bowel, an odd-skipped homolog, functions in the terminal pathway during Drosophila embryogenesis

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The terminal genes of Drosophila specify non-segmented regions of the larval body that are derived from the anterior and posterior regions of the early embryo. Terminal class genes include both maternal-effect loci (typified by the receptor tyrosine kinase torso) that encode components of a signal transduction cascade and zygotic genes (e.g. tailless and huckebein) that are transcribed at the poles of the embryo in response to the local activation of the pathway. We have characterized a zygotic gene, bowel, that was identified as a zinc finger homolog of the pair-rule segmentation gene odd-skipped. bowel transcripts are initially expressed at both poles of the blastoderm embryo and in a single cephalic stripe. This pattern depends upon torso and tailless activity, but is not affected in huckebein mutants. We isolated and sequenced five mutations that affect the bowel protein, including a nonsense mutation upstream of the zinc fingers and a missense mutation in a putative zinc-chelating residue. bowel mutants die as late embryos with defects in terminal derivatives including the hindgut and proventriculus. Our results indicate that the developmental roles of odd-skipped and bowel have diverged substantially, and that bowel represents a new member of the terminal hierarchy that acts downstream of tailless and mediates a subset of tailless functions in the posterior of the embryo.

Keywords: Drosophila/embryogenesis/gut development/terminal gene/zinc finger

Introduction

The Drosophila body plan is established during embryogenesis through interacting regulatory networks that are initiated by distinct maternal gene products. Along the anterior–posterior axis, the central segmented domain is dependent upon morphogen gradients encoded by bicoid and nanos, which initiate a transcriptional cascade of zygotic segmentation gene expression that subdivides the embryo into metameric repeats. In contrast, the embryonic termini (anterior and posterior poles) are specified by a different mechanism; rather than establishing maternal transcription factor gradients, the terminal genes encode components of a signal transduction pathway (reviewed by St Johnston and Nüsslein-Volhard, 1992; Duffy and Perrimon, 1994). In this pathway, a localized ligand that is dependent upon the torso-like gene (Savant-Bhonsale

and Montell, 1993; Martin et al., 1994) and probably encoded by trunk (Casanova et al., 1995) activates the receptor tyrosine kinase encoded by torso (Sprenger et al., 1989). Acting through a phosphorylation cascade that includes the kinases Ras1 (Lu et al., 1993) and D-raf (Ambrosio et al., 1989) and additional components such as Sos (Lu et al., 1993), the pathway results in the localized activation of the zygotic genes tailless (tll) and huckebein (hkb) at the poles of the early embryo (Casanova, 1990; Pignoni et al., 1990; Weigel et al., 1990). tll and hkb in turn mediate terminal functions by regulating genes further downstream in the pathway, including fork head (fkh) (Weigel et al., 1989, 1990) and the Drosophila Brachyury homolog, Trg (Kispert et al., 1994).

Terminal gene activity is required for the formation of structures derived from the poles of the wild-type embryo. These include the labrum and acron at the anterior and all elements posterior to the seventh abdominal segment, including the eighth abdominal segment, telson, hindgut, Malpighian tubules and posterior midgut. Most of these structures lack any overt metameric organization and are not initially specified through the action of the segmentation hierarchy. However, despite the overall distinction, there are certain links between genes in the segmentation and terminal hierarchies. One role of the terminal system is to limit segmentation to the central region of the embryo by preventing the inappropriate expression of segmentation genes at the posterior pole (Mahoney and Lengyel, 1987; Strecker et al., 1989; Casanova, 1990; Steingrímsson et al., 1991). At the anterior end of the embryo, interactions between bicoid and the terminal system are necessary to specify the acron and labrum (Frohnhöfer and Nüsslein-Volhard, 1986; Pignoni et al., 1992); in bicoid mutants, the anterior develops as a secondary posterior pole, which represents the terminal default state. Also, many segmentation genes are expressed in terminally derived structures during embryogenesis, where they may perform 'secondary' roles distinct from their segmentation functions. For example, cells in the hindgut express hairy (Ingham et al., 1985), fushi tarazu (Krause et al., 1988) and engrailed (Kornberg et al., 1985), while Kruppel expression in the anlage of the Malpighian tubules (Gaul et al., 1987) is controlled by zygotic terminal genes and is required for subsequent development of these organs (Gaul and Weigel, 1990).

We describe here our molecular and genetic analysis of bowel (bowl), one of two Drosophila genes isolated through sequence homology to the segmentation gene odd-skipped (odd) (M.Hart et al., in preparation). odd is one of the pair-rule genes required to establish portions of every other segment. In common with most pair-rule genes, odd-skipped encodes a putative transcriptional regulator (with four C2H2 zinc fingers) and is expressed
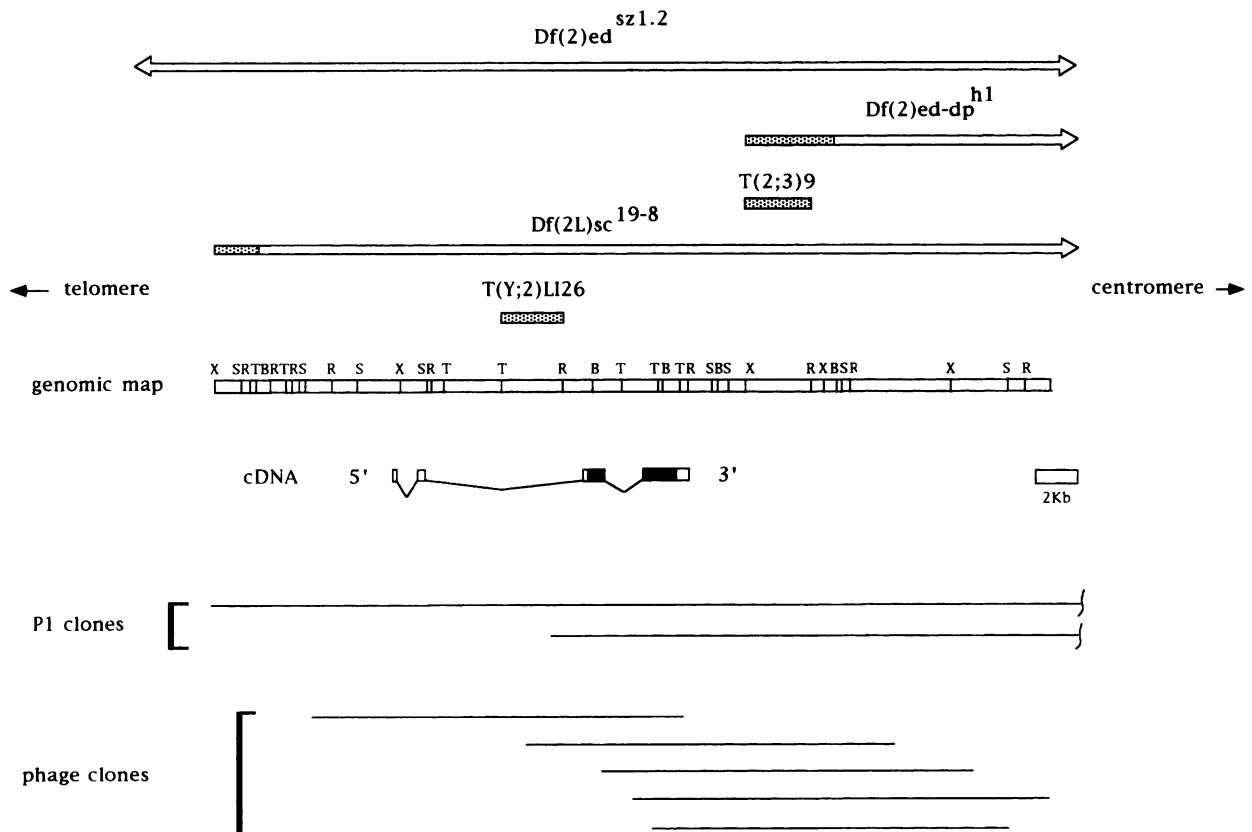
Fig. 1. Molecular organization of the *bowl* region. *bowl* genomic DNA is represented by seven overlapping clones (from top to bottom: P1 clones P31-26 and P31-94 and lambda clones B8, B4, B1, B13 and B7). The *bowl* transcription unit is shown diagramatically below the genomic restriction map; untranslated and protein-coding regions are represented by open and solid boxes, respectively. Chromosomal lesions mapped to the *bowl* region are indicated above the genomic map. Arrows indicate the direction of the deletions and open bars represent genomic sequences that are removed. Hatched bars represent regions of uncertainty. Restriction enzymes: B, *Bam*HI; R, *Eco*RI; S, *Sal*I; T, *Sac*I; X, *Xho*I.

in the appropriate 'pair-rule' pattern of seven transverse stripes at the cellular blastoderm stage of embryogenesis (Coulter *et al.*, 1990). Phenotypic analyses indicate that *odd* is required during segmentation to prevent the inappropriate expression of other pair-rule and segment-polarity genes (DiNardo and O'Farrell, 1987; Coulter and Wieschaus, 1988; Baumgartner and Noll, 1990; Mullen and DiNardo, 1995). Our studies of *bowel* indicate a developmental role that is clearly distinct from *odd*. While both genes are essential for embryogenesis and encode proteins with remarkably similar zinc fingers, *bowel* is initially expressed at both poles of the embryo in response to the terminal gene cascade, and *bowl* mutations primarily affect terminally derived structures including the gut. Our results indicate that *bowel* is a novel terminal gene that functions downstream of *tailless*.

Results

Structural organization and sequence of bowel

The *bowel* (*bowl*) gene is one of two *Drosophila* zinc finger genes that were identified during screens for *odd-skipped* (*odd*) cDNAs (M.Hart *et al.*, in preparation). Two overlapping *bowl* cDNA clones of 3.0 kb and 1.35 kb were isolated using an *odd* probe during these initial screens. We have subsequently isolated two additional *bowl* cDNA clones of 3.3 kb each from a different *Drosophila* embryonic cDNA library using a *bowl* cDNA

subclone as a probe. Although these clones are roughly comparable in size with the 3.4 kb *bowl* transcript identified on Northern blots (see Figure 3), a primer extension experiment using 0–8 h embryonic mRNA as template indicated that the longest cDNA clone lacked ~100 bp from the 5' end of the transcript (data not shown). We therefore employed the RACE (rapid amplification of cDNA ends) strategy (Frohman *et al.*, 1988) to characterize the 5' end of the *bowl* transcript. Consistent with the primer extension result, we isolated a RACE clone that extends 101 bp 5' of the longest *bowl* cDNA; this region includes a small (78 bp) 5' exon not present in the largest cDNAs (see below).

Five overlapping lambda phage genomic clones covering ~32 kb were isolated from an EMBL 4 genomic library using *bowl* cDNAs as probes (Figure 1). In addition, we obtained and partially mapped two P1 phage clones covering >80 kb of the *bowl* region from a P1 genomic library (Smoller *et al.*, 1991). Restriction map comparisons and Southern analysis of cDNA and genomic clones (not shown) indicated that the *bowl* gene contains three introns and encodes a putative primary transcript of ~14 kb. The first two introns (0.8 kb and 8.2 kb) are located in the 5' untranslated region, while the third (2.0 kb) falls within the coding sequence and disrupts the second zinc finger.

Figure 2 shows a composite of the DNA sequence of *bowl*, including the complete cDNA sequence and partial genomic sequence. The putative *bowl* transcription initi-

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tccacagtgcactaagctagttttagtgccaccgctcgctactcgrcgcttgtctctttctacgcatttctcagcccgcgattctctcga
ttctctcttttttccattcgggccctgtttcttgggttcgtggctcAGTCTTCGAGAGTTGATCGTGGCCAACGCACCTGCGCGCGGATCG   45
CTGGTAATAGCTGTTGGAAAAGTGAACTGCATTgtgagtgcccctcgctctatatctatctgtatctttcgccgccgatgtatcttattg   78
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TATTAACAGAAGTCTTCAAACAAATACAACAAGAAAAAACAACATACAATTCAAGTGCCACTTAAATGA^ATAAATTACTACTTTAAAAA   173
CCAAAACAATGCCAAGCATTTCAAAGCTTACAAACTGACAAAAAACCAGCAAAATCATATTGAAACAACTACTTGGATACTTTAGGACGC   263
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.................ttatttatttcgcttttctgttctatccctgtctcggcagTTCCAACGGAATGTGAAGAGCCCCTCCAATATA   344

      M  P  T  E  S  S  S  S  E  I  S  G  G  G  G  A  I  P  M  L  R  P  S  R  M  D  Q  F  M    30
ATGCCCACGGAAAGCTCATCCAGCGAGATCTCCGGCGGAGGAGGCGGTGCGATACCCATGCTGCGACCTTCCCGGATGGATCAGTTCATG   434

   N  S  M  A  A  A  A  A  V  G  G  G  L  P  G  A  A  D  R  N  G  G  S  G  G  S  D  G    60
AACTCGATGGCGGCGGCAGCGGCGGCGGTGGGTGGTGGCGGTCTTCCTGGTGCGGCGGATAGGAACGGCGGATCCGGAGGATCGGATGGT   524

   G  S  Q  N  G  N  G  D  S  R  N  S  S  A  S  R  I  S  A  Y  E  T  Q  L  A  Y  Q  Q  H  L    90
GGCTCCCAGAATGGCAACGGCGATTCGCGCAACTCATCGGCCAGCCGGATCTCAGCGTATGAAACACAGCTGGCCTATCAGCAGCATTTG   614

   A  G  L  H  G  P  P  P  P  P  P  P  S  H  H  R  E  I  S  A  F  V  P  V  L  P  T  G  K  V   120
GCCGGACTGCATGGTCCGCCGCCACCGCCACCACCATCGCATCATCGCGAGATCTCTGCCTTTGTGCCGGTTCTGCCCACCGGAAAGGTG   704

   R  P  G  S  N  S  N  Y  E  I  I  A  M  M  A  D  K  R  K  E  L  A  L  R  E  A  A  A  A  A   150
CGCCCGGGTAGTAATTCCAATTACGAAATCATTGCCATGATGGCGGACAAGCGAAAGGAGTTGGCCCTAAGGGAGGCGGCTGCGGCGGCG   794

   A  M  L  G  R  G  P  G  G  P  G  G  P  G  V  P  P  P  G  V  L  Y  G  P  A  G  V  P  P  P   180
GCAATGTTAGGACGAGGTCCTGGAGGACCCGGCGGTCCTGGTGTGCCACCGCCTGGAGTGTTGTACGGACCAGCTGGCGTGCCACCTCCG   884

   P  Y  L  T  G  P  G  P  S  P  T  G  A  G  S  S  P  F  P  P  G  A  A  A  A  L  F  P  P   210
CCATATCTCACCGGACCGGGTCCATCGCCCACGGGAGCCGGAAGTTCTCCATTTCCACCAGGAGCCGCCGCCGCAGCTCTATTTCCACCT   974

   G  L  G  P  G  M  H  A  G  L  D  R  R  L  L  R  A  P  G  R  A  S  R  P  K  K  Q  F  I  C   240
GGCCTGGGTCCTGGAATGCATGCCGGACTCGATAGGCGCCTGCTACGTGCTCCTGGTCGGGCATCGCGGCCTAAAAAACAGTTCATCTGC   1064

   K  F  C  N  R  Q  F  T  K  S  Y  N  L  L  I  H  E  R  T  H  T  D  E  R  P  Y  S  C  D  I   270
AAGTTTTGCAATCGACAATTCACCAAGTCGTACAATCTGCTCATCCACGAGAGGACACACACGGACGAGCGGCCCTATTCCTGTGATATC   1154

   C  G  K  A  F  R  R  Q  D  H  L  R  D  H  R                                               285
TGCGGCAAGGCGTTCCGGCGACAGGATCACCTGAGGGATCACAGgtgagtcgaggttggggtacaagtaatcacaaaaggggggcagtgtc   1198
tgctcgggtaattgccatcaggcctggagttactctttgtgctcaaagtgtgaacgaatggcgaataatggaataccctgtaaaggcaaa
ggatattagatcatgtaatttattgcttactaggcagtttatacacacattctgaaca...................gcccacgctat

                                    Y  I  H  S  K  E  K  P  F  K  C  T  E  C  G  K    301
aacaataaagtctaattgtttctccacttgtattcgcccagGTATATTCACTCCAAGGAGAAACCCTTCAAATGCACGGAATGCGGCAAG   1247

   G  F  C  Q  S  R  T  L  A  V  H  K  I  L  H  M  E  E  S  P  H  K  C  P  V  C  S  R  S  F   331
GGATTCTGCCAATCGAGAACCTTGGCTGTCCACAAGATCCTGCACATGGAGGAATCACCCCACAAGTGCCCCGTCTGCAGTCGATCATTC   1337

   N  Q  R  S  N  L  K  T  H  L  L  T  H  T  D  H  K  P  Y  E  C  S  S  C  G  K  V  F  R  R   361
AATCAGCGCTCCAACCTGAAGACCCATCTGCTCACCCACACGGATCACAAGCCCTACGAGTGCTCTTCATGCGGCAAAGTTTTCCGCCGT   1427

   N  C  D  L  R  R  H  A  L  T  H  A  V  G  E  V  N  S  G  D  Y  V  D  V  G  E  E  D  E  A   391
AACTGCGATCTACGACGCCATGCCTTGACCCATGCAGTGGGTGAGGTCAACTCCGGGGACTATGTGGATGTGGGCGAAGAGGATGAGGCC   1517

   R  N  L  S  G  D  E  E  D  S  L  L  E  V  D  S  P  R  Q  S  P  V  H  N  L  G  E  S  G  G   421
AGAAATTTAAGTGGCGACGAGGAGGATTCGTTGCTGGAAGTGGACTCGCCTCGGCAGTCGCCAGTTCACAATTTGGGCGAGTCTGGAGGA   1607

   S  G  E  K  S  E  S  E  R  M  R  L  K  R  K  A  A  I  D  H  E  E  S  E  E  E  F  D  D  F   451
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   D  E  E  E  L  Q  D  L  P  R  V  H  K  L  P  R  E  E  D  D  D  F  D  P  E  D  E  E  Q   481
GACGAGGAAGAGGAACTGCAGGATCTGCCAAGAGTACACGATTTGCCCAGGGAAGAGGACGAACGATTTGATCCCGAGGACGAGGAGCAG   1787

   A  E  V  A  L  V  A  R  F  Q  A  S  K  A  A  A  T  S  Q  S  S  S  V  G  T  K  P  E  R   511
GCAGAAGTGGCACTAGTGGCGCGTTTCCAGGCGAGTAAGGCTGCCGCCACATCGCAATCATCGTCGTCAGTGGGCACCAAGCCGGAACGC   1877

   Q  G  V  T  H  C  H  H  E  G  G  E  T  Y  T  M  R  P  H  G  E  K  H  Q  E  E  P  G  N  S   541
CAGGGAGTCACCCACTGTCATCATGAGGGTGGTGAAACCTACACAATGCGGCCACATGGCGAGAAACATCAGGAGGAACCCGGGAACTCT   1967

   G  I  A  S  L  P  V  P  P  S  F  V  R  Y  S  V  P  P  G  A  A  G  P  P  P  A  P  P  G  A   571
GGCATTGCCAGCCTACCCGTTCCACCTTCCTTTGTGCGATATTCTATGCCACCTGGGGCAGCTGGACCGCCGCCTGCAAACCCTGGAGCT   2057

   P  P  P  T  H  Q  H  P  G  H  P  H  L  L  P  P  N  G  D  P  Y  L  P  I  L  H  V  R  R  D   601
CCTCCGCCAACCCACCAGCACCCAGGACATCCACACTTACTCCCACCCAACGGGGATCCCTATTTGCCCATTCTGCACGTTCGACGCGAC   2147

   L  H  H  K  S  L  N  L  S  K  A  G  V  P  P  P  P  H  T  P  P  T  I  I  T  Q  P  E  S  G   631
TTGCACCACAAGAGCTTGAACCTTTCGAAGGCTGGTGTTCCACCGCCACCACATACACCACCCACTATAATAACCCAGCCGGAGTCGGGT   2237

   K  P  L  N  Q  P  L  H  S  P  H  E  A  M  P  S  F  L  G  S  I  P  M  R  K  R  I  L  P  A   661
AAACCACTCAATCAACCTTTGCATTCACCGCATGAAGCTATGCCCAGCTTCCTGGGATCGATACCCATGCGAAAGAGGATTCTTCCGGCA   2327

   P  T  L  D  L  M  D  P  H  H  H  P  G  L  G  Q  R  T  F  V  D  S  P  S  I  Y  A  L  I. M   691
CCGACCTTGGACTTGATGGATCCGCACCACCATCCTGGATTGGGTCAAAGGACCTTTGTGGATAGTCCCAGCATATATGCTTTGAATATG   2417

   S  R  H  P  P  R  Q  L  L  G  K  P  P  S  T  E  T  S  G  A  T  T  E  K  G  P  P  V  P  A   721
TCGCGACATCCCCCCAGACAGCTTCTGGGAAAACCACCGAGTACGGAAACAAGTGGAGCCACCACCGAAAAGGGACCACCAGTGCCAGCA   2507

   P  P  I  A  P  P  P  A  P  P  R  R  T  G  F  S  I  E  D  I  M  R  R                        744
CCGCCCATAGCACCACCGCCTGCACCACCGGAGAAGAACAGGGTTCAGCATTGAGGACATAATGAGGCGTTAGAAGTAAACCGAGAAAGAA   2597
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ATTAATAATGAAAAACAAttgcgtgcctttttttcttgaagt
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Fig. 2. Nucleotide and amino acid sequences of the *bowl* gene. The sequence of the *bowl* transcript is indicated by upper-case letters and represents a composite of the embryonic cDNAs and the 5' RACE clone. Additional genomic sequences are shown in lower-case and include regions upstream of the transcription initiation site, downstream of the polyadenylation site, plus partial sequences of the three introns. The inferred amino acid sequence is indicated above the cDNA sequence. The cDNA and protein sequences are numbered from the putative transcription start site and initiator methionine, respectively.

ation site identified by the RACE clone is flanked by the arthropod transcription 'initiator' sequence TCAGT (Cherbas and Cherbas, 1993). At the 3' end, poly(A) tails were present in both of the longest (3.3 kb) cDNA clones at a common site 24 bp downstream of a consensus polyadenylation signal (AATAAA).

The cDNA sequence contains a single long open reading frame (ORF) of 2232 bp. The putative initiation codon at nucleotide 345 of the cDNA sequence represents the first in-frame ATG and occurs in a context (TATA<u>ATG</u>) that more closely matches the *Drosophila* translation start consensus sequence (C/A)AA(A/C)<u>ATG</u> (Cavener, 1987) than the next five potential initiator codons. The ORF encodes a 744 residue protein with a predicted molecular weight of 79.8 kDa and an estimated isoelectric point of 6.9 (120 basic versus 86 acidic residues). The most notable feature of the protein is the presence of five tandem zinc fingers near the middle of the sequence (residues 237–376). Besides matching the consensus for C2H2 class zinc fingers, these show a remarkable degree of similarity to the zinc fingers encoded by the *odd-skipped* and *sob* genes (M.Hart *et al.*, in preparation), with 84% identity (94/112 residues) between the first four fingers of *bowl* and *odd* (which has only four fingers) and 93% identity (130/140 residues) between the five zinc fingers of *bowl* and *sob*.

In addition to the potential DNA binding motif represented by the zinc fingers, the Bowl protein includes at least three potential transcriptional activation domains. These include two proline-rich regions near the N-terminus (residues 156–210) and C-terminus (residues 546–646), which contain 35% and 30% proline, respectively, and an acidic domain (residues 381–470), located immediately following the zinc fingers, that contains 40% glutamate or aspartate. Additional features of the protein include homopolymeric stretches of alanine (residues 34–39, 146–151 and 202–206), glycine (residues 11–15 and 41–44) and proline (residues 96–102).

Expression of bowel during embryogenesis

Northern blot analysis of RNA from various embryonic stages was used to determine the mobility and relative abundance of *bowl* transcripts during development. To avoid potential cross-reaction with homologous genes such as *odd* and *sob*, a 'zinc-fingerless' cDNA subclone derived from the 3' end of the *bowl* gene was used as a probe. As shown in Figure 3A, a single transcript of 3.4 kb was first detected in 2–4 h embryos. The level of this transcript increased in subsequent stages and reached a peak at 8–12 h, followed by a reduction in 12–24 h embryos. The absence of detectable transcript in 0–2 h embryos suggests that no maternal *bowl* messages are deposited in the early embryo. The presence of a single transcript size at all subsequent stages indicates that neither alternative splicing nor differential promoter usage is likely to contribute significantly to the regulation of *bowl* expression, although we cannot rigorously exclude the possibility of multiple mRNA isoforms with similar mobilities and/or relatively low abundance.

We used whole mount *in situ* hybridization to wild-type embryos to determine the spatial distribution of *bowl* transcripts. For these experiments, an antisense RNA probe was synthesized from the same cDNA subclone as was used to probe the Northern blot. Consistent with the
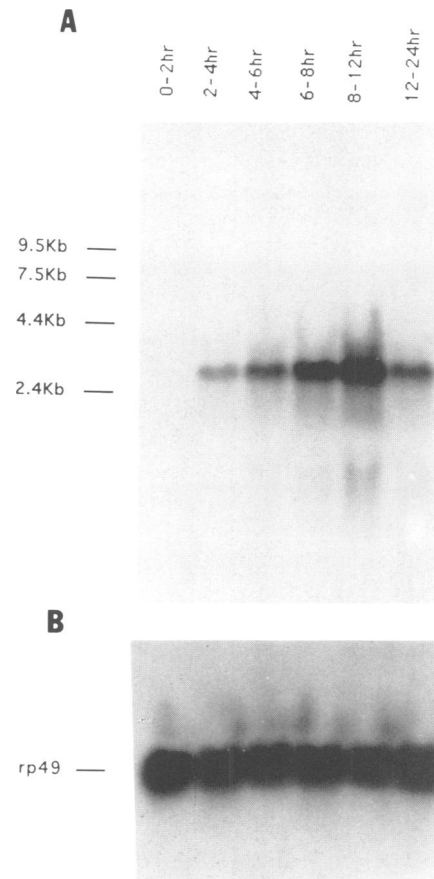
Fig. 3. Temporal expression of *bowl* during embryogenesis. (**A**) Total RNA from the indicated embryonic stages was Northern-blotted and probed with a 1.2 kb *Pst*I–*Eco*RI fragment (pB-P) subcloned from the 3' end of *bowl* cDNA pB. The migration of size standards is indicated on the left. (**B**) As a control for loading, the blot was re-probed with ^{32}P-labeled rp49 cDNA.

Northern results, *bowl* expression was first detected at the cellular blastoderm stage (late stage 5; stages according to Campos-Ortega and Hartenstein, 1985). Near the end of cellularization, *bowl* transcripts accumulate in three distinct domains, corresponding to the anterior and posterior poles of the embryo plus a single transverse 'stripe' in the gnathocephalic region of the blastoderm (Figure 4A). At the posterior terminus, a strong 'cap' of expression extends from the pole at 0% egg length (EL) to ~10% EL. The transverse stripe, approximately six cells wide, is centered at 67% EL at the dorsal midline and 76% EL at the ventral midline. At the anterior pole, expression is detectable in a region covering ~85–100% EL. Staining in this domain is weaker and appears less uniform than the posterior cap or anterior stripe, with the intensity appearing maximal just posterior to the anterior tip of the embryo and trailing to a poorly defined posterior limit.

Subsequent to the blastoderm stage, the initial *bowl* pattern changes to reflect the morphological rearrangements associated with gastrulation and germ band extension (Figure 4B–E). The posterior domain, which corresponds to the anlagen of the hindgut and posterior midgut (PMG), moves dorsally and anteriorly as gastrulation commences (stage 6), and follows the amnioproctodeal invagination into the interior during germ band elongation (stages 7–9). In the anterior, cells from the ventral region
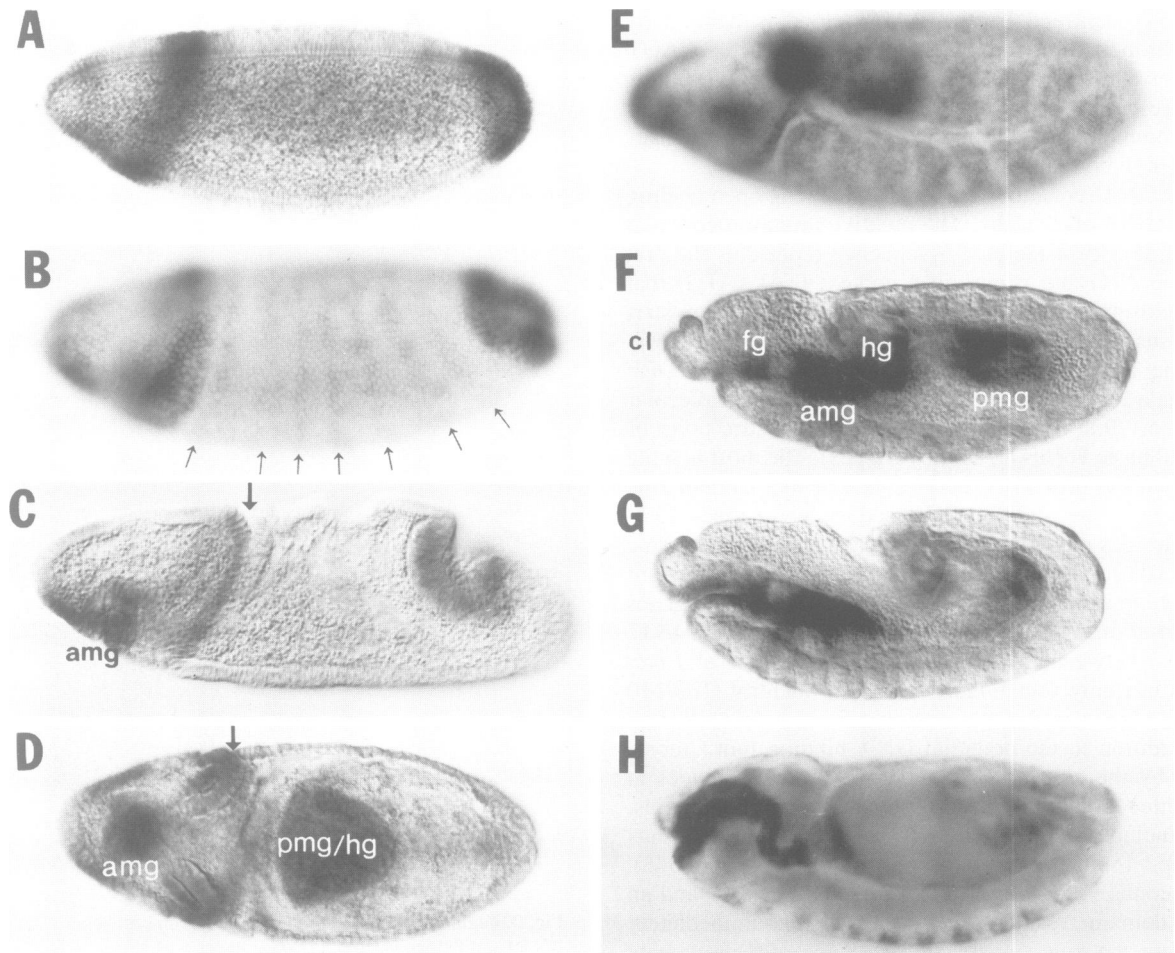
Fig. 4. Spatial distribution of *bowl* transcripts during embryogenesis. Fixed wild-type embryos were hybridized with a digoxygenin-labeled antisense RNA probe synthesized from cDNA subclone pB-P (see Materials and methods). Embryos were photographed with Normarski (A, C, D, F and G) or bright-field (B, E and H) optics. All embryos are oriented with anterior to the left and dorsal side up, except for the dorsal view in (D). (A) Stage 5 (cellular blastoderm). *bowl* transcripts accumulate at both the anterior and posterior poles and in a broad anterior stripe. (B) Stage 6 (early gastrulation). In addition to the terminal domains, seven faint stripes in the central region are also apparent (arrows). Note that the cephalic furrow is visible just posterior to the *bowl* anterior stripe. (C) Stage 7 (gastrulation complete). The *bowl* posterior domain follows the amnioproctodeal invagination, and cells from the anterior domain have begun to invaginate with the anterior midgut. The arrow indicates the position of the cephalic furrow. (D) Stage 8 (rapid phase of germ band elongation). Expression is apparent in the anterior midgut (which lies out of the focal plane), posterior midgut and hindgut. (E) Stage 9 (rapid phase of germ band elongation complete). Note that *bowl* is expressed in 14 broad stripes in the germ band. (F) Stage 11 (fully extended germ band). Transcripts have accumulated in cells in the stomodeal invagination and, at lower levels, the clypeolabrum. The anterior stripe is no longer apparent, and *bowl* expression has ceased in the region connecting the hindgut and posterior midgut. (G) Stage 12 (germ band retraction). *bowl* expression in the hindgut is undetectable and the expression in the posterior midgut has diminished. (H) Stage 14 (beginning of head involution). *bowl* transcripts are no longer detected in the midgut, but persist in the foregut. Abbreviations: amg, anterior midgut; pmg, posterior midgut; hg, hindgut; fg, foregut; cl, clypeolabrum.

of the *bowl* domain follow the invaginating primordium of the anterior midgut (AMG); this region extends caudally as the elongating germ band reaches its maximum extent (stage 10). At the beginning of gastrulation the cephalic furrow forms at ~65% EL, the posterior boundary of the transverse *bowl* stripe, and a fraction of the cells in the stripe become incorporated into the deepening cephalic furrow. Expression in this stripe declines progressively as germ band elongation proceeds and eventually vanishes as the cephalic furrow disappears.

Additional changes in the transcript pattern become apparent subsequent to gastrulation and germ band elongation (Figure 4E–H). At the anterior of the embryo, two additional domains appear during stages 9–10: cells in the invaginating stomodeum show strong expression, and cells at the tip of the procephalon that correspond to the future clypeolabrum express *bowl* transcripts at lower levels

(Figure 4E). Although high levels persist in the hindgut and posterior midgut, expression decreases in the intervening region, which includes the Malpighian tubule primordium (Figure 4F). By the time germ band retraction commences (early stage 12; Figure 4G), *bowl* expression has decreased substantially in the PMG and almost disappeared from the hindgut. In contrast, cells in the AMG and foregut (stomodeal invagination) continue to express *bowl* at a high level, and transcript levels in the clypeolabrum have increased. Subsequently, *bowl* expression in the midgut decreases, becoming undetectable by the beginning of head involution (stage 14), but persists strongly in the foregut (Figure 4H).

In addition to the obvious *bowl* expression in the terminal regions and the cephalic stripe, we observed seven transverse stripes, each approximately two to three cells wide, in the trunk region of early gastrulae

(Figure 4B). This initial striped pattern, which corresponds to a double-segment periodicity characteristic of the pair-rule genes, was barely detectable in most embryos, and the phasing of these stripes was not determined. During germ band elongation, the pattern evolves rapidly to a single-segment periodicity: transcripts accumulate in additional, interstitial stripes in rows of cells that previously had no detectable expression, so that by the end of the rapid phase of germ band elongation (stage 9), 14 equivalent stripes are apparent (Figure 4E). By this stage, both the intensity and the width of the stripes have notably increased, such that each spans six to eight cells (covering from two-thirds to three-quarters of each segment). Although the *odd-skipped* stripes undergo a similar doubling during early gastrulation, *odd* is expressed in a lower level in the 14 stripes than in the initial pair-rule stripes, and the *odd* stripes remain relatively narrow (one to three cells wide) at the end of germ band extension.

Regulation of *bowel* expression by terminal class genes

The accumulation of *bowel* transcripts at the poles of the blastoderm is reminiscent of zygotic terminal class genes that are activated by the terminal cascade. To investigate the role of the terminal hierarchy in the initial expression of *bowl*, we used *in situ* hybridization to examine the initial *bowl* transcript pattern in embryos mutant for the maternal-effect gene *torso* (*tor*) and the zygotic genes *tailless* (*tll*) and *huckebein* (*hkb*). Although no alterations were noted in *hkb* mutants (data not shown), the lack of either *tor* or *tll* function was found to cause significant changes in the expression of *bowl* (Figure 5). In both mutants (i.e. homozygous *tll* embryos or embryos derived from homozygous *tor* mothers), the most dramatic effect was the complete absence of *bowl* expression at the posterior pole of the embryo. Because *tll* expression requires the activity of *tor* (Pignoni *et al.*, 1992), the effect of *tor* on *bowl* in the posterior is probably mediated through *tll*. In the terminal hierarchy, both *tll* and *hkb* function downstream of *tor*, and it has been inferred that the two genes together mediate all terminal functions of *tor* in the posterior (Weigel *et al.*, 1990). Because the posterior cap of *bowl* strictly requires *tll* but not *hkb*, *bowl* appears to lie downstream of *tll* alone. This contrasts with the posterior expression of *fkh* and *Trg*, which are affected by both *tll* and *hkb* (Weigel *et al.*, 1990; Kispert *et al.*, 1994).

In the anterior of *tor* and *tll* mutant embryos, *bowl* transcripts were detected in a single strong cap at the pole, unlike the initial pattern observed in wild-type (a diffuse, weakly staining cap plus a cephalic stripe) (Figure 5A and C). This domain appeared somewhat broader in *tll* embryos than in *tor* due to differences in its posterior extent (~75% versus 80% EL, respectively). Although in both *tor* and *tll* mutants the intensity initially appears uniform, in each case the cap subsequently retracts to a stripe via loss of transcripts from the anterior pole. Interestingly, the timing of this retraction is not the same in the two mutants, occurring by the end of cellular blastoderm in *tll* embryos (Figure 5D) but subsequent to gastrulation in *tor* mutants (Figure 5B). Both the intensity of the cap and its subsequent retraction to a stripe suggest that it results from the anterior expansion of the cephalic

stripe normally present in wild-type. Although other explanations are possible, this proposed expansion would be similar to behavior reported for the head-specific gap gene *orthodenticle* (*otd*), which is normally expressed as a discrete stripe by the end of the cellular blastoderm stage but extends to the anterior pole in *tor* mutants (Finkelstein and Perrimon, 1990).

Cytogenetic mapping and mutagenesis of *bowel*

The expression of *bowel* in the embryonic termini and its regulation by terminal class genes indicated that the gene might mediate some aspect(s) of terminal development. To test this possibility, we undertook a genetic analysis of the *bowl* locus. This began with a cytogenetic analysis to localize the gene and identify associated chromosomal aberrations, followed by a screen to isolate new point mutations.

Initially, the *bowl* gene was localized to the 24C region of polytene chromosomes by *in situ* hybridization (data not shown). We obtained several deficiency and translocation stocks that affect this region (de Belle *et al.*, 1993) and used a combination of genomic Southern blotting and RFLP analysis to determine which lesions remove and/or break in the vicinity of the *bowl* gene. We determined that the *bowl* transcription unit is completely removed by the deficiencies $Df(2)ed^{sz1.2}$ and $Df(2L)sc^{19-8}$, based upon the failure of either chromosome to contribute any RFLP pattern (see Materials and methods), whereas the transcription unit is intact in deficiencies $Df(2)ed\text{-}dp^{h1}$ and $Df(2L)M11$. We also mapped breakpoints associated with four different chromosomal rearrangements. These include a breakpoint of $Df(2L)sc^{19-8}$ located ~10 kb upstream of the transcription start site, the breakpoint of translocation $T(Y;2)L26$ within the second *bowl* intron, and two breakpoints, associated with $Df(2)ed\text{-}dp^{h1}$ and $T(2;3)9$, that map to a common restriction fragment 5 kb downstream of the *bowl* polyadenylation site (see Figure 1). Based upon previous cytogenetic characterizations (de Belle *et al.*, 1993) and our identification of the $Df(2L)sc^{19-8}$ and $Df(2)ed\text{-}dp^{h1}$ breakpoints, we concluded that the *bowl* transcription unit maps to polytene band region 24C2-5 and is oriented with the 5' end toward the telomere and the 3' end toward the centromere.

The results presented above indicated that the *bowl* transcription unit resides in a discrete cytogenetic interval of ~30 kb, between the distal breakpoints of deficiencies $Df(2L)sc^{19-8}$ and $Df(2)ed\text{-}dp^{h1}$. Furthermore, the *bowl* transcription start site and a portion of the 5' untranslated region are separated from the protein-coding sequence by the translocation $T(Y;2)L26$, indicating that this breakpoint disrupts *bowl* function. A single point mutation, $l(2)c^{E17}$, has been mapped to this interval and reportedly fails to complement $T(Y;2)L26$ (de Belle *et al.*, 1993). However, our analysis indicated that an unrelated secondary lethal mutation was associated with this allele. We removed the linked mutation and found that homozygotes were partially viable. Given the lethality associated with other *bowl* mutations (see below), this result indicates that the *E17* mutation is hypomorphic.

On the assumption that *bowl* is an essential gene, we conducted an F2 mutagenesis screen to isolate recessive lethal point mutations. From a total of 10 800 ethyl methanesulfonate (EMS)-mutagenized chromosomes, we
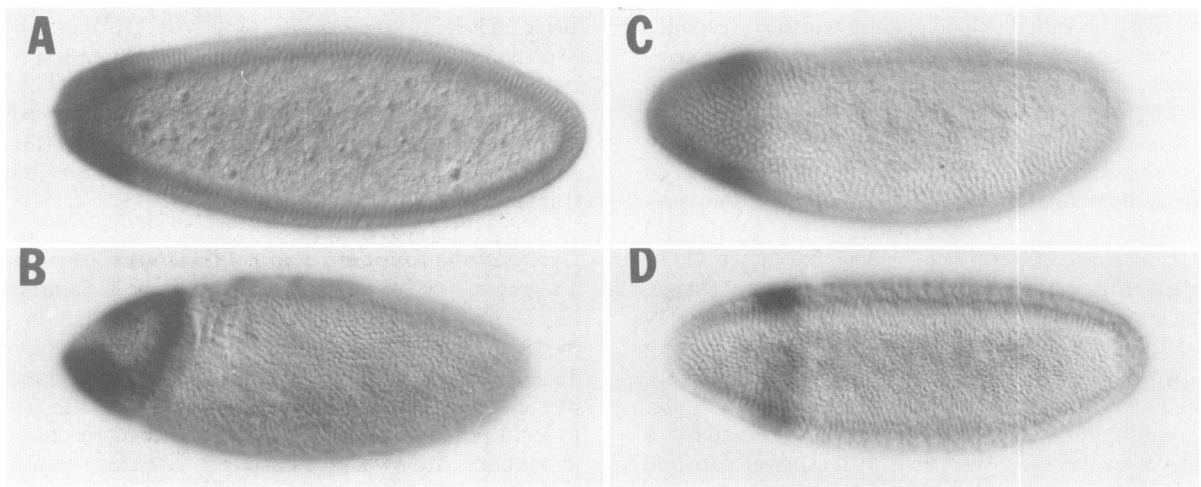
Fig. 5. bowl expression in terminal mutants. Whole mount embryos collected from tor^PM51 homozygotes or tll^L10/TM3 heterozygotes were hybridized to the digoxygenin-labeled bowl probe as in Figure 4. Embryos are oriented with anterior to the left and dorsal side up. In tor mutants at the cellular blastoderm (**A**) and gastrula (**B**) stages, bowl expression at the posterior pole was undetectable. At the anterior, bowl transcripts initially accumulate in a broad cap covering 80–100% EL (**A**) that retracts to a stripe at 80% EL during gastrulation (**B**). In tll mutants (**C** and **D**), bowl expression at the posterior pole is also undetectable, while a broad anterior cap covering 75–100% EL at the early cellular blastoderm stage (**C**) resolves into a stripe at 75% EL during subsequent cellularization (**D**); this is located at a position equivalent to the anterior stripe in wild-type embryos.

Table I. Sequence alterations associated with bowel mutations

Allele	AA residue	Wild type AA (codon)	Mutation AA (codon)	Comments
bowl[1]	232	Ser (TCG)	Stop (TAG)	five residues upstream of 1st finger
bowl[2]	284	His (CAC)	Tyr (TAC)	His_{20} of 2nd zinc finger
bowl[3]	261	Thr (ACG)	Met (ATG)	1st finger, residue 25
bowl[4]	279	Asp (GAT)	Asn (AAT)	2nd finger, residue 15
bowl[5]	343	Thr (ACC)	Ile (TCC)	4th finger, residue 23
	345	Thr (ACG)	Lys (AAG)	4th finger, residue 25
	609	Leu (CTT)	Pro (CCT)	233 residues downstream of fingers
bowl[6]	none			
bowl[7]	ND			

The protein-coding region of each allele was amplified, cloned and sequenced as described in Materials and methods. Zinc finger residues are numbered according to the scheme: $X_{1-3}C_4X_{5-6}C_7X_{8-10}F_{11}X_{12-16}L_{17}X_{18-19}H_{20}X_{21-23}H_{24}X_{25-28}$ (Desjarlais and Berg, 1992). ND, not determined.

obtained 87 lethal mutations that failed to complement deficiency Df(2)ed^sz1.2. Seven mutations (bowl^1-7) mapped to the interval between the distal breakpoints of deficiencies Df(2L)sc^19-8 and Df(2)ed-dp^h1, and each of these also failed to complement T(Y;2)L26. Complementation inter se indicated that all seven mutations are allelic. One of the alleles, bowl^7, could not be maintained as a balanced stock due to a linked dominant female sterile mutation on the mutagenized chromosome and was not further characterized.

Based upon complementation to the pre-existing hypomorphic allele (E17), we classified bowl mutants into three groups. The potential null alleles, bowl^1 and bowl^2, behaved similarly to Df(2)ed^sz1.2 or Df(2L)sc^19-8 (2–10% adult viability when heterozygous in trans with l(2)c^E17). Moderate alleles include bowl^5 (20–30% viability in trans to l(2)c^E17) and bowl^3 and bowl^4 (50–60% viability in trans to l(2)c^E17). A weak allele, bowl^6, fully complemented l(2)c^E17. These results confirmed that the E17 allele represents a weak hypomorph, given that the weakest newly induced mutation (bowl^6) failed to complement other new bowl alleles.

Sequence analysis of bowel mutants

To confirm that the mutant phenotypes associated with these alleles are caused by mutations in bowl, the protein-coding regions were amplified, cloned and sequenced (see Materials and methods). Five of the six alleles analyzed were associated with nonsense or missense mutations (Table I). Interestingly, these are not randomly distributed: all five alleles encode mutant proteins in which the zinc fingers are either altered (bowl^2-bowl^5), or, in the case of the nonsense mutation (bowl^1), absent from the truncated protein. Besides indicating the importance of this potential DNA binding motif for bowl function, these data appear consistent with our phenotypic classification (see above). The apparent null mutations either eliminate the zinc fingers (bowl^1) or substitute one of the zinc-chelating histidines (bowl^2) in the second finger, which would likely disrupt the folding of the motif. The moderate alleles change non-chelating residues in the first (bowl^3), second (bowl^4) or fourth (bowl^5) fingers; these presumably have less severe effects on the function of the protein, possibly by reducing or altering the binding specificity of the Bowl protein. Indeed, the bowl^4 mutation affects one of the four
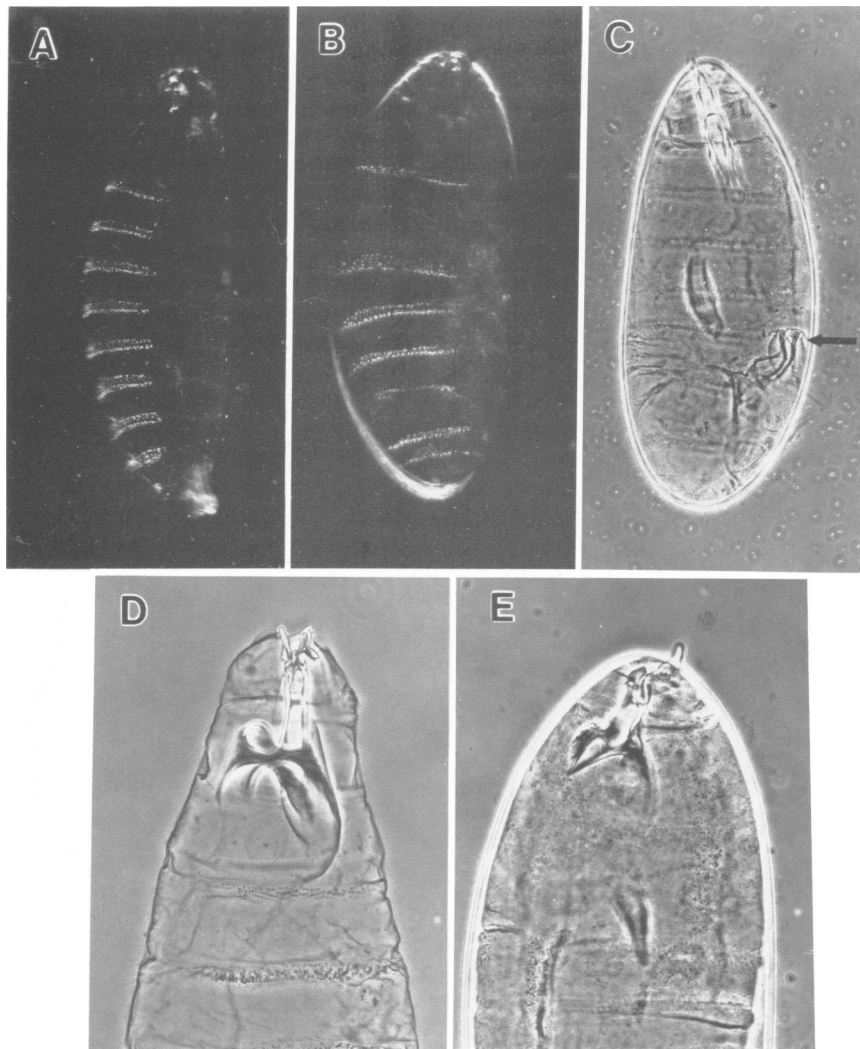
Fig. 6. Cuticle defects associated with *bowl* mutations. Dark-field (A and B) and phase contrast (C–E) photomicrographs of cuticle preparations of first instar larvae or late mutant embryos are shown. Segmentation patterns of (**A**) wild-type and (**B**) a *bowl¹/bowl³ trans*-heterozygote. In the mutant, the second abdominal denticle belt is missing and the sixth abdominal denticle belt is defective, whereas the remaining abdominal segments appear normal. (**C**) A *bowl¹* homozygous embryo showing a 'tail-up' phenotype. The position of the posterior spiracles and Filzkorper are indicated (arrow). (**D** and **E**) Cephalopharyngeal structure of wild-type (D) and a *bowl¹/bowl² trans*-heterozygote (E). Compared with the wild-type embryo, the lateralgräten are shortened and the ventral arm is reduced in the mutant. Note that each of the three types of cuticular abnormalities illustrated shows variable expressivity, and the segmentation defect in particular exhibits low penetrance. However, each phenotype was observed with more than one *bowl* allele and was also confirmed in various *trans*-heterozygous combinations.

positions (13, 15, 16 and 19; see Table I legend) that are most intimately involved with DNA binding in other C2H2 zinc fingers (Nardelli *et al.*, 1991; Pavletich and Pabo, 1991, 1993). With *bowl³* or *bowl⁵*, the affected residue(s) lies adjacent to the second histidine (position 24), at or near the C-terminal end of the putative DNA recognition α-helix. However, in the case of *bowl⁵*, it is conceivable that neither of the two finger mutations identified is significant, given the formal possibility that the reduced function of this allele is solely due to a third change (Leu609 to Pro) downstream of the zinc fingers. Finally, the weakest mutation analyzed (*bowl⁶*) appears to leave the protein-coding region intact. Preliminary genetic characterization (not shown) suggests that this allele might be associated with a chromosomal translocation, although a breakpoint in the vicinity of *bowl* has not been mapped.

bowel functions in embryonic gut development

Animals homozygous for our newly induced *bowl* mutations fail to develop to adulthood. The only exception is *bowl⁶*, in which escapers were occasionally observed. Four of the mutations (*bowl¹*, *bowl²*, *bowl³* and *bowl⁵*) cause embryonic lethality. The remaining mutation, *bowl⁴*, exhibited embryonic semi-lethality: ~50% of the homozygous embryos hatch and die as larvae or pupae. Mutants homozygous or *trans*-heterozygous for the embryonic lethal alleles appear to die as late embryos, since most major morphogenetic movements (germ band elongation, segmentation, head involution) were observed and the larval cuticle was secreted.

Analysis of ectodermal cuticular structures revealed three specific abnormalities in *bowl* homozygotes. Anteriorly, derivatives of at least two of the gnathal
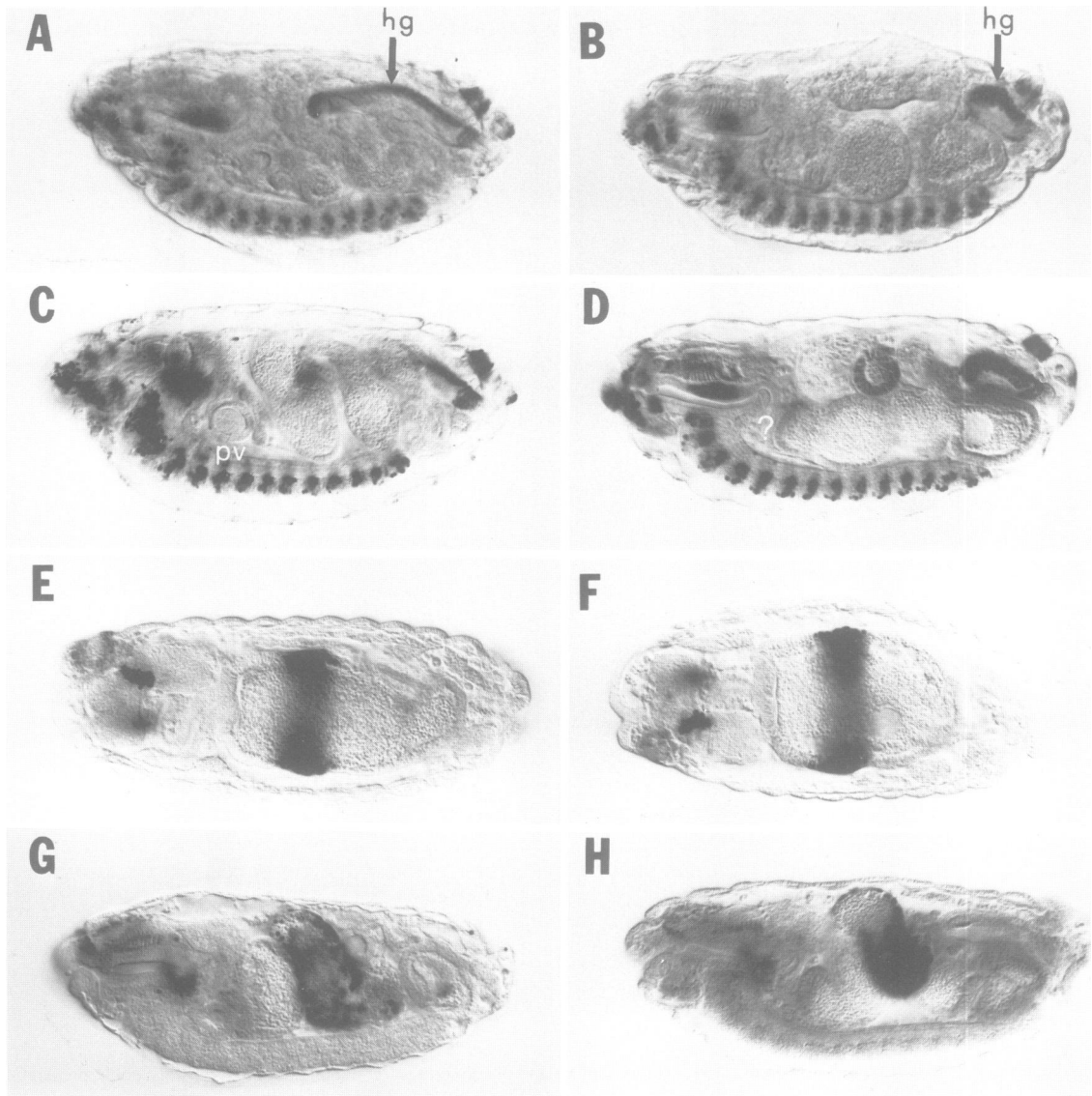
Fig. 7. Embryonic gut development in *bowl* mutants. Embryos were stained with engrailed (A–D) and labial (E–H) antibodies to mark appropriate regions of the hindgut and midgut. Equivalently staged wild-type (A, C, E and G) and *bowl*[1] mutant embryos (B, D, F and H) are shown. Similar phenotypes were observed with other *bowl* alleles (data not shown). (**A and B**) During ventral nerve cord retraction (stage 17), the wild-type hindgut is an elongated tube extending anteriorly and dorsally from the anus (A). In contrast, the hindgut is drastically reduced in the *bowl*[1] embryo (B). (**C and D**) In wild-type embryos (stage 17), a well-developed proventriculus (pv) forms at the junction between the foregut and the midgut (C). In *bowl*[1] mutant embryos at this stage, the characteristic 'heart-shaped' structure is completely absent (D). Engrailed staining of the hindgut, which is out of the focal plane, was used to positively identify the mutant embryos. (**E and F**) At stage 14, labial expression in the middle portion of the midgut appears identical in wild-type (E) and *bowl*[1] (F). (**G and H**) At stage 17, labial is expressed in the second chamber of the convoluted midgut in wild-type embryos (G). In *bowl*[1] mutants (H), this expression is apparently normal, but the midgut chambers are displaced dorsally. Homozygous *bowl*[1] embryos were identified by the reduced hindgut phenotype under Nomarski optics, and these results were further confirmed via double labeling with anti-engrailed and anti-labial antibodies.

segments (mandible and maxilla) were affected. Specifically, compared with wild-type (Figure 6D), the lateral-gräten were shortened and the ventral arm reduced (Figure 6E). Posteriorly, structures including the eigth abdominal segment, anal pads, posterior spiracles, tuft and Filzkorper were present and apparently normal, but were frequently displaced dorsally and anteriorly, resulting in a 'tail-up' phenotype (Figure 6C) that reflects incomplete germ band retraction. This phenotype ranged from as many as three abdominal segments remaining dorsally to a slightly dorsal location of only the most posterior cuticular structures (posterior spiracles and Filzkorper). Finally, in the trunk region, even-numbered denticle belts were occasionally

missing (Figure 6B), with A2 and A6 appearing more sensitive than A4 and A8. However, the penetrance of these segmentation defects was low compared with the terminal abnormalities.

Only a portion of the *bowl*-expressing cells in the termini give rise to cuticular structures. During embryogenesis, *bowl* is expressed in the primordia of all three parts of the digestive tract (foregut, midgut and hindgut); these structures are not readily observed in cuticle preparations. Therefore, we examined the internal morphology in whole mounts of *bowl* mutants to assess the role of the *bowl* gene in gut development. The anterior portion of the embryonic gut is derived from the anterior midgut (AMG)

and stomodeal (foregut) invaginations whereas the posterior portion (PMG and hindgut) develops from a single proctodeal invagination. In *bowl* mutants, all three invaginations appeared to initiate and proceed normally during early embryogenesis. In contrast, mutant embryos did exhibit defects in regions of the mature alimentary tract, most notably the hindgut and proventriculus (Figure 7).

To aid in visualizing the hindgut, we used an antibody to the tissue-specific marker, *engrailed*. In wild-type embryos, *engrailed* is expressed in the hindgut primordium during germ band extension and persists through the remainder of embryogenesis. By stage 16 (ventral nerve cord shortening), the hindgut is an elongated tube extending longitudinally from the anal pad at the posterior to 50% EL on the dorsal side (Figure 7A), where it connects to the midgut. In *bowl* mutants at this stage, the hindgut is substantially reduced in size, extending less than half the normal length (Figure 7B).

The proventriculus, or gastric valve, forms where the midgut (endoderm) and foregut (ectoderm) connect. In wild-type embryos at stage 16 (Figure 7C), the fully developed proventriculus exhibits a heart shape with an internal, esophageal portion encompassed by an external 'funnel' that connects to the midgut; both layers appear to be ectodermally derived (Tepass and Hartenstein, 1994). In *bowl* mutants, the esophagus appears normal, but no structure resembling the proventriculus was apparent (Figure 7D).

To further characterize midgut development, we used antibodies to *labial*, which is expressed in the PMG primordium at germ band extension and subsequently accumulates in the second chamber of the midgut at the end of dorsal closure (Figure 7E). No gross midgut defects were observed in *bowl* mutant embryos. The fusion of the anterior and posterior midgut and subsequent constriction of the midgut appeared to proceed normally (Figure 7F). Notably, during stage 16 the four midgut chambers were arranged somewhat differently in *bowl* mutants compared with wild-type (Figure 7G and H). This altered configuration probably reflects displacement of the midgut resulting from its attachment to the shortened hindgut.

Finally, we examined the morphology of the foregut in *bowl* mutants. In the wild-type embryo, the foregut has a complicated origin and development (Skaer, 1993). The distal portion of the pharynx and esophagus are derived from the stomodeal invagination at germ band extension (stage 10) while the proximal pharynx and atrium develop from the clypeolabrum, hypopharyngeal lobe and gnathal segments during head involution. From anterior to posterior, the fully developed foregut consists of the atrium, pharynx and esophagus, and joins the midgut at the proventriculus. In *bowl* mutants, the atrium, pharynx and esophagus were apparently normal, and we could trace the foregut from the anterior opening to the distal tip of the esophagus.

Discussion

The *bowel* (*bowl*) gene is one of two loci identified as homologs of the pair-rule gene *odd-skipped* (*odd*) on the basis of a highly conserved zinc finger domain (M.Hart *et al.*, in preparation). In view of the possibility that *bowel*

might share similar or redundant functions with *odd*, we have undertaken a molecular and genetic analysis of the *bowl* locus. For this report, we have determined the structure, sequence and cytogenetic location of the *bowl* transcription unit; characterized the expression of *bowl* mRNA during embryogenesis and examined the regulatory effects of mutations in selected terminal class genes; and isolated, sequenced and phenotypically characterized point mutations in the *bowl* locus. Our results indicate that the functions of *bowel* during embryogenesis are distinct from *odd* despite the structural similarities between the two genes.

Embryonic functions of bowel

The lethality associated with *bowl* mutations indicates an essential role for the gene during embryogenesis. The most prominent morphological defects in late *bowl* embryos involve internal structures that are derived from regions of the blastoderm where *bowl* is initially expressed. At the cellular blastoderm stage, the *bowl* transcript pattern includes domains at both poles of the embryo plus a stripe that lies anterior to the cephalic furrow. Cells in the *bowl* posterior domain become incorporated into the proctodeal invagination, which ultimately develops into the posterior portion of the gut including the hindgut and posterior midgut (PMG). At the anterior, *bowl*-expressing cells give rise to the anterior midgut (AMG) and stomodeal invaginations from which the AMG and foregut are derived. In *bowl* mutants, the reduced hindgut phenotype in the posterior and the absence of a proventriculus in the anterior region of the gut indicate the importance of *bowl* expression in both termini. The *bowl* anterior stripe overlaps two of the three gnathal segment primordia (mandibular and maxillary); cells from this region undergo considerable movement during later embryonic stages, eventually incorporating into the foregut and giving rise to portions of the cephalopharyngeal apparatus including mouth hooks, ventral arm and lateralgräten. In *bowl* mutants, defects in the latter two structures, which are derived from either the mandibular or maxillary segments, presumably reflect a function of the *bowl* stripe.

An additional posterior defect associated with *bowl* mutants is the 'tail-up' syndrome. This defect is reminiscent of the mutant phenotypes of at least three other loci (*u-shaped*, *tailup* and *hindsight*; Nüsslein-Volhard *et al.*, 1984; Wieschaus *et al.*, 1984), and indicates an incomplete retraction of the germ band during embryogenesis. Although the cellular and molecular mechanisms involved in this process are not understood, tail-up phenocopies have been generated by laser ablation of a discrete region of the blastoderm located just anterior to the proctodeal primordium (Jürgens, 1987). Because the implicated cells are likely to express *bowl*, it will be interesting to determine whether this aspect of the phenotype reflects a specific function of *bowl* in these cells, and whether interactions with other loci in the 'tail-up' group are involved.

The apparent correspondence between the early expression and mutant phenotype of *bowl* suggests that the gene may play a role in the initial specification of terminal regions of the embryo. However, because expression persists through subsequent embryonic stages, and because the precise period(s) when *bowl* function is required have not been rigorously defined, a role in the maintenance or

elaboration of cell fate cannot be ruled out. In addition, many of the cells that express *bowl* give rise to structures that appear unaffected by *bowl* mutations. For example, the midgut and most of the structures derived from the foregut appear normal in *bowl* mutants even though *bowl* is expressed in the primordia of these tissues. Similarly, although the hindgut is clearly abnormal, the residual structure seen in mutants suggests that some hindgut cells may not require *bowl*. While the limited extent of these defects might be attributed to residual function in the *bowl* mutants analyzed, this is unlikely given that the strongest alleles probably eliminate the ability of the Bowl protein to bind DNA, and deficiency homozygotes that completely lack the *bowl* gene still have a midgut and partial hindgut (Harbecke and Lengyel, 1995). We conclude that the consequences of *bowl* expression are not the same for all cells, even within a given blastoderm domain. Such behavior is reminiscent of the zygotic gene *tailless* (*tll*), whose posterior expression domain is somewhat broader than the region affected in *tll* mutants (Skaer, 1993), and contrasts with the *Drosophila T-related gene* (*Trg*), which is also necessary for hindgut development (Kispert *et al.*, 1994). Whereas *Trg* has been proposed to function as a hindgut selector gene on the basis of its hindgut-specific expression and phenotype, a similar role appears unlikely for *bowl*.

Examination of enhancer-trap lines indicates that the developing gut epithelium becomes divided into a surprisingly large number of discrete domains along its length (Murakami *et al.*, 1994). While each domain or 'compartment' is defined by the expression of various marker genes, these do not appear to correspond to obvious morphological units. A possible explanation for the relatively limited gut defects in *bowl* mutants is that *bowl* is required in only a subset of these compartments. While it is doubtful that *bowl* expression *per se* could define a given compartment, *bowl* might function in concert with other, spatially restricted factors in the combinatorial specification of these domains.

bowl and terminal class genes

The expression of *bowel* at the poles of the early embryo, the alterations in this pattern in embryos mutant for the terminal class genes *torso* and *tll*, and the defects in terminally derived structures seen in *bowl* mutants indicate that *bowl* functions as a mediator of terminal gene activity. The non-metameric embryonic termini are established through the activity of maternal-effect genes that encode components of a signal transduction/phosphorylation cascade (Sprenger and Nüsslein-Volhard, 1993) that acts through the *torso* receptor. The ligand-dependent activation of *torso* results in the transcription of two primary zygotic terminal/gap genes, *tll* and *huckebein* (*hkb*), in overlapping regions at the poles of the embryo. At the posterior pole, expression of both genes is apparently necessary and sufficient to mediate *torso* function, since the *tll hkb* double mutant (but neither single mutant) mimics the full spectrum of *tor* posterior defects (Weigel *et al.*, 1990), and the ectopic development of posterior structures in *tor* gain-of-function mutants is dependent on *tll* and *hkb* activity (Klingler *et al.*, 1988; Weigel *et al.*, 1990). The functions of *tll* and *hkb*, which encode transcription factors in the steroid receptor and C2H2 zinc finger families,

respectively (Pignoni *et al.*, 1990; Brönner *et al.*, 1994), are mediated through the activation and repression of other, subordinate zygotic genes (e.g. *fkh*; Weigel *et al.*, 1990) which, in many cases, also encode transcription factors. Our results indicate that *bowl* is one such factor.

The posterior expression of *bowl* strictly requires *tor* and *tll*, but appears to be independent of *hkb* function. Although it is not certain that *tll* regulates *bowl* directly, these results implicate *bowl* as a mediator of *tll* (but not *hkb*) function, and also suggest that the posterior phenotype of *bowl* mutants should not be more extensive than that of *tll* mutants. Indeed, the effects of *bowl* mutations are much less severe than loss of *tll* function, which eliminates essentially all posterior ectodermal derivatives and also causes blastoderm fate map alterations that are characterized by the deletion of A8 and posterior shifts of the remaining trunk segments (Mahoney and Lengyel, 1987). Examination of both the cuticle phenotype and the expression of *engrailed* and *odd-skipped* in *bowl* mutants (data not shown) indicate that a similar shift in the fate map does not occur. Thus, *bowl* does not appear to mediate the posterior repression of segmentation genes that is disrupted in *tll* mutants.

The *fork head* (*fkh*) and *Trg* genes are also required for hindgut development. While these could conceivably function as regulators or mediators of *bowl* function in the posterior, we believe they more likely act either in parallel with *bowl* or in independent pathways, rather than in a hierarchical sequence. In contrast to *bowl*, the posterior expression of both *Trg* and *fkh* is affected by mutations in *hkb*. These results exclude any simple model whereby *bowl* is expressed solely in response to either *fkh* or *Trg*. Indeed, preliminary *in situ* hybridization analysis of *fkh* and *bowl* expression in *bowl* and *fkh* mutants, respectively (data not shown), indicates that the initial expression of each gene is independent of the other (similar results have been reported for *Trg* and *fkh*; Kispert *et al.*, 1994). While mutations in *fkh* result in the appearance of ectopic head structures in the hindgut region (Jürgens and Weigel, 1988), a similar homeotic transformation is not apparent in *bowl* mutants. However, as with *bowl*, portions of the hindgut may be unaffected in *fkh* mutants, and further genetic analysis should reveal whether the two genes function together in a common pathway, or independently mediate distinct aspects of hindgut development. The greater severity of the *Trg* mutant phenotype, in which the hindgut (together with the anal pads) appears to be completely absent, suggests that *Trg* expression does not strictly require *bowl*.

In contrast to the posterior, specification of the anterior pole of the embryo involves interactions between the terminal system and the anterior morphogen encoded by the maternal-effect gene *bicoid*. As with other zygotic terminal genes (e.g. *tll*; Pignoni *et al.*, 1992), the regulation of *bowl* at the anterior of the embryo is clearly distinct from that at the posterior. In the wild-type blastoderm, asymmetry is manifested by both the relatively weak and non-uniform domain at the anterior pole and the presence of the strong cephalic stripe. Also, while both features appear to be affected by mutations in either *tor* or *tll*, the situation is more complex than the all-or-nothing behavior of the posterior domain. Based upon our data, we propose that the anterior cap and cephalic stripe are differentially

regulated, with *tor* and *tll* activating (and *bcd* modulating) expression in the anterior cap but repressing expression of the stripe (which must require *bcd* for activation). This aspect of *bowl* regulation, together with the location of the *bowl* stripe in wild-type and the gnathal defects seen in *bowl* mutants, suggests that *bowl* has properties in common with the head-specific gap genes (e.g. *otd*; Finkelstein and Perrimon, 1990) as well as zygotic terminal genes.

Finally, whereas *tor* and *tll* mutations have equivalent effects on the posterior expression of *bowl*, the anterior pattern is differentially affected. In both mutants, an initially uniform anterior cap of expression retracts to a stripe as transcripts are cleared from the anterior pole, but the retraction occurs significantly earlier in *tll* than in *tor* mutants. Thus, while the absence of *tll* function could account for the initial derepression at the anterior of either mutant, *tor* activity appears to be capable of initiating the secondary repression (i.e. retraction) through a *tll*-independent mechanism (although this can occur later even in the absence of *tor* function). While it is possible that some other zygotic gene regulated by *tor* is responsible, this behavior could also be mediated by interactions between *tor* and *bcd* (i.e. phosphorylation) that are independent of zygotic transcription, as has been proposed for the secondary repression of *hunchback* and other zygotic genes at the anterior pole (Ronchi *et al.*, 1993).

Relationship between *bowel* and *odd-skipped*

Although the sequence conservation between *odd* and *bowl* suggests the possibility of functional similarities, the two genes are expressed in different patterns during embryogenesis, and their mutant phenotypes are clearly distinct. Nevertheless, both genes encode putative transcription factors likely to share at least some aspect(s) of function at the molecular level. Given that the two genes do not have significant homology outside the zinc fingers, any functional relatedness might be limited to similarities in nucleotide binding specificity, and possibly common or partially overlapping regulatory targets *in vivo*. As a pair-rule gene, the presumed function of *odd* is to repress segmentation genes in the odd-numbered segments; potential targets include *engrailed* (DiNardo and O'Farrell, 1987; Coulter and Wieschaus, 1988), *paired* (Baumgartner and Noll, 1990; Gutjahr *et al.*, 1993), and *wingless* and *fushi tarazu* (Mullen and DiNardo, 1995). Although it is possible that these (or other) segmentation genes are also targeted by *bowl* in the embryonic termini, the *bowl* phenotype does not suggest any obvious link between the terminal and segmentation hierarchies such as the posterior fate map shift characteristic of *tll* embryos (see above), and any such regulation would most likely occur after fate map specification at cellular blastoderm.

In contrast to its terminal functions, the striped expression of *bowl* in the presumptive trunk of the embryo suggests a possible direct role in segmentation that might be analogous to that of *odd*. Indeed, the overall pattern of *bowl* stripes is reminiscent of *odd*, given that both genes undergo a transition during gastrulation from a pair-rule pattern (seven stripes) to a segment-polarity-like pattern (14–15 stripes), and it is likely that the expression patterns overlap in these (and possibly other) regions. However, the *bowl* stripes appear relatively weak at this stage, and

neither the frequency nor severity of trunk defects in mutants is sufficient to categorize *bowl* as a bona fide segmentation gene. Interestingly, the defects that are observed in *bowl* mutants preferentially affect the even-numbered segments, in contrast to the odd-numbered defects associated with *odd* mutants.

The low penetrance of the *bowl* segmentation defects, together with the absence of even-numbered segment defects in *odd* mutants (despite the persistent post-blastoderm expression of *odd* within every segment), might be explained by functional redundancy between *odd* and *bowl*. An analogous situation has been reported for the segmentation genes *sloppy paired 1* (*slp1*) and *sloppy paired 2* (*slp2*). Mutations in *slp1* result in a pair-rule phenotype, whereas double mutants of *slp1* and *slp2* exhibit a segment-polarity phenotype (Grossniklaus *et al.*, 1992). However, our analysis of the segmentation defects in *odd bowl* double mutants (data not shown) has revealed no obvious synergism. A second *odd*-homolog, *sob*, might represent a better candidate for redundancy, given that its zinc fingers most closely match those of *bowl* and its expression pattern is most similar to *odd* (M.Hart *et al.*, in preparation). Resolution of this issue will require future characterization of double and triple mutant combinations involving all three homologs.

Materials and methods

cDNA and genomic cloning

bowl cDNA clones pB (3.0 kb) and pC (1.4 kb) were isolated from a 0–3 h embryonic cDNA library (Poole *et al.*, 1985) using an *odd-skipped* genomic probe (M.Hart *et al.*, in preparation). Additional cDNA clones (LW-1 and LW-2; 3.3 kb each) were obtained by screening a *Drosophila* embryonic cDNA library (Stratagene) with a 1.1 kb *Eco*RI–*Eco*RV subclone (pB-RV) from the 5' end of clone pB. Genomic lambda clones were isolated from a *Drosophila* EMBL4 library (courtesy of P.Schedl) using cDNA clone pB as a probe. P1 clones were isolated from a *Drosophila* library (Smoller *et al.*, 1991) using pB as a probe, and were a generous gift of D.Smoller and D.Hartl. cDNA and genomic subclones were inserted into Bluescript (Stratagene). Plasmid DNAs were isolated using the alkaline lysis method (Maniatis *et al.*, 1982) and sequenced (Sanger *et al.*, 1977) as double-stranded templates using the T3 and T7 primers and internal synthetic oligonucleotides as necessary.

RNA analysis

RNA was prepared as described (Chomczynski and Sacchi, 1987) from staged wild-type embryos that had been frozen on dry ice and stored at −70°C. For Northern analysis, total RNA (45 µg per sample) was electrophoresed on a 1% formaldehyde–agarose gel, transferred to Biotrans(+) nylon membrane (ICN Biomedicals) and UV cross-linked. Hybridization to ^{32}P-labeled probes was carried out at 65°C in Church buffer (Church and Gilbert, 1984) followed by washes in 0.2× SSC/ 0.1% SDS at 65°C.

For primer extension, mRNA from 0–8 h embryos was fractionated through a single round of oligo(dT) column selection and analyzed by Northern blot to verify its integrity. A *bowl* primer (5'GTAAGCTTTG-AAATGCTTGGCATTG3'; complementary to cDNA residues 180–204) was ^{32}P-end-labeled, annealed to 1 µg mRNA, and elongated with reverse transcriptase at 42°C for 60 min. The reaction product was analyzed on a sequencing gel together with a DNA sequence ladder generated using the same primer with an appropriate genomic clone.

5' end RACE

5' end RACE (rapid amplification of cDNA ends) was carried out according to the procedure of Frohman *et al.* (1988) with minor modifications. First, strand cDNA was synthesized with AMV reverse transcriptase according to the Promega Technical Bulletin, using 0.5 µg of a *bowl* 5' primer (5'-CTATCGAGTCCGGCATG-3'; complementary to cDNA residues 993–1009) and 5 µg of 0–8 h wild-type mRNA as template. After removal of excess primer and poly(A) tail addition, PCR

amplification was performed for 40 cycles (94°C/40 s; 57°C/1 min; 72°C/3 min) with 20 pmol each of a XhoI–SalI-tagged dT-adapter primer (5'-GACTCGAGTCGACTTTTTTTTTTTTTTTTTT-3') and the internal bowl primer used for primer extension (see above), followed by a final 15 min extension at 72°C. Southern analysis of half the reaction was used to verify the presence of the appropriately sized band; the rest was subsequently digested with HindIII and XhoI, cloned into Bluescript, and sequenced.

Drosophila stocks

The torso (tor^{PM51}) and tailless (tll^{L10}) alleles were obtained from the Bloomington Drosophila Stock Center (Indiana University). The huckebein allele (hkb^2) was provided by S.Sugiyama and W.Gehring. The wild-type strain (Canton-S) was obtained from J.Eissenberg. Df(2)ed$^{sz1.2}$, Df(2L)M11 (Reuter and Szidonya, 1983), Df(2L)ed-dp^{h1} and Df(2)sc$^{19-8}$ (Szidonya and Reuter, 1988) were provided by T.Schupbach. l(2)c^{E17} (de Belle et al., 1993) and translocations T(2;3)9 (Hilliker and Trusis-Coulter, 1987) and T(Y;2)L26 [Gatti and Pimpinelli, 1983; note that this is listed as T(Y;2)L126 by de Belle et al., 1993] were provided by J.S.de Belle and M.B.Sokolowski.

Southern and RFLP analysis

Genomic DNA was isolated from adult flies using the procedure of Ish-Horowicz et al. (1979). Southern blots were prepared by standard methods (Maniatis et al., 1982) using four or five fly equivalents of DNA per lane and hybridized with ^{32}P-labeled probes in ECL (Amersham) hybridization solution plus 0.5 M NaCl at 42°C; filters were washed twice (30 min each) at 65°C in 0.2× SSC, 0.1% SDS.

We determined that bowl sequences are removed by Df(2)ed$^{sz1.2}$ and Df(2)sc$^{19-8}$, but not Df(2)ed-dp^{h1} or Df(2)M11, using RFLPs (for EcoRI, SalI and XhoI) in the vicinity of bowl; these were identified by comparing Southern patterns of DNA from balanced deficiency stocks (Df/CyO) with an isogenized wild-type strain (cn bw sp). For deficiencies that delete the locus, hemizygosity was verified by outcrossing to the wild-type strain and analyzing the Southern patterns of DNA from each progeny class: Df/cn bw sp flies showed only the wild-type pattern when probed with the appropriate bowl genomic fragment, while CyO/cn bw sp flies, as expected, were heterozygous (i.e. showed a combined RFLP pattern). Similar analyses using the other deficiencies showed that both progeny classes were heterozygous, indicating that bowl sequences were not eliminated in these lines.

Mutagenesis

Ethyl methanesulfonate (EMS) mutagenesis of an isogenized (pr cn bw) chromosome was performed according to Grigliatti (1986) using a conventional F2 lethal screen. After mutagenized males were outcrossed to a CyO balancer, single F1 males (pr cn bw*/CyO) were mated to Df(2)ed$^{sz1.2}$ cn bw/CyO females. Stocks of mutagenized chromosomes that failed to complement the deficiency were established by mating balanced F2 siblings carrying the pr marker.

To eliminate possible secondary mutations, six of the bowl alleles (bowl$^{1-6}$) were crossed to a homozygous viable second chromosome marked with dp and cl. Recombinants between dp and cl (i.e. dp$^+$ cl pr$^+$ cn$^+$ bw$^+$ progeny) were tested over deficiency Df(2)ed$^{sz1.2}$ to confirm the presence of the bowl mutation. Phenotypic characterization of bowl homozygotes was performed using these recombinant lines, in which ~80% of the mutagenized second chromosome has been replaced.

Sequence analysis of bowel mutants

The protein-coding region of the bowl gene was PCR-amplified from genomic DNA using two sets of primers specific for the third and fourth exons. Amplified fragments were subcloned and sequenced using primers spanning the coding region at ~200 bp intervals. For each base substitution identified in the mutants, clones from at least two independent amplifications were sequenced to eliminate PCR artifacts. For reference, the same regions of the mutagenized (pr cn bw) and balancer (CyO) chromosomes were analyzed using DNA from the isogenized parental stock and Df(2)ed$^{sz1.2}$/CyO, respectively. Sixteen sequence polymorphisms between these chromosomes were identified.

Amplification of mutant alleles was performed using DNA isolated from either heterozygous (bowl/CyO) adults (for bowl^2, bowl^5 and bowl^6) or single homozygous mutant embryos (for bowl^1, bowl^3 and bowl^4). For the former method, clones derived from mutant chromosomes were distinguished from the balancer based upon known sequence polymorphisms. For the single embryo method, mutants were marked with even-skipped (eve) and identified by scoring the eve segmentation phenotype in late embryos. Individual embryos were homogenized in

50 µl embryo lysis buffer (50 mM KCl, 10 mM Tris, pH 8.0, 2.5 mM MgCl$_2$, 0.9% Tween 20, 1% SDS and 1 mg/ml proteinase K), frozen at −70°C for 10 min and incubated at 37°C for 48 h. The homogenate was phenol/chloroform extracted, DNA was precipitated with ethanol, and one-fifth of the sample was used in each PCR reaction.

Embryo methods

For whole mount in situ hybridization or antibody staining, staged embryos were dechorionated in bleach and fixed for 20 min in heptane/ formaldehyde (equal volumes heptane and 4% formaldehyde/50 mM EGTA/1× PBS). After removal of the aqueous phase, embryos were devitellinized by vigorous shaking with methanol/EGTA (90%/50 mM), washed several times with methanol, and stored in ethanol at −20°C. bowl transcripts were detected by in situ hybridization (Tautz and Pfeifle, 1989) using reagents provided in the Genius Kit (Boehringer Mannheim). A digoxygenin-labeled, anti-sense RNA probe for bowl transcripts was synthesized using linearized subclone pB-P (see Figure 3 legend) as template. Following synthesis, the probe was hydrolyzed in 0.1 M Na$_2$CO$_3$, pH 10.2, at 60°C for 1 h.

Antibody staining was performed using the Vectastain Elite ABC-peroxidase system and DAB substrate kit (Vector Laboratories). Mono-clonal mouse anti-en hybridoma culture supernatant (Patel et al., 1989; a gift of I.Duncan) was used without dilution. Polyclonal rabbit anti-labial antibody (Diederich et al., 1989; a gift of T.Kaufman) was used at a dilution of 1:200. Secondary biotinylated anti-mouse and anti-rabbit IgG (Vector Laboratories) were used at concentrations of 1:1000 and 1:500 respectively. Embryos were dehydrated and mounted in Canada Balsam/methyl salicylate (70%/30%).

Cuticle preparations of late embryos were prepared as described (Wieschaus and Nüsslein-Volhard, 1986).

Acknowledgements

References

Ambrosio,L., Mahowald,A.P. and Perrimon,N. (1989) Requirement of the Drosophila raf homolog for torso function. Nature, 342, 288–291.

Baumgartner,S. and Nol,M. (1990) Network of interactions among pair-rule genes regulating paired expression during primordial segmentation of Drosophila. Mech. Dev., 33, 1–18.

Brönner,G., Chu-LaGraff,Q., Doe,C.Q., Cohen,B., Weigel,D., Taubert,H. and Jäckle,H. (1994) Sp1/egr-like zinc-finger protein required for endoderm specification and germ-layer formation in Drosophila. Nature, 369, 664–668.

Campos-Ortega,J.A. and Hartenstein,V. (1985) The Embryonic Development of Drosophila melanogaster. Springer-Verlag, Berlin.

Casanova,J. (1990) Pattern formation under the control of the terminal system in the Drosophila embryo. Development, 110, 621–628.

Casanova,J., Furriols,M., McCormick,C.A. and Struhl,G. (1995) Similarities between trunk and spätzle, putative extracellular ligands specifying body pattern in Drosophila. Genes Dev., 9, 2539–2544.

Cavener,D.R. (1987) Comparison of the consensus sequence flanking translational start sites in Drosophila and vertebrates. Nucleic Acids Res., 15, 1353–1361.

Cherbas,L. and Cherbas,P. (1993) The arthropod initiator: the capsite consensus plays an important role in transcription. Insect Biochem. Mol. Biol., 23, 81–90.

Chomczynski,P. and Sacchi,N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem., 162, 156–159.

Church,G.M. and Gilbert,W. (1984) Genomic sequencing. *Proc. Natl Acad. Sci. USA*, **81**, 1991–1995.

Coulter,D.E. and Wieschaus,E. (1988) Gene activities and segmental patterning in *Drosophila*: analysis of *odd-skipped* and pair-rule double mutants. *Genes Dev.*, **2**, 1812–1823.

Coulter,D.E., Swaykus,E.A., Beran-Koehn,M.A., Goldberg,D., Wieschaus,E. and Schedl,P. (1990) Molecular analysis of *odd-skipped*, a zinc finger encoding segmentation gene with a novel pair-rule expression pattern. *EMBO J.*, **9**, 3795–3804.

de Belle,J.S., Sokolowski,M.B. and Hilliker,A.J. (1993) Genetic analysis of the *foraging* microregion of *Drosophila melanogaster*. *Genome*, **36**, 94–101.

Desjarlais,J.R. and Berg,J.M. (1992) Redesigning the DNA-binding specificity of a zinc finger protein – a data base-guided approach. *Protein Struct. Funct. Genet.*, **12**, 101–104.

Diederich,R.J., Merrill,V.K., Pultz,M.A. and Kaufman,T.C. (1989) Isolation, structure, and expression of *labial*, a homeotic gene of the Antennapedia Complex involved in *Drosophila* head development. *Genes Dev.*, **3**, 399–414.

DiNardo,S. and O'Farrell,P.H. (1987) Establishment and refinement of segmental pattern in the *Drosophila* embryo: spatial control of *engrailed* expression by pair-rule genes. *Genes Dev.*, **1**, 1212–1225.

Duffy,J.B. and Perrimon,N. (1994) The *torso* pathway in *Drosophila*: lessons on receptor tyrosine kinase signaling and pattern formation. *Dev. Biol.*, **166**, 380–395.

Finkelstein,R. and Perrimon,N. (1990) The *orthodenticle* gene is regulated by *bicoid* and *torso* and specifies *Drosophila* head development. *Nature*, **346**, 485–488.

Frohman,M.A., Dush,M.K. and Martin,G.R. (1988) Rapid production of full-length cDNAs from rare transcripts: amplification using a single gene-specific oligonucleotide primer. *Proc. Natl Acad. Sci. USA*, **85**, 8998–9002.

Frohnhöfer,H.G. and Nüsslein-Volhard,C. (1986) Organization of anterior pattern in the *Drosophila* embryo by the maternal gene *bicoid*. *Nature*, **324**, 120–125.

Gatti,M. and Pimpinelli,S. (1983) Cytological and genetic analysis of the Y chromosome of *Drosophila melanogaster*. I. Organization of the fertility factors. *Chromosoma*, **88**, 349–373.

Gaul,U. and Weigel,D. (1990) Regulation of *Kruppel* expression in the anlage of the Malpighian tubules in the *Drosophila* embryo. *Mech. Dev.*, **33**, 57–67.

Gaul,U., Seifert,E., Schuh,R. and Jäckle,H. (1987) Analysis of *Kruppel* protein distribution during early *Drosophila* development reveals posttranscriptional regulation. *Cell*, **50**, 639–647.

Grigliatti,T. (1986) Mutagenesis. In Roberts,D.B. (ed.), *Drosophila: A Practical Approach*. IRL Press, Oxford, pp. 39–58.

Grossniklaus,U., Kurth Pearson,R. and Gehring,W.J. (1992) The *Drosophila sloppy paired* locus encodes two proteins involved in segmentation that show homology to mammalian transcription factors. *Genes Dev.*, **6**, 1030–1051.

Gutjahr,T., Frei,E. and Noll,M. (1993) Complex regulation of early *paired* expression: initial activation by gap genes and pattern modulation by pair-rule genes. *Development*, **117**, 609–623.

Harbecke,R. and Lengyel,J.A. (1995) Genes controlling posterior gut development in the *Drosophila* embryo. *Roux's Arch. Dev. Biol.*, **204**, 308–329.

Hilliker,A.J. and Trusis-Coulter,S.N. (1987) Analysis of the functional significance of linkage group conservation in *Drosophila*. *Genetics*, **117**, 233–244.

Ingham,P.W., Howard,K.R. and Ish-Horowicz,D. (1985) Transcription pattern of the *Drosophila* segmentation gene *hairy*. *Nature*, **318**, 439–445.

Ish-Horowicz,D., Pinchin,S.M., Schedl,P., Artavanis-Tsakonas,S. and Mirault,M.E. (1979) Genetic and molecular analysis of the 87A7 and 87C1 heat-inducible loci of *D. melanogaster*. *Cell*, **18**, 1351–1358.

Jürgens,G. (1987) Segmental organization of the tail region in the embryo of *Drosophila melanogaster*. *Roux's Arch. Dev. Biol.*, **196**, 141–157.

Jürgens,G. and Weigel,D. (1988) Terminal versus segmental development in the *Drosophila* embryo: the role of the homeotic gene *fork head*. *Roux's Arch. Dev. Biol.*, **197**, 345–354.

Kispert,A., Herrmann,B.G., Leptin,M. and Reuter,R. (1994) Homologs of the mouse *Brachyury* gene are involved in the specification of posterior terminal structures in *Drosophila*, *Tribolium*, and *Locusta*. *Genes Dev.*, **8**, 2137–2150.

Klingler,M., Erdelyi,M., Szabad,J. and Nüsslein-Volhard,C. (1988) Function of *torso* in determining the terminal anlagen of the *Drosophila* embryo. *Nature*, **335**, 275–277.

Kornberg,T., Siden,I., O'Farrell,P. and Simon,M. (1985) The *engrailed* locus of *Drosophila*: in situ localization of transcripts reveals compartment-specific expression. *Cell*, **40**, 45–53.

Krause,H.M., Klemenz,R. and Gehring,W.J. (1988) Expression, modification, and localization of the *fushi tarazu* protein in *Drosophila* embryos. *Genes Dev.*, **2**, 1021–1036.

Lu,X., Chou,T.B., Williams,N.G., Roberts,T. and Perrimon,N. (1993) Control of cell fate determination by p21ras/Ras1, an essential component of *torso* signaling in *Drosophila*. *Genes Dev.*, **7**, 621–632.

Mahoney,P.A. and Lengyel,J.A. (1987) The zygotic segmentation mutant *tailless* alters the blastoderm fate map of the *Drosophila* embryo. *Dev. Biol.*, **122**, 464–470.

Maniatis,T., Fritsch,E.F. and Sambrook,J. (1982) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Martin,J.R., Raibaud,A. and Ollo,R. (1994) Terminal pattern elements in *Drosophila* embryo induced by the *torso-like* protein. *Nature*, **367**, 741–745.

Mullen,J.R. and DiNardo,S. (1995) Establishing parasegments in *Drosophila* embryos: roles of the *odd-skipped* and *naked* genes. *Dev. Biol.*, **169**, 295–308.

Murakami,R., Shigenaga,A., Kawano,E., Matsumoto,A., Yamaoka,I. and Tanimura,T. (1994) Novel tissue units of regional differentiation in the gut epithelium of *Drosophila*, as revealed by P-element-mediated detection of enhance. *Roux's Arch. Dev. Biol.*, **203**, 243–249.

Nardelli,J., Gibson,T.J., Vesque,C. and Charnay,P. (1991) Base sequence discrimination by zinc-finger DNA-binding domains. *Nature*, **349**, 175–178.

Nüsslein-Volhard,C., Wieschaus,E. and Kluding,H. (1984) Mutations affecting the pattern of the larval cuticle of *Drosophila melanogaster*. I. Zygotic loci on the second chromosome. *Roux's Arch. Dev. Biol.*, **193**, 267–282.

Patel,N.H., Martin-Blanco,E., Coleman,K.G., Poole,S.J., Ellis,M.C., Kornberg,T.B. and Goodman,C.S. (1989) Expression of *engrailed* proteins in arthropods, annelids, and chordates. *Cell*, **58**, 955–968.

Pavletich,N.P. and Pabo,C.O. (1991) Zinc finger-DNA recognition: crystal structure of a Zif268-DNA complex at 2.1 Å. *Science*, **252**, 809–817.

Pavletich,N.P. and Pabo,C.O. (1993) Crystal structure of a five-finger GLI-DNA complex: new perspectives on zinc fingers. *Science*, **261**, 1701–1707.

Pignoni,F., Baldarelli,R.M., Steingrímsson,E., Diaz,R.J., Patapoutian,A., Merriam,J.R. and Lengyel,J.A. (1990) The *Drosophila* gene *tailless* is expressed at the embryonic termini and is a member of the steroid receptor superfamily. *Cell*, **62**, 151–163.

Pignoni,F., Steingrímsson,E. and Lengyel,J.A. (1992) *Bicoid* and the terminal system activate *tailless* expression in the early *Drosophila* embryo. *Development*, **115**, 239–251.

Poole,S.J., Kauvar,L.M., Drees,B. and Kornberg,T. (1985) The *engrailed* locus of *Drosophila*: structural analysis of an embryonic transcript. *Cell*, **40**, 37–43.

Reuter,G. and Szidonya,J. (1983) Cytogenetic analysis of variegation suppressors and a dominant temperature-sensitive lethal in region 23-26 of chromosome 2L in *Drosophila melanogaster*. *Chromosoma*, **88**, 277–285.

Ronchi,E., Treisman,J., Dostatni,N., Struhl,G. and Desplan,C. (1993) Down-regulation of the *Drosophila* morphogen bicoid by the torso receptor-mediated signal transduction cascade. *Cell*, **74**, 347–355.

Sanger,F., Nicklen,S. and Coulson,A.R. (1977) DNA sequencing with chain-terminating inhibitors. *Proc. Natl Acad. Sci. USA*, **74**, 5463–5467.

Savant-Bhonsale,S. and Montell,D.J. (1993) *torso-like* encodes the localized determinant of *Drosophila* terminal pattern formation. *Genes Dev.*, **7**, 2548–2555.

Skaer,H. (1993) The alimentary canal. In Bate,M. and Martinez-Arias,A. (eds), *The Development of Drosophila melanogaster*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 941–1012.

Smoller,D.A., Petrov,D. and Hartl,D.L. (1991) Characterization of bacteriophage P1 library containing inserts of *Drosophila* DNA of 75–100 kilobase pairs. *Chromosoma*, **100**, 487–494.

Sprenger,F. and Nüsslein-Volhard,C. (1993) The terminal system of axis determination in the *Drosophila* embryo. In Bate,M. and Martinez-Arias,A. (eds), *The Development of Drosophila melanogaster*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 365–386.

Sprenger,F., Stevens,L.M. and Nüsslein-Volhard,C. (1989) The *Drosophila* gene *torso* encodes a putative receptor tyrosine kinase. *Nature*, **338**, 478–483.

St Johnston,D. and Nüsslein-Volhard,C. (1992) The origin of pattern and polarity in the *Drosophila* embryo. *Cell*, **68**, 201–219.

Steingrímsson,E., Pignoni,F., Liaw,G.J. and Lengyel,J.A. (1991) Dual role of the *Drosophila* pattern gene *tailless* in embryonic termini. *Science*, **254**, 418–421.

Strecker,T.R., Halsell,S.R., Fisher,W.W. and Lipshitz,H.D. (1989) Reciprocal effects of hyper- and hypoactivity mutations in the *Drosophila* pattern gene *torso*. *Science*, **243**, 1062–1066.

Szidonya,J. and Reuter,G. (1988) Cytogenetic analysis of the *echinoid* (*ed*), *dumpy* (*dp*) and *clot* (*cl*) region in *Drosophila melanogaster*. *Genet. Res.*, **51**, 197–208.

Tautz,D. and Pfeifle,C. (1989) A non-radioactive *in situ* hybridization method for the localization of specific RNAs in *Drosophila* embryos reveals translational control of the segmentation gene *hunchback*. *Chromosoma*, **98**, 81–85.

Tepass,U. and Hartenstein,V. (1994) The development of cellular junctions in the *Drosophila* embryo. *Dev. Biol.*, **161**, 563–596.

Weigel,D., Jürgens,G., Kuttner,F., Seifert,E. and Jäckle,H. (1989) The homeotic gene *fork head* encodes a nuclear protein and is expressed in the terminal regions of the *Drosophila* embryo. *Cell*, **57**, 645–658.

Weigel,D., Jürgens,G., Klingler,M. and Jäckle,H. (1990) Two gap genes mediate maternal terminal pattern information in *Drosophila*. *Science*, **248**, 495–498.

Wieschaus,E. and Nüsslein-Volhard,C. (1986) Looking at embryos. In Roberts,D.B. (ed.), *Drosophila: A Practical Approach*. IRL Press, Oxford, UK, pp. 199–228.

Wieschaus,E., Nüsslein-Volhard,C. and Jürgens,G. (1984) Mutations affecting the pattern of the larval cuticle of *Drosophila melanogaster*. III. Zygotic loci on the X-chromosome and fourth chromosome. *Roux's Arch. Dev. Biol.*, **193**, 296–307.

Note added in proof

The GenBank accession number for the *bowel* sequence is U58282.