsequently narrow, as the expression of each gene is extinguished in single rows of cells in the regions of overlap. Sharply defined expression domains of each gene remain, leaving rows of cells expressing neither gene in between [47–49]. The stripes of *ftz* and *eve* expression are no longer symmetric, but have anterior margins that express strongly and are sharply defined, while the posterior margins are less well defined [50]. Normally, the stripes of expression of each gene narrow to the same extent, and so remain equal in width and evenly spaced. However, in mutant embryos that either partially inactivate eve [48,51], or that hyperactivate ftz [49], the spacing of the stripes becomes unequal. For instance, in *eve* mutant embryos, ftz stripes remain symmetric, lack sharp borders [51], and the metameres that subsequently form are spaced unevenly [48,49] (Fig. 2). These observations suggest that the regular spacing of the segmental primordia that is characteristic of normal embryos is dependent upon the mutually antagonistic activities of the eve and ftz proteins. The immediate response of *ftz* transcription to ectopic synthesis of eve protein suggests that these activities may be direct [52].

In summary, transcription factors produced in successive waves lead to the periodic expression of a set of seven

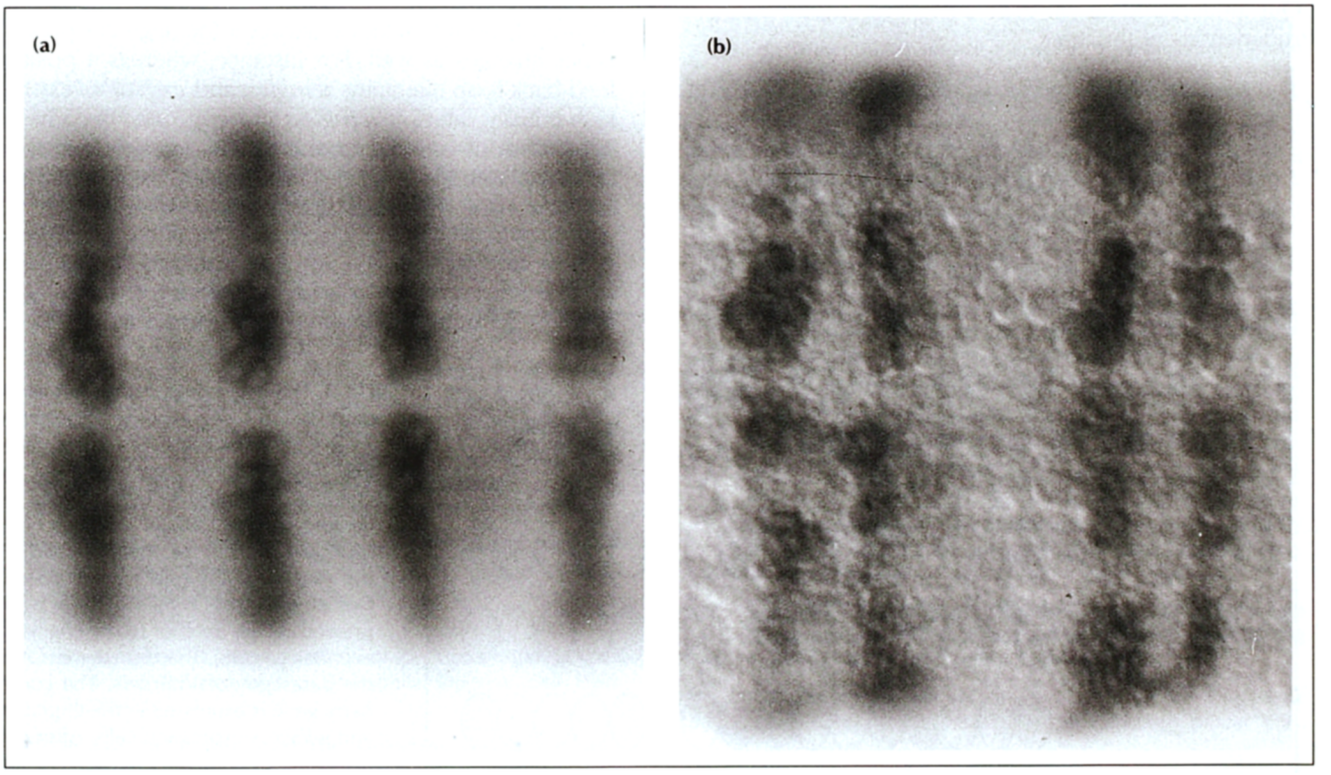

Fig. 2. Distribution of *hedgehog* RNA in the **(a)** wild-type and **(b)** *even-skipped* mutant embryo. A ventral view of the gnathal and thoracic region of germ band extended embryos after *in situ* hybridization reveals that the stripes of *hedgehog* RNA are evenly spaced in the wild type, but are unevenly spaced in $eve^{3.77.17}$ mutant embryos.

pair-rule genes. The regularity of the final pattern is, in part, a consequence of the finely tuned mechanisms that produce eve and ftz proteins in functionally equivalent quantities. This process transduces a pattern established by at least five different genes (*bcd*, *giant*, *hunchback*, *Krüppel*, and *knirps*) to a more refined one directed by only two. Presumably, any irregularities in the spacing of the initial domains of expression of the pair-rule genes can be corrected by these mutually antagonistic interactions.

Refining the pattern after cellularization

The segment polarity genes elaborate the final step in the sequential process that controls successively finer aspects of segmental patterning. These genes assume control of the segmentation process as cellularization of the embryo is completed and gastrulation commences. In contrast to the gap and pair-rule gene products, all of which are transcription factors that function in nuclei in an acellular environment, the segment polarity gene products are diverse in character and mediate communication between the newly formed cells of the embryo. Some of the segment polarity gene products are likely to be nuclear transcription factors (engrailed [53,54], gooseberry [55], and sloppy-paired [56]), while others are protein kinases (fused [57] and shaggy [58]), membrane-associated (patched [59,60] and armadillo [61]), or are secreted (wingless [62,63] and, perhaps, hedgehog [64]). Our understanding of their roles and relationships is rudimentary at this time.

The segment polarity genes are expressed in various different patterns in the newly cellularized embryo (Fig. 3). Several (*fused* [57], *shaggy* [58], *cubitus interruptus-Dominant* [65,66], and *armadillo* [67]) are expressed ubiquitously or in broad domains. Others (*engrailed* [54,68], *wingless* [69], *hedgehog* [70], *patched* [59,60], and *gooseberry* [55]) are expressed in segmentally reiterated stripes. The gene *wingless* is expressed in the cells lacking either ftz or eve protein, *engrailed* and *hedgehog* are expressed in the most anterior cell of each of the ftz and eve stripes. The *patched* gene is expressed in the cells not expressing *engrailed*, and *gooseberry* is expressed in the *engrailed*- and *wingless*-expressing cells. These patterns of expression are thought to be a consequence of a combination of positive and negative influences from the pair-rule gene products, although the details of the interactions are yet uncertain. The critical point is that *engrailed*, *wingless*, *hedgehog*, *patched*, and *gooseberry* expression appears to be inaugurated by the pair-rule hierarchy in a manner that is independent of any other segment polarity gene products.

Shortly following the completion of cellularization and gastrulation, pair-rule gene expression fades, while expression of the segment polarity genes continues. At this stage of development, interactions among the segment polarity gene products themselves determine the expression patterns of the segment polarity genes. For example, *wingless* and *engrailed* become dependent upon each other for their continued and stable expression [71–74]. Communication between *engrailed*- and *wingless*-expressing cells that foster such interactions are thought to involve most of the other segment polarity genes [6,72,75–77], although the complexity of this multi-step process has so far obscured a clear understanding of its nature. A further complicating aspect is that as the embryo matures, the patterns of *wingless* and *engrailed* expression change [69,78], and the nature of the interactions between the segment polarity genes changes as well. For instance, whereas a positive feed-back loop maintains *wingless* and *engrailed* expression initially, later on *wingless* and *engrailed* become independent of each other and become dependent upon other interactions [74,79]. The complete catalog of these interactions is still being assembled.

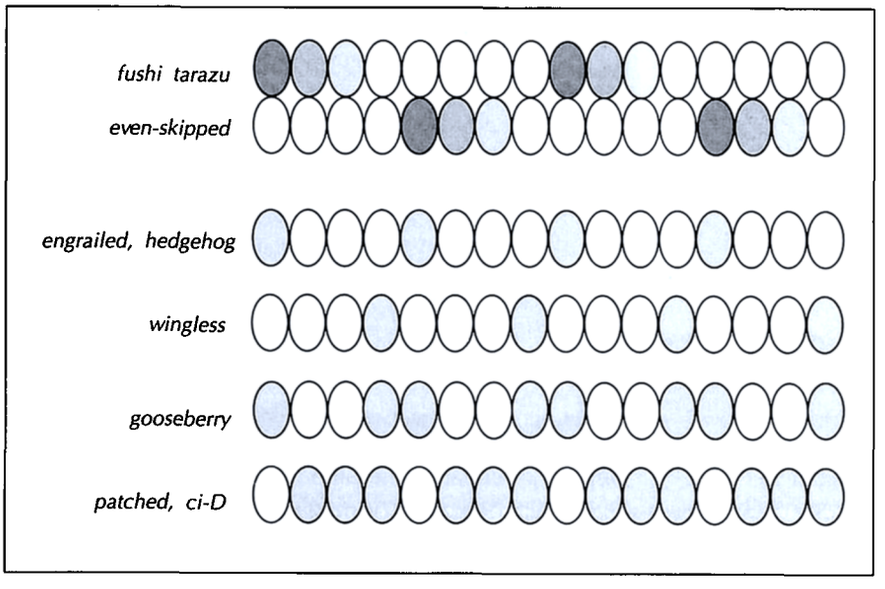

Fig. 3. The domains of expression of the pair-rule and segment polarity genes. At cellular blastoderm stage, *engrailed* and *hedgehog* are expressed in the cells that contain the highest level (indicated by the darkest shading) of either *fushi tarazu* or *even-skipped* — the most anterior cells of their respective stripes. The *wingless* gene is expressed in cells anterior to the *engrailed*-expressing cells, thereby demarcating the parasegmental borders. The *gooseberry* gene is expressed in the *engrailed*- and *wingless*-expressing cells. After gastrulation, the domains of *patched* and *cubitus interruptus-Dominant* (*ci-D*) expression resolve into stripes, complementing the pattern of *engrailed* and *hedgehog* expression.

A critical issue that remains to be determined is how position is defined within the cellularized epithelium, since it is obvious that the mechanisms involving diffusion-generated gradients of transcription factors cannot operate in the cellular environment of the older embryo. We believe that the developmental unit in which positional parameters are defined is the parasegment. Indications from studies of *engrailed* expression in *Drosophila* and other arthropods suggest that the parasegment border is established as a stable lineage restriction even as cellularization is being completed and gastrulation begins [80,81••]. The coincidence of this border with both the anterior, well defined limits of eve and ftz stripes and with the juxtaposition of *wingless*- and *engrailed*-expressing cells suggests that generating and fixing this border is what much of the segmentation gene machinery is designed to accomplish. However, the parameter that measures position within the parasegment remains mysterious. We know that wingless protein diffuses from *wingless*-expressing cells [62,63,82], and it has been suggested that a diffusion gradient of wingless protein might provide an instructive measure of position relative to the parasegment border [83]. However, such models appear to have been invalidated by the observation that uniform expression of *wingless* can largely rescue the phenotype of *wingless* mutant embryos [84••]. It therefore remains an unanswered question whether and how the principles gleaned from the protein gradients of bcd, nanos, hunchback, giant, Krüppel, and knirps in the pre-cellular embryo will apply to other proteins that function within the cellularized environment that follows.

Conclusions

Elegant and incisive studies have identified many of the genes and molecular functions involved in organizing the early *Drosophila* embryo. Ongoing efforts to understand the mechanisms involved at a more detailed molecular level, and to elucidate how the cellular epithelia and organs of the developing animal are organized embody the promise that more of the longstanding riddles of development will soon be solved.

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