# ARTHROPOD SEGMENTATION: BEYOND THE *DROSOPHILA* PARADIGM

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Abstract | Most of our knowledge about the mechanisms of segmentation in arthropods comes from work on *Drosophila melanogaster*. In recent years it has become clear that this mechanism is far from universal, and different arthropod groups have distinct modes of segmentation that operate through divergent genetic mechanisms. We review recent data from a range of arthropods, identifying which features of the *D. melanogaster* segmentation cascade are present in the different groups, and discuss the evolutionary implications of their conserved and divergent aspects. A model is emerging, although slowly, for the way that arthropod segmentation mechanisms have evolved.

MACROEVOLUTION
Evolutionary processes that lead to significant morphological change. This usually refers to processes that occur above the species level.

LIFE HISTORY
The sum of the morphological stages and the ecological environments that an organism goes through during its life.

BILATERIANS
Members of the animal kingdom that have bilateral symmetry — the property of having two similar sides, with definite upper and lower surfaces, and anterior and posterior ends.

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The gene networks that generate segments in arthropods provide an excellent model for understanding MACROEVOLUTION at the molecular level. Arthropods share a basic body plan, but elaborate a wide diversity of morphologies that affect segment form and number. They also have different modes of embryogenesis that are adaptations to different LIFE-HISTORY strategies, and these differences often affect how and when segments are generated in relation to other processes of embryogenesis.

Segmentation is also of interest because it has either evolved repeatedly in several animal phyla, or has been lost entirely in many others. Molecular phylogenies identify three distinct lineages of BILATERIAN animals[1]. Each of these lineages contains one of the three principal segmented phyla — the vertebrate chordates within the DEUTEROSTOMES, the annelid worms within the LOPHOTROCHOZOANS and the arthropods within the ECDYSOZOANS. This raises developmental and evolutionary questions. To what extent are the mechanisms that underlie the development of segmented body plans similar across phylogenetically diverse bilaterian phyla? And, if there are similarities, do they reflect a common evolutionary origin of segmentation or the convergent recruitment of similar developmental mechanisms during evolution? Put simply, and perhaps naively, was the bilaterian common ancestor a segmented animal, or have segmented body plans evolved multiple times?

Interest in these questions has been stirred recently by the discovery that some arthropods seem to pattern their posterior segments using genetic mechanisms that are similar to those that operate in vertebrates during SOMITOGENESIS[2,3]. It is therefore timely to review our understanding of segment patterning across the arthropods.

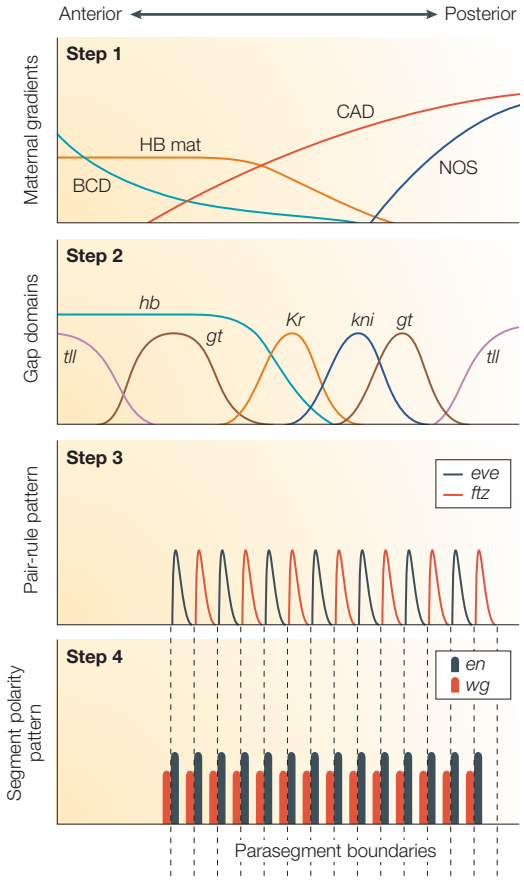
In the 1980s large-scale genetic screens in the fruitfly *Drosophila melanogaster* identified about 40 genes that are necessary to generate a normal segmentation pattern. Subsequent studies showed that these genes function in a hierarchy — they encode a cascade of interacting transcription factors that generate progressively finer patterns of gene expression in the BLASTODERM-stage embryo[4] (BOX 1). Until recently most studies of segment patterning in arthropods other than *D. melanogaster* have been limited to descriptions of gene-expression patterns. Tests for function have been limited to a few small-scale genetic screens[5–7] and one or two attempts at gene knockdown. The advent of effective RNAi methods[8] has meant that the function, as well as the expression, of segmentation genes can be examined in a wide range of arthropods, and successful transgenesis in some new 'model' species indicates that

Box 1 | **Segmentation in *Drosophila melanogaster***

**Steps 1 and 2**

***From maternal signals to gap domains.*** **Oogenesis provides the *Drosophila melanogaster* egg with a 'ready mix' cytoplasm, so that segmentation can proceed rapidly after fertilization. Maternal transcripts of the segmentation genes *caudal* (*cad*) and *hunchback* (*hb*) are ubiquitously distributed. *bicoid* (*bcd*) is localized at the anterior pole of the egg, and a complex of proteins and RNAs (the pole plasm) is localized at the posterior. After fertilization, translation of localized maternal transcripts, and the diffusion of their protein products, generates protein gradients along the egg (step 1 in the figure). BCD is both a transcriptional activator of *hb* and a translational repressor of *cad* mRNA. Nanos (NOS), a key component of germ plasm, represses translation of maternal *hb* RNA. The resulting egg contains the HB protein in the anterior half (maternal HB, HB mat), and long-range gradients of both BCD (high at the anterior) and CAD (high at the posterior).**

**At the same time, signals that are embedded in the egg-shell by localized populations of** FOLLICLE CELLS **activate a transmembrane receptor, Torso, at the poles of the egg. Together, the signals from these maternal proteins activate a small set of zygotic 'gap' genes (*tailless* (*tll*), *giant* (*gt*), *Krüppel* (*Kr*) and *knirps* (*kni*)) at specific positions along the anterior–posterior axis of the egg (step 2). Interactions between the gap genes refine their expression further, but because these proteins are free to diffuse in the egg syncytium, the distribution of each gap protein is graded around its peak site of synthesis.**




**Step 3**

***From gap domains to pair-rule pattern.*** **The pattern of gap-gene expression is aperiodic. The concentrations of their transcription factor products, together with inputs from maternal proteins, give nuclei a unique axial identity. At least three of the pair-rule genes (the primary pair-rule genes *hairy* (*h*), *runt* (*run*) and *even skipped* (*eve*)) interpret these identities to generate periodic stripes of gene expression. Again, transcriptional interactions between these primary pair-rule genes and the other genes that they regulate (for example, *fushi tarazu* (*ftz*) and *paired* (*prd*)) refine the domains of expression until the edges of each stripe are defined to single-cell resolution (step 3 in the figure). The boundaries of the pair-rule stripes predict the boundaries of parasegments.**

**Step 4**

***From pair-rule pattern to stable boundaries.*** **The pattern of pair-rule gene expression is transient. However, as cell boundaries form, the activities of the pair-rule proteins (again, mostly transcription factors) result in the activation of the segment polarity genes. Odd- and even-numbered parasegments express different combinations of pair-rule genes, but produce the same output of segment polarity gene expression in every segment. The segment polarity pattern is stable, and at least for some genes (notably *engrailed* (*en*)) it will persist into the adult. The boundary between *en*-expressing cells and their anterior neighbours (which express *wingless* (*wg*)) becomes the parasegment boundary. Segment boundaries are established slightly later, posterior to *en*-expressing cells.**

misexpression and reporter-gene studies will soon be widely applicable[9–11].

Understanding the genetic basis of segmentation in a wider range of arthropods is important for two reasons. First, it might help us to understand how the fly genetic model for segmentation evolved, and second, it might reveal similarities between arthropod and vertebrate genetic mechanisms of segmentation that are obscured, or no longer exist, in the DERIVED mode of segmentation that is seen in *D. melanogaster*.

In this review we examine what recent functional studies have taught us about the conservation, or otherwise, of genes and gene networks that operate in each tier of the segmentation cascade of *D. melanogaster*. We then highlight the limitations of the 'candidate-gene' approach, and consider the lessons that might be learned from the segmentation mechanisms that operate in vertebrates. We conclude by outlining some important questions for future research in this field.

DEUTEROSTOMES
One of the three main branches of the bilaterian animals. The deuterostomes include chordates, hemichordates and echinoderms.

LOPHOTROCHOZOANS
One of the three main branches of the bilaterian animals. Lophotrochozoans include annelids, molluscs, flatworms and several other smaller phyla.

ECDYSOZOANS
One of the three main branches of the bilaterian animals. Ecdysozoans are characterized by an unciliated integument, and grow by ecdysis, or moulting. They include nematodes, arthropods and many other smaller phyla.

SOMITOGENESIS
The process of progressive formation, during embryogenesis, of metameric mesodermal units (somites) that represent the precursor structures of dermis, skeletal muscles and the axial skeleton.

BLASTODERM
The layer of cells that completely surrounds an internal mass of yolk in an arthropod embryo.

FOLLICLE CELLS
The somatic cells in *Drosophila melanogaster* that surround the oocyte; they provide patterning signals to the oocyte and secrete the egg-shell.

DERIVED
Having undergone significant evolutionary change relative to the ancestral state.

## A segmented body plan

A segmented body plan is a defining characteristic of the arthropods, and is almost certainly a trait that was ancestral to the whole phylum. All arthropod embryos pass through a segmented GERM-BAND stage that, at the morphological level at least, seems to be remarkably conserved, and has been referred to as the 'phylotypic stage'[12]. Embryonic events either side of this stage are much less conserved, presumably as a result of directional selection for divergent life histories. Interestingly, the segmentation genes that function at the bottom of the *D. melanogaster* segmentation cascade, just before and during the phylotypic stage, seem to be conserved across the arthropods (FIG. 1). These genes — which include homologues of the *D. melanogaster* SEGMENT POLARITY GENES *engrailed* (*en*), *wingless* (*wg*) and *hedgehog* (*hh*), and encode proteins that have a range of functions — establish definitive segment (or, to be precise, PARASEGMENT[13] (BOX 1)) boundaries. They show similar patterns of expression in diverse arthropods[14–21] and constitute an evolutionarily conserved regulatory cassette[22,23]. Exactly why the segment polarity genes are so conserved has been the subject of some debate[15,21].

Divergent embryology before the phylotypic stage indicates that the genetic networks that function towards the top of the segmentation cascade in *D. melanogaster* might be much less conserved. In particular, the syncytial context of segmentation in *D. melanogaster* is a derived characteristic that is shared with only some other groups among the higher (HOLOMETABOLOUS) insects. Most arthropods do not pattern all of their segments while the embryo is still a SYNCYTIUM (BOXES 1,2), which raises the question as to whether local transcription factor gradients could be operating as they do in *D. melanogaster*.

Most arthropods pattern their posterior segments sequentially from a cellularized growth zone (BOX 2), a trait that is thought to be primitive to the arthropods[24]. In this review, species in which segmentation occurs by sequential addition from a posterior growth zone are referred to as 'sequentially segmenting' arthropods, but it is important to note that these species show significant embryonic differences in relation to one another, as well as to *D. melanogaster* (BOX 2). The term 'SHORT GERM', which is often used to describe such arthropods, is misleading when referring to those arthropods — such as the centipede *Strigamia maritima* — in which segments are generated from a large pool of cells, rather than a small embryonic primordium. Therefore, we prefer not to use it as a generalized term.

Much of the available data on segmentation mechanisms relates to insects, but even among these, some groups are poorly represented. The more derived, holometabolous insect orders are relatively well-sampled (data exist for 4 out of 11 orders), particularly with the development of *Tribolium castaneum* as a powerful model for functional studies in Coleoptera (beetles)[8,25,26]. However, it is clear that even within this group there is a wide diversity of segmentation mechanisms, perhaps reaching an extreme in the polyembryonic wasp *Copidosoma floridanum*[27]. The more basal, HEMIMETABOLOUS insects are represented by descriptive studies in just a handful of species, and by functional studies in just two — the orthopteran *Gryllus bimaculatus*[28–30] (a cricket) and the hemipteran *Oncopeltus fasciatus*[31–34] (the milkweed bug) (FIG. 2). The diversity of Crustacea has barely been sampled, and functional data are so far available only for the brine shrimp *Artemia franciscana*[35].

The insects and crustaceans together — referred to as the Pancrustacea — are thought to constitute one of three principal monophyletic lineages within the arthropods (the traditional view that insects and crustaceans form two closely allied but distinct monophyletic clades is not supported by molecular data)[36,37]. The other two main lineages are the chelicerates (represented here by studies of spiders[2,3,14,38,39] and mites[40]) and the myriapods. Myriapods are no longer thought to be closely related to the insects, but instead are an ancient lineage in their own right[37]. They are represented here by studies on segmentation in centipedes[17,41,42] and millipedes[20].

How pancrustaceans, myriapods and chelicerates are related remains unclear, but the hope is that by encompassing appropriate representatives of all three of these clades (FIG. 2), comparative studies might reveal which aspects of the segmentation machinery represent ancestral characteristics of the arthropods.

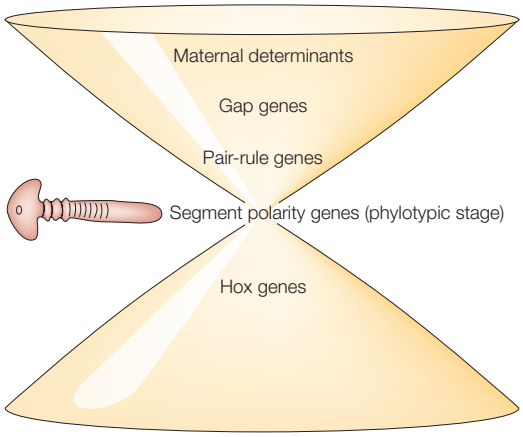



Figure 1 | **Conservation of the segmentation cascade in arthropods.** The well-studied segmentation cascade of *Drosophila melanogaster* represents a derived mechanism compared with that of other arthropods. The degree of conservation of genes that function in successive steps of the segmentation cascade are represented by the width of the hourglass. The earliest stage of the cascade, axis determination by maternal gradients, has diverged significantly between arthropod groups. Gap-gene homologues can be found in all arthropods, but their function in segmentation is variable. Pair-rule patterning has been described in several arthropods, but it is not clear whether this is an ancestral feature or one that has evolved convergently. The best-conserved stage is the one in which segmental boundaries are defined by the interaction of segment polarity genes. The expression of these genes coincides with the so-called 'phylotypic stage' of arthropods — the segmented germ band[12,21]. Later on in the cascade, when axial identity is conferred by Hox genes, arthropod groups diverge again, with genes of the Hox family being expressed at different axial levels in different species.

GERM BAND
In an arthropod embryo, this is the differentiated portion, which has a distinct anterior–posterior axis, and is where the segmentation process takes place.

SEGMENT POLARITY GENES
A group of genes that define different parts of each segmental repeat. When segment polarity genes are mutated the normal number of segments is formed, but these show internal pattern replication and polarity reversals.

PARASEGMENT
The initial segmental unit that is formed during the segmentation process. The final segment boundaries lie in the middle of the parasegments.

HOLOMETABOLOUS
Insects for which the life cycle includes distinct larvae, pupae and (usually) winged adults.

SYNCYTIUM
A population of nuclei that are not separated by cell membranes. It is typical of the developing blastoderm in *Drosophila melanogaster*.

SHORT GERM
A mode of insect development in which anterior segments are patterned in the blastoderm, with posterior segments forming sequentially from a cellularized growth zone after gastrulation.

HEMIMETABOLOUS
Insects for which the life cycle includes several larval stages, ending in a sexually mature, winged adult, without going through a pupal stage.

### Top of the hierarchy: the maternal effect genes

In *D. melanogaster* maternal cues trigger the patterning of the early embryo. Maternal transcripts are loaded into the oocyte and are specifically targeted to the anterior (*bicoid*; *bcd*)[43–46] or posterior (*nanos*; *nos*)[47–49] poles by the cytoskeletal machinery[4,50]. At fertilization these maternally provided transcripts are translated to form the source of protein gradients that initiate anterior–posterior (A–P) patterning[4,50] (BOX 1).

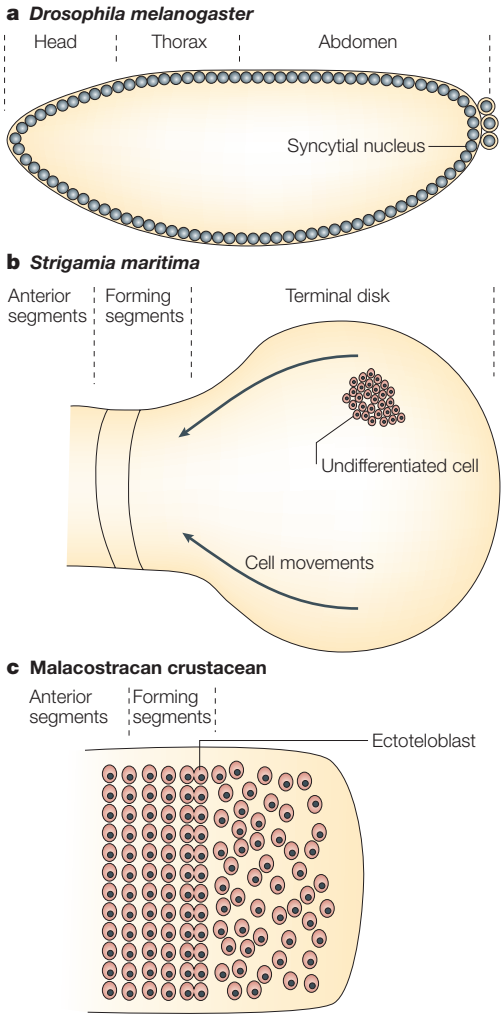
***Conservation and divergence of anterior patterning.*** At the anterior the BCD protein activates transcription of downstream segmentation genes[44]. Despite its central role in patterning *D. melanogaster*, an anterior gradient of the maternally derived BCD protein was probably a new invention of the higher Diptera[51–56]. *bcd* genes have been isolated from several *Drosophila* species, from houseflies[51], and from other 'higher flies' of the suborder Cyclorrhapha[52]. However, no *bcd* gene has been isolated from species other than those from the Diptera[53,55], and none has been found in the genome of the mosquito *Anopheles gambiae*, a distant relative within the Diptera. Instead, at the location where the *bcd* gene resides in the Hox cluster of *D. melanogaster*, there are only genes that are related to *zerknullt* (*zen*), the neighbour of *bcd* in the *D. melanogaster* genome, which is a derived *Hox3* gene[53,54]. Comparison of the sequences of the Dipteran *zen* and *bcd* genes indicates that *bcd* arose within the higher Diptera (basal Cyclorrhapha) by rapid sequence divergence of a *zen* gene, and by the acquisition of new DNA targets through a mutation in the homeodomain that gives BCD a recognition sequence that is similar to that of Orthodenticle (OTX), another homeodomain-containing transcription factor[52–55].

## Box 2 | Diverse cellular mechanisms of segmentation in arthropods

**In *Drosophila melanogaster*, all segments are patterned more or less simultaneously while the blastoderm is still syncytial (panel a; also see BOX 1). By contrast, most other arthropods pattern a small number of segments at the blastoderm stage, and then add posterior segments consecutively from a growth zone. The first patterned segments include at least the three anterior segments: the antennal segment, the intercalary segment (which is the second antennal segment in crustaceans) and the mandibular segment. These three segments are sometimes referred to as the naupliar segments, and are the only ones that are present in the larval stages of many crustacean groups. In many insects, in addition to the naupliar segments, two to five other segments are formed in the blastoderm stage, including up to three thoracic segments. Little is known about the mechanism behind the formation of anterior segments. It is possible that they are generated by a mechanism that is distinct from those that function during the formation of more posterior segments.**

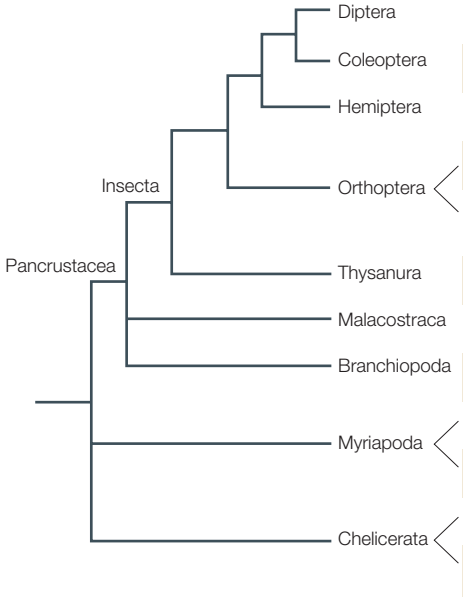
**In most arthropods posterior segments are generated in a cellular environment. In some cases these segments arise from a small population of posterior cells in the blastoderm, which proliferate later to generate the tissue from which segments are patterned (this occurs, for example, in *Artemia franciscana* and other branchiopod crustaceans, and in most 'short germ' insects). In other cases (such as in the centipede *Strigamia maritima*, panel b) a blastodisc that contains many thousands of apparently undifferentiated cells persists after the completion of anterior segmentation, and posterior segments are generated sequentially from this population by a combination of proliferation and cell rearrangement.**

**In MALACOSTRACAN crustaceans, cells of the germ band organize into a square array, each row of which will give rise to a single segment through a stereotyped series of polarized cell divisions (panel c). Sometimes these rows are generated by the aggregation of cells from a preformed blastodisc (for example, in *Parhyale hawaiiensis* and other AMPHIPODS). In many cases they are generated by the sequential divisions of ectoteloblasts — stem cells that lie at the posterior of the germ band.**




MALACOSTRACANS
A subclass of crustaceans that includes shrimps, lobsters and sandhoppers.

AMPHIPODS
An order of malacostracan crustaceans that includes beachhoppers.




| Species | Available techniques |
|---|---|
| Fruitfly *Drosophila melanogaster* | G, R, T |
| Flour beetle *Tribolium castaneum* | G, R, T |
| Milkweed bug *Oncopeltus fasciatus* | G, R |
| Cricket *Gryllus bimaculatus* | G, R, T |
| Grasshopper *Schistocerca gregaria* *Schistocerca americana* | G |
| Firebrat *Thermobia domestica* | G |
| Amphipod *Parhyale hawaiiensis* | G, T |
| Brine shrimp *Artemia franciscana* | G, R |
| Garden centipede *Lithobius atkinsoni* | G |
| Coastal centipede *Strigamia maritima* | G |
| Spider mite *Tetranychus urticae* | G |
| Spider *Cupiennius salei* | G, R |

Figure 2 | **Phylogenetic relationships between the arthropod species discussed in this article.** The experimental techniques that are available for each organism are also shown. G, gene-expression data through RNA *in situ* hybridization experiments; R, RNA interference; T, germline transgenesis.

Details are emerging about how the embryo is patterned in some insects that lack *bcd*. In the beetle *T. castaneum*, RNAi knockdown phenotypes indicate that maternally derived OTX1 and Hunchback (HB) proteins cooperate to carry out a role that is analogous to BCD during embryogenesis[26,56]. *Tribolium castaneum* differs from *D. melanogaster* in that it forms its abdominal segments sequentially in a cellular environment, but is similar to *D. melanogaster* in that its anterior segments are patterned in a syncytium[57,58]. Therefore, the proposal that a maternally derived anterior gradient is operating in this arthropod seems feasible. It is not known how gradients of these two maternal proteins are generated in the beetle — in both cases, their maternal RNAs are initially ubiquitously distributed in the egg[26,56,59]. However, the transcripts for two other transcription factors — *eagle* (*eg*) and *pangolin* (*pan*) — are maternally localized to the anterior pole in *T. castaneum*, which indicates that the molecular machinery required to localize mRNAs to the anterior pole predated the recruitment of *bcd* to anterior patterning[60]. Whether *eg* and *pan* are involved in A–P patterning in *T. castaneum* is unclear[60]; neither is known to have such a role in *D. melanogaster*.

The mechanism of anterior patterning in *T. castaneum* need not necessarily represent the ancestral state for insects, let alone all arthropods. Many other arthropods do not pattern their anterior segments in a syncytium. For example, cellularization occurs early in the embryonic development of the grasshopper *Schistocerca gregaria*[61], before any segment patterning. An anterior gradient might therefore not be required in arthropods that show sequential segmentation from a posterior growth zone, given that only a few anterior segments are patterned in the blastoderm[62,63] (BOX 2). Indeed, earlier experimental studies indicate that anterior patterning gradients are widely used only among the higher (holometobolous) insects[63]. In most hemimetabolous insects, the anterior pole of the egg seems to have no specific role in patterning the embryo[63].

## Caudal and Nanos in embryonic patterning

In *D. melanogaster*, the Caudal (CAD) and NOS proteins both show graded distributions in the blastoderm, with levels that are high at the posterior and decrease anteriorly[49,64–66]. Both are required for normal segmentation at the posterior of the germ band (BOX 1). Because *cad* RNA is provided both maternally and zygotically, but is not initially localized[65], genetic screens were slow to reveal the full role of *cad* during segment patterning.

There is now evidence that homologues of both *nos* and *cad* might be involved in patterning more anterior segments in sequentially segmenting arthropods.

**caudal.** RNAi experiments in two sequentially segmenting insects — *T. castaneum*[35] and the cricket *G. bimaculatus*[28] — and in one crustacean (*A. franciscana*[35]) have revealed an essential role for *cad*, not only in patterning posterior segments, but also in the formation of the entire segmented trunk. In *G. bimaculatus*[28] and *T. castaneum*[35], the most extreme knockdown phenotypes eliminate all but the PRE-GNATHAL SEGMENTS. In *A. franciscana*, knockdown of *cad* in the newly hatched nauplius blocks formation of all new segments[35] (BOX 2). These experiments indicate that *cad* might ancestrally have been involved in the formation of all trunk segments, but that this function has been lost in *D. melanogaster*, perhaps by the acquisition of the long-range morphogen *bcd*.

**nanos.** Evidence for the role of *nos* in other insects is less direct. In the locust *Schistocerca americana* (a species closely related to *S. gregaria*), the maternally derived mRNA of the *nos* orthologue[62] is restricted to the posterior of the early embryonic primordium, and localization of the NOS protein is consistent with a role in the translational repression of *hb* posterior to a gap-like domain of expression in the gnathal segments. Although it has not been directly demonstrated, the likelihood of this interaction is supported by the observation that *S. americana* *hb* mRNA retains well-conserved NOS response elements in its 3′ UTR.

In *D. melanogaster* NOS acts on maternal *hb* mRNA[47–49]. However, in *S. americana* maternal HB is provided as protein, and might be involved in defining the extent of the BLASTODISC[62], rather than in A–P patterning. The role of NOS is more likely to be in regulating zygotically transcribed *hb* RNA[62]. So the maternal provision of *hb* mRNA and its translational regulation by NOS could be a feature that evolved subsequently to the divergence of flies and grasshoppers.

PRE-GNATHAL SEGMENTS
The gnathal segments comprise the mandibular, maxillary and labial head segments of insects. The pre-gnathal segments lie anterior to these segments. The number of pre-gnathal segments has been debated, but probably includes at least three: the ocular, antennal and intercalary segments.

BLASTODISC
An undifferentiated single-cell layer in an arthropod embryo that ultimately gives rise to all embryonic structures.

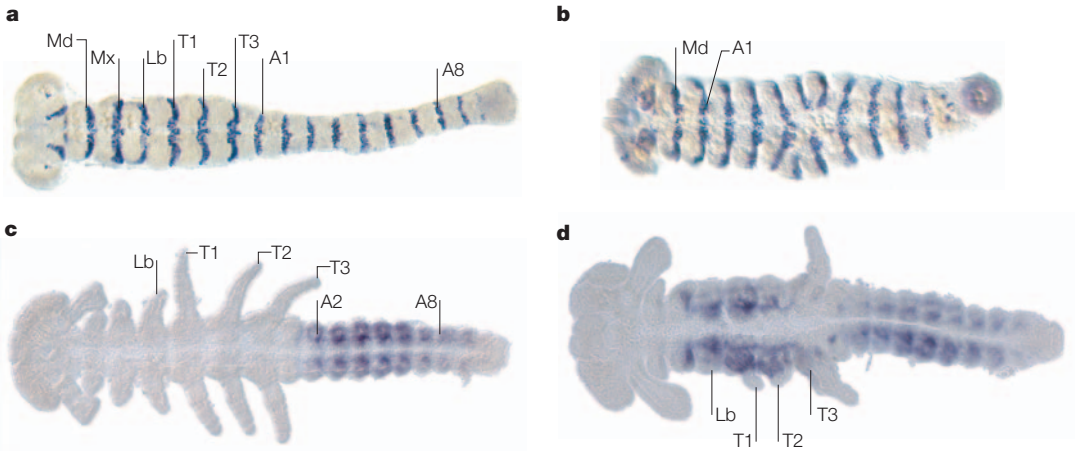



Figure 3 | **A comparison of *hunchback* expression and function in two sequentially segmenting insects.** **a**,**b** | Panel **a** shows the wild-type *Tribolium castaneum* embryo; panel **b** shows the consequences of parental RNAi (pRNAi) that is targeted against *hunchback* in the *T. castaneum* embryo. pRNAi results in a canonical gap phenotype: the loss of maxillary (Mx), labial (Lb) and thoracic (T) segments. The mandibular (Md) segment is still present and the development of posterior abdominal (A) segments remains largely unaffected. Embryos are stained for *engrailed* expression. Hox gene expression in *hunchback* pRNAi embryos was not examined in this study, but homeotic transformations might be obscured by the gap phenotype. **c**,**d** | Panel **c** shows *abdominal A* (*abdA*) expression in the wild-type *Oncopeltus fasciatus* embryo; panel **d** shows *abdA* expression in the *hunchback* pRNAi *O. fasciatus* embryo. In *O. fasciatus*, gnathal and thoracic segments do form following *hunchback* pRNAi, but are transformed towards abdominal identity. Note the highly reduced labium and T1/T2 legs in the *hunchback* pRNAi embryo in panel **d**. Abdominal segments form normally, although they seem to be compacted in embryos that show strong pRNAi phenotypes (not shown). In *O. fasciatus*, the transformation of gnathal and thoracic segments to abdominal identity is correlated to the ectopic expression of *abdA*, which indicates that this Hox gene is usually repressed in the anterior by *hunchback*, although possibly indirectly. The anterior is to the left. Panels **a** and **b** are reproduced, with permission, from *Nature* REF. 26 © (2003) Macmillan Magazines Ltd. Images kindly provided by Reinhard Schröder, Universität Tübingen, Germany. Panels **c** and **d** are reproduced, with permission, from REF. 32 © (2004) Company of Biologists. Images kindly provided by Thom Kaufman, Indiana University, USA.

During later embryogenesis, *S. americana nos* is no longer expressed in the segments that appear sequentially as the embryonic primordium elongates, which indicates that its role is limited to the earliest stages of embryonic patterning, and that it has no specific function in patterning posterior segments[62].

Therefore the emerging picture is that both *nos* and *cad* had an ancestral role in A–P patterning in insects and at least some crustaceans, but the extent of their influence, the identity of their regulatory targets and the nature of their roles during early development might have diverged significantly in different derived lineages. Whether an anterior patterning gradient was used in ancestral insect lineages seems less clear.

## The gap genes

In *D. melanogaster*, the gap genes are the direct targets of maternal patterning information, and establish a series of molecularly distinct regions along the A–P axis of the blastoderm[4,67] (BOX 1). The transcription factors that they encode have two distinct roles at this stage of development. In combination with maternal factors, they regulate pair-rule genes through segment-specific enhancers[68]. In addition, they function with downstream segmentation genes to regulate the initial activation of HOX GENES in region-specific patterns[69,70]. In later development most of the gap genes are re-used in many other patterning processes[71].

***Functional analysis of gap-gene orthologues.*** Orthologues of the *D. melanogaster* gap genes are relatively easy to identify in other arthropods (except *knirps* (*kni*), for which orthologues are hard to distinguish from genes that encode other *kni*-related transcription factors). For three genes — *hb*, *Krüppel* (*Kr*) and *giant* (*gt*) — both the expression patterns and functions of the orthologues have been examined in sequentially segmenting insects[25,26,30,32,33].

On the basis of expression patterns alone one might conclude that the role of these gap genes has generally been conserved during insect evolution, with the caveat that there have been shifts in their precise domains of expression, and, in particular, posterior shifts within the lineage that leads to the *Drosophila* genus[25]. However, RNAi experiments indicate a more complex picture[25,26,30,32,33]. These gap genes seem to have a broadly conserved role in the regulation of Hox genes, but their knockdown does not always result in a true 'segment gap' phenotype, as seen in *D. melanogaster* — that is, in the failure of segments to form in the region where the gap gene is normally expressed. Rather, segments might form, but have abnormal identity. In these cases, corresponding and interpretable shifts in Hox gene-expression domains are detected by *in situ* hybridization on embryos in which these genes have been knocked down by RNAi[25,30,32] (FIG. 3). This is particularly clear in the case of the *T. castaneum Kr* gene, for which both RNAi and mutant data are

HOX GENES
A family of homeodomain transcription factors that are conserved across bilaterian animals; they are expressed in sequence along the A–P axis and are involved in conferring axial identity.

available — the *T. castaneum jaws* mutant has now been characterized as a null allele of *Kr*[95].

It is not always clear whether the variation across species reflects real differences in the function of gap-gene homologues or differences in the interpretation of phenotypes. Because some segments are taking on abnormal identity, whereas others are deleted, it is not easy to identify the specific segments that are lost. The penetrance of RNAi phenotypes might also vary from species to species — for example, incomplete knockdown could be an issue with the interpretation of RNAi against *hb* in *G. bimaculatus*[30].

Several of these studies have reported an effect of knockdown on the formation of segments that appear by sequential addition from a posterior growth zone[25,30,32]. Embryos treated with RNAi are often truncated owing to the loss or compaction of abdominal segments, with posterior abdominal segments being affected even when they lie outside the ectodermal expression domain of the gene[25,30,32]. How gap-gene homologues mediate these apparent 'long-range' effects remains unclear.

***Evolution of gap-gene function.*** Taken at face value, the available data indicate that the function of individual gap-gene homologues has diverged significantly in different insect lineages. If this is correct, then the regulation of pair-rule genes must also have changed radically during insect evolution. Indeed, a recent study in *A. gambiae* revealed the existence of different combinations of gap repressors for homologous pair-rule stripes, indicating that there has been divergence even within the Diptera[72]. Perhaps in those cases in which gap-gene homologues do not seem to function as true gap genes, other unidentified genes have analogous roles.

One possibility is that in the ancestor of the insects the homologues of *D. melanogaster* gap genes had a role in the regulation of Hox genes and, through this, in the specification of segment identity, but not in the regulation of segmentation genes. It will be interesting to see whether gap-gene homologues are involved in the process of segment generation in arthropods other than insects. At present we have almost no data to address this.

Among the many other developmental roles of the *D. melanogaster* gap genes, one is particularly intriguing in the context of this review. This is the role of *hb* and *Kr* during neurogenesis[71]. These two genes are among a set of transcription factors that are expressed in a stereotyped temporal sequence in neuroblasts, where they define the temporal identity of the neuroblast progeny. The expression of *hb*, the first gene of the sequence, is followed slightly later by *Kr* — a sequence that corresponds to the A–P order of the expression of these genes in the *D. melanogaster* blastoderm[71]. Expression of *hb* and *Kr* in neuroblasts is widely conserved among arthropods[30,32,33,73], and a temporal sequence related to that seen in *D. melanogaster* has been observed in centipedes, which comprise a distantly related arthropod lineage (A.D.C. and A. Stollewerk, unpublished observations). Perhaps these genes were recruited from neural patterning to function in segment specification in arthropods, where segments form in a temporal A–P sequence. Later, in the lineage leading to *Drosophila*, some of these genes (notably *hb* and *Kr*) might then have been expressed in the right place and at the right time to be recruited into regulating pair-rule gene homologues.

## Pair-rule genes

The repetitive segment pattern of *D. melanogaster* is generated in the blastoderm, shortly before cellularization, by transcriptional regulation of the pair-rule genes (BOX 1). The 14 parasegments of the *D. melanogaster* embryo are defined by 7 stripes of *even skipped* (*eve*) expression that alternate with 7 stripes of *fushi tarazu* (*ftz*) expression[4].

Every arthropod that has been examined expresses at least one homologue of a *D. melanogaster* pair-rule gene in a pattern that is consistent with a role in segmentation[17,31,38–40,42,74–79]. However, this does not mean that 'pair-rule patterning' is conserved in all arthropods. It is not clear whether a double-segment repeat is involved in the patterning of segments in all arthropods.

We distinguish two distinct aspects of pair-rule patterning. The transcriptional network that generates the classic pair-rule stripes is only half the story (BOX 1, step 3). The final segment pattern is generated in a second step of transcriptional computation, in which the pair-rule 'codes' of the even- and odd-numbered parasegments establish a pattern that recurs identically in every segment: the initial expression of the segment polarity genes (BOX 1, step 4).

In *D. melanogaster* many of the pair-rule genes do not provide only the inputs for this computation — their promoters also contain regulatory elements that drive segmentally repeated expression as part of the output[4,80]. In this sense, genes such as *eve* are both pair-rule and segment polarity genes.

Conserved expression of a pair-rule gene during segmentation does not mean that all aspects of *D. melanogaster* pair-rule patterning are conserved. We have noted already that the expression of some segment polarity genes is widely conserved among the arthropods[14–21]. If the segment polarity network is more ancient than the pair-rule network, then the most widely conserved roles of some pair-rule genes might lie at the level of defining the single-segment repeat, not the double-segment pre-pattern.

***Insects.*** Individual genes of the pair-rule class have diverged significantly in their expression patterns and function during arthropod evolution. For example, homologues of the pair-rule gene *eve* have different expression patterns in different insect species. In *T. castaneum* an *eve* homologue shows pair-rule expression, with broad pair-rule stripes splitting to form segmental stripes[78]. Inhibition of *eve* function generates pair-rule segmental defects, confirming that *eve* in this insect functions in the generation of a double-segment repeat[81]. However, in other insects

Box 3 | **Notch signalling in vertebrate somitogenesis and arthropod segmentation**

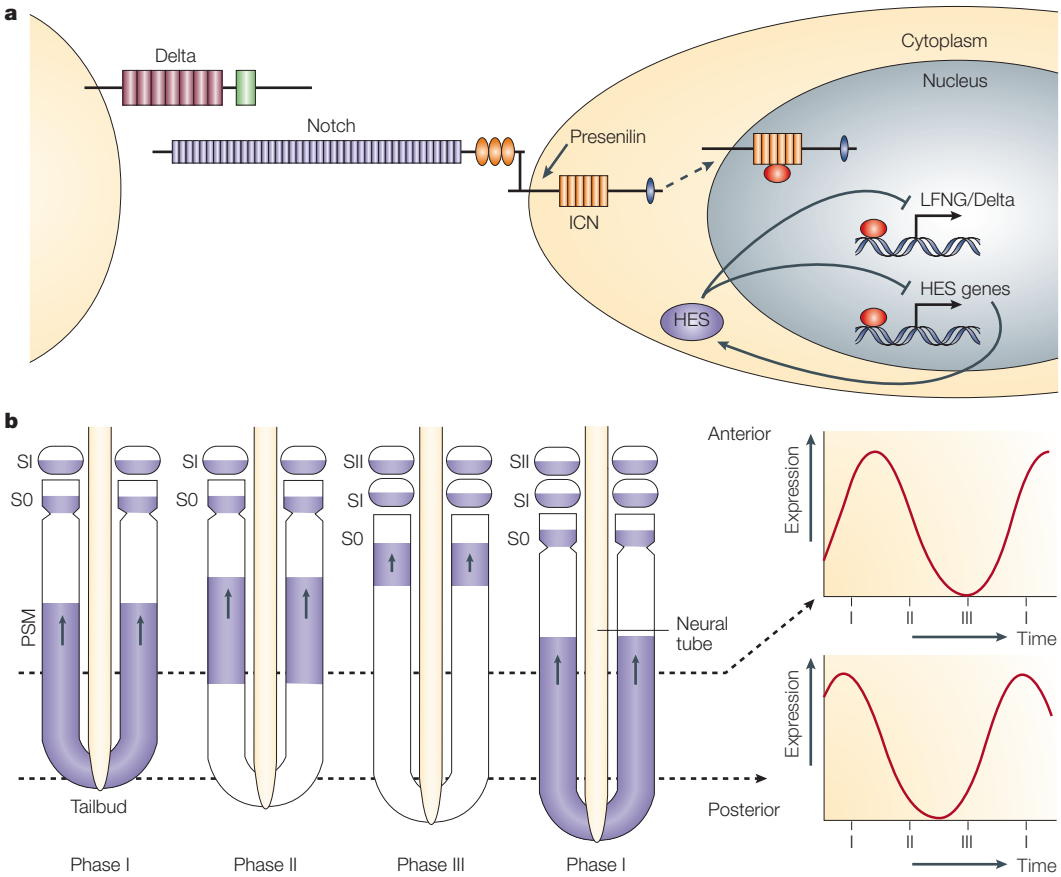
### Vertebrate somitogenesis

A segmentation clock, generated by a Notch-signalling-based oscillator, is central to somitogenesis in vertebrates[90,94]. The Notch pathway is activated by a signal from the ligand Delta in an adjacent cell. Notch then activates several downstream target genes, including those that encode transcription factors from the hairy/enhancer of split (HES) family, Lunatic fringe (LFNG) and Delta. HES family proteins repress their own expression and that of other Notch pathway genes (*Lfng* or *Delta*) (panel **a**). These regulatory interactions result in oscillations in the levels of the products of these genes within individual cells, which appear as anteriorly progressing waves of expression in the presomitic mesoderm (PSM; panel **b**). Each wave of expression precedes the formation of one somite. The extent to which intracellular versus extracellular (Notch) negative regulatory feedback loops set the period of these oscillations is still debated. Intercellular Notch signalling might couple oscillations in neighbouring cells. Opposing and antagonistic retinoic acid and fibroblast growth factor (FGF) gradients form a 'determination front'. Oscillations cease and somites are patterned in this region of the PSM — cells that fall below a particular threshold of FGF signalling during the period of one oscillation form a somite.

Recent data[91] support a role for Wnt signalling upstream of both Notch signalling (the expression of *Axin2*, a suppressor of Wnt signalling, oscillates in the PSM of mouse embryos) and the posterior FGF gradient (in mouse embryos there is a posterior gradient of WNT3A). Data from zebrafish[92] indicate that the Cdx genes (homologues of *D. melanogaster caudal*) are also downstream targets of Wnt signalling during morphogenesis of the posterior body.

### Segmentation of basal arthropods

Recent work on segmentation in the spider *Cupiennius salei* indicates that a Notch-based segment-generating mechanism functions in this species as well[2]. Notch and Delta, two of the central players in the vertebrate segmentation clock, are expressed in a segmental pattern before overt segment formation, and their disruption causes segmentation defects. In addition, the disruption of two downstream targets of Notch signalling, *Presenilin* (*Psn*) and *Suppressor of Hairless* (*Su(H)*), causes severe segmentation defects. Preliminary data indicate that in the centipede *Strigamia maritima*, Notch target genes are involved in early segmentation. Their dynamic expression patterns indicate the existence of an underlying cycling mechanism (REF. 43; A.D.C., unpublished observations). There is also now evidence from other arthropods that Wnt signalling and *caudal* are crucial for sequential segmentation. However, there is currently no direct evidence for travelling waves or that gene expression oscillates in arthropods, nor is there evidence that the regulatory interactions between Notch, Wnt and caudal family genes resemble those seen in vertebrates. ICN, intracellular domain of Notch; S0, newly forming somite; SI/II, formed somites.



species, including a hemipteran (*O. fasciatus*)[31], and a parasitic hymenopteran (*C. floridanium*)[76], *eve* homologues are expressed in segmental stripes. By contrast, in *S. americana* an *eve* homologue is not expressed in stripes at all, but only in a broad posterior domain[77]. A homologue of the pair-rule gene *ftz* is similarly not expressed in stripes in *S. gregaria*[82], but does seem to be in the primitive insect *Thermobia domestica*[83]. There is molecular evidence that the protein domains required for efficient pair-rule function in *D. melanogaster* FTZ are missing in the *Schistocerca spp.* homologue[84]. Interestingly, *ftz* is expressed in a pair-rule pattern in *T. castaneum*[85], but its expression is not necessary for segmentation.

Notwithstanding changes in the role of individual pair-rule genes, it seems likely that the generation of segments by subdivision of a transient double segmental unit is ancestral to the insects, or at least most of them. Pair-rule expression has been recorded for *hairy* (*h*)[79] and *eve*[78] in *T. castaneum*, and for a *paired box gene 3/7* (*pax3/7*) in *S. americana*[75].

***Non-insect arthropods.*** The situation in arthropods other than insects is not yet clear. Expression of pair-rule gene homologues is consistent with a role in segmentation in a wide range of arthropods, including chelicerates[38–40] and myriapods[17,42]. However, in most cases, the expression data have been interpreted as showing segmentally repeated stripes, not pair-rule patterns[38]. One exception is the expression of a *pax3/7* homologue in the PROSOMA of the spider mite *Tetranychus urticae*, which is pair-rule, even though expression of the same gene in the OPISTHOSOMA seems to be segmental[40]. However, in another chelicerate, the spider *Cupiennius salei*, expression of a *pax3/7* homologue seems to be in segmental, not pair-rule, stripes[38].

Another exception is the myriapod *S. maritima* (BOX 2B), which is a GEOPHILOMORPH centipede that generates a large number of segments as an embryo[86]. Several genes, including a homologue of the pair-rule gene *odd skipped* (*odd*), reveal that initial patterning of the entire trunk involves a double-segment repeat that is subsequently subdivided to generate individual segments[42].

The existence of pair-rule patterning in both centipedes and insects, two distantly related classes of arthropods, could be taken as evidence that a double-segment repeat pattern is ancestral to arthropods. However, geophilomorph centipedes are a derived group even among the myriapods, and might be a special case. It is possible that the geophilomorphs as a group have evolved a segment-doubling step to increase segment numbers[42,87]. However, one observation argues against this. Geophilomorphs share with all centipedes the constraint that no matter how much segment numbers vary they always possess an even number of trunk segments (including the segment carrying the poison claw plus an odd number of leg-bearing segments)[88]. If, as we have suggested, this constraint reflects the initial generation of double-segment units, then this trait too would be ancestral to the centipedes.

Whether or not geophilomorphs are exceptional among myriapods, it seems extremely unlikely that pair-rule expression of genes in *S. maritima* is regulated by a series of gap genes that are analogous to those in *D. melanogaster* — all the available evidence indicates that something more akin to an oscillator is active during segmentation (see below). It is also far from clear whether the resolution of the pair-rule stripes to yield a single-segment repeat is homologous in any way to what happens in *D. melanogaster*[42,87].

## Beyond the *Drosophila* paradigm

***Parallels with vertebrate somitogenesis.*** Until recently, comparative studies of segmentation in arthropods have focused on the homologues of *D. melanogaster* segmentation genes. The inherent problem with this candidate-gene approach is that genes will be overlooked if their role in segmentation has been lost or replaced by a novel mechanism in *D. melanogaster*. Recent discoveries in chelicerates and myriapods indicate that genes of the Notch signalling pathway fall into exactly this category[2,3,89].

Notch signalling is not thought to be involved in the primary segmentation process in *D. melanogaster*. However, in vertebrates, somite patterning uses a segmentation clock, or oscillator, which is dependent on the function of hairy/enhancer of split (HES) family transcription factors, and genes of the Notch pathway (BOX 3a). In spiders[2,3] and in the centipede *Strigamia maritima* (A.D.C. *et al.*, unpublished observations) Notch pathway genes show patterned expression very early in the segmentation process, before expression of segment polarity genes. In spiders, Notch signalling is required for segment formation, and for the resolution of patterned expression of *h*, which is itself the homologue of a *D. melanogaster* pair-rule gene[2,3]. In the centipede, the expression patterns indicate that the expression is dynamic, with many cycles of gene expression generating more than 40 trunk segments. These data would be consistent with the existence of a Notch-dependent oscillator that generates the primary segment pattern in myriapods and chelicerates (BOX 3b). This model is attractive because it explains how posterior segments can arise in the cellular environment of sequentially segmenting arthropods. By analogy with vertebrates, homologues of the primary pair-rule genes — and in particular *h* — might function within the clock mechanism, or downstream of it[2,3,39].

At present there is no evidence that the Notch signalling pathway is involved in the formation of posterior segments in the germ bands of insects, and for *T. castaneum* there are unpublished (but cited[89]) claims that it is not. However, there is equally no evidence that the homologues of *D. melanogaster* gap genes function during segment patterning in chelicerates and myriapods.

The discovery of similarities between the mechanisms that control posterior sequential segmentation in

PROSOMA
The anterior part of the body in chelicerates, including the head, the mouthparts and the walking legs.

OPISTHOSOMA
The posterior part of the body in chelicerates. It does not include any walking legs.

GEOPHILOMORPHS
A group of centipedes, normally soil dwelling, that are characterized by a long, thin body made up of many segments (27–191).

Box 4 | **Unresolved questions and future avenues of research**

**The study of *Drosophila melanogaster* is limited in its ability to answer general questions about arthropod segmentation and its evolutionary history. However, the study of an increasing number of arthropods, using a wider range of approaches, will allow many new questions to be addressed. The advent of RNAi and transgenesis in non-model organisms will also facilitate this process. Some of the key areas that need to be addressed are as follows:**

- **We understand very little about the cellular dynamics of the 'growth zone' in sequentially segmenting arthropods (BOX 2). A much clearer understanding of the basic embryology of some insects is required before gene-expression patterns can be properly interpreted. In many cases, reliable fate maps are desperately needed. Cell-labelling experiments will be important to generate such maps.**
- **Which signalling pathways are involved in posterior elongation? Are Notch, Wnt or fibroblast growth factor pathways involved in basally branching insects and other arthropods?**
- **Is an oscillator involved in the generation of new segments in the growth zone? Are gene or protein levels oscillating, and if so what is the primary oscillator? Cell-labelling experiments and reporter constructs can provide more data. The development of techniques for reporting gene expression in live embryos will also be invaluable for tracking dynamic gene expression.**
- **Is head segmentation achieved through a separate mechanism to that generating trunk segmentation? Are there two separate mechanisms in 'intermediate germ band' arthropods?**
- **What is the role of the mesoderm in generating posterior segments? Are the mesoderm and the ectoderm patterned independently? Is the mesoderm required for the segmentation of the ectoderm, or *vice versa*? These questions are of particular interest, given the similarities that are observed between posterior segmentation in some arthropods and the segmentation of the presomitic mesoderm in vertebrates. The control of segmentation in the mesoderm has been largely ignored.**

some arthropods and that control somitogenesis in vertebrates indicates further candidate genes for which the expression pattern should be examined in arthropods. Wnts and fibroblast growth factors (FGFs) are such candidates. In vertebrates, FGFs are involved in establishing a wavefront[90] that is defined by a threshold level of signalling, below which oscillations of the clock cease. The intensity of signalling is graded from a posterior source, so the level of signalling defines the position at which somites are stably patterned. Wnt signalling functions upstream of the Notch-signalling-dependent segmentation clock, the FGF-dependent wavefront, and posteriorly expressed *cad*-related genes during somitogenesis[90–92] (BOX 3b). Wnt signalling is also known to be involved in the A–P patterning of other deuterostomes[93]. There are already data showing that wingless function is needed for sequential segmentation in both *G. bimaculatus*[29] and *O. fasciatus*[34], but its specific role in this process is unclear.

## Conclusions

Our quest to understand the evolution of arthropod segmentation mechanisms is still in its infancy; some of the important questions that remain unanswered are outlined in BOX 4. However, several general conclusions can already be made.

The definitive segmentation of arthropods reflects the conserved expression of *en* and other segment polarity genes — a role that these genes presumably acquired before the radiation of the main arthropod groups. Homologues of some of the *D. melanogaster* pair-rule genes were also involved in the segmentation of the arthropod common ancestor, but exactly which genes were involved, and whether that animal used a pair-rule segmental pre-pattern, remains unclear.

The role of maternal factors in *D. melanogaster* is not representative, even of all insects. A maternally derived anterior gradient might have evolved more than once in insects, but the use of BCD for this purpose is an invention of the higher Diptera. The involvement of *cad* in segment patterning, or at least growth of the segmenting primordium, is probably a characteristic that is ancestral to arthropods. The ancestral role of *cad* probably extended more anteriorly than it does in *D. melanogaster*, to the development of most or all trunk segments.

In a range of insects, homologues of several of the gap genes are involved in regionalization of the early embryo. A role in regulating Hox gene expression seems to be broadly conserved, but it remains unclear when they acquired the role of instructing the downstream expression of segmentation genes.

An alternative mechanism of segment generation might be operating in the trunk regions of chelicerates and myriapods. This involves the Notch signalling pathway, which might indicate an ancestral role for an arthropod segmentation clock that is at least analogous to that operating in vertebrates.

Opinions differ as to how conserved the basic mechanisms of segmentation will prove to be, but it is already evident that there has been significant divergence during arthropod evolution in the function of some of the best-known genes of the *D. melanogaster* segmentation cascade. One must be careful to avoid the assumption that the *D. melanogaster* pattern in some way represents an evolutionary endpoint, and that other species represent intermediate stages in a progression towards this endpoint. More data will be required, and from a wider range of arthropods, before we can say with any certainty what the ancestral mechanism of arthropod segmentation might have been, and how it has been modified in different groups.

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Competing interests statement
The authors declare no competing financial interests.

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