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Maternal activation of gap genes in the hover fly *Episyrphus*

Steffen Lemke[1], Stephanie E. Busch[1,*], Dionysios A. Antonopoulos[2], Folker Meyer[2], Marc H. Domanus[2] and Urs Schmidt-Ott[1,†]

SUMMARY

The metameric organization of the insect body plan is initiated with the activation of gap genes, a set of transcription-factor-encoding genes that are zygotically expressed in broad and partially overlapping domains along the anteroposterior (AP) axis of the early embryo. The spatial pattern of gap gene expression domains along the AP axis is generally conserved, but the maternal genes that regulate their expression are not. Building on the comprehensive knowledge of maternal gap gene activation in *Drosophila*, we used loss- and gain-of-function experiments in the hover fly *Episyrphus balteatus* (Syrphidae) to address the question of how the maternal regulation of gap genes evolved. We find that, in *Episyrphus*, a highly diverged *bicoid* ortholog is solely responsible for the AP polarity of the embryo. *Episyrphus bicoid* represses anterior zygotic expression of *caudal* and activates the anterior and central gap genes *orthodenticle*, *hunchback* and *Krüppel*. In *bicoid*-deficient *Episyrphus* embryos, *nanos* is insufficient to generate morphological asymmetry along the AP axis. Furthermore, we find that *torso* transiently regulates anterior repression of *caudal* and is required for the activation of *orthodenticle*, whereas all posterior gap gene domains of *knirps*, *giant*, *hunchback*, *tailless* and *huckebein* depend on *caudal*. We conclude that all maternal coordinate genes have altered their specific functions during the radiation of higher flies (Cyclorrhapha).

KEY WORDS: *Episyrphus*, Evolutionary development, Bicoid, Gap genes

INTRODUCTION

In insects, the initiation of differential zygotic gene expression along the anteroposterior (AP) axis of the embryo begins with the activation of gap genes (Davis and Patel, 2002; Pankratz and Jäckle, 1993). The sequence of gap gene expression domains along the embryonic AP axis is generally conserved across insects, but the maternal genes that initiate their expression differ between higher taxa (Brent et al., 2007; Goltsev, 2004; Bucher and Klingler, 2004; Cerny et al., 2008; Liu and Patel, 2010; Lynch et al., 2006a; Lynch et al., 2006b; Marques-Souza et al., 2008; Olesnicky et al., 2006; Pultz et al., 2005; Schröder, 2003; Schröder et al., 2000; Sommer and Tautz, 1991; Stauber et al., 2002; Wolff et al., 1995). How the maternal factors evolve and become exchanged is not well understood.

In *Drosophila*, the differential expression of gap genes in the early embryo rests on *torso*, *bicoid*, *hunchback*, *caudal* and *nanos*. These genes are active in distinct portions of the syncytial embryo and ensure pattern formation at the poles through the terminal gap genes *huckebein* and *tailless*, head segmentation through head gap genes such as *orthodenticle*, *empty spiracles* and *buttonhead*, and trunk segmentation through the canonical gap genes *hunchback*, *Krüppel*, *knirps* and *giant* (Furriols and Casanova, 2003; Pankratz and Jäckle, 1993; Rivera-Pomar and Jäckle, 1996; St Johnston and Nüsslein-Volhard, 1992; Surkova et al., 2008).

The maternal gene products of *bicoid*, *torso*, *hunchback* and *caudal* provide partially redundant input for the activation of gap genes in their proper domains. The homeodomain transcription factor Bicoid is expressed in an anterior-to-posterior gradient (Berleth et al., 1988; Driever and Nüsslein-Volhard, 1988a; Driever and Nüsslein-Volhard, 1988b; Gregor et al., 2007a; Gregor et al., 2007b; Spirov et al., 2009) and contributes to the activation of all gap genes (Driever et al., 1989; Eldon and Pirrotta, 1991; Finkelstein and Perrimon, 1990; Gao et al., 1996; Gaul and Jäckle, 1987; Hülskamp et al., 1990; Kraut and Levine, 1991; Liaw and Lengyel, 1993; Ochoa-Espinosa et al., 2009; Pignoni et al., 1992; Rivera-Pomar et al., 1995; Rivera-Pomar et al., 1996; Tautz, 1988; Walldorf and Gehring, 1992; Wimmer et al., 1995). The receptor tyrosine kinase Torso signals symmetrically at both poles of the egg and activates the terminal gap genes *huckebein* and *tailless* by locally counteracting ubiquitous repressors (Casanova and Struhl, 1993; Jiménez et al., 2000; Paroush et al., 1997; Sprenger and Nüsslein-Volhard, 1992). In the absence of Torso, the posterior domains of *huckebein* and *tailless* are missing but, because of the activating input by Bicoid, their anterior domains are only reduced (Brönner and Jäckle, 1991; Pignoni et al., 1992). In the absence of Bicoid, the head gap gene domains are missing and zygotic anterior *hunchback* expression is substituted by a mirror image duplication of the Torso-dependent posterior domain (Finkelstein and Perrimon, 1990; Tautz, 1988; Walldorf and Gehring, 1992; Wimmer et al., 1995). The central and posterior domains of the trunk gap genes *Krüppel*, *knirps* and *giant* are shifted towards the anterior pole in Bicoid-deficient embryos (Eldon and Pirrotta, 1991; Gaul and Jäckle, 1987; Kraut and Levine, 1991), but they persist because of the maternal activities of *hunchback* and *caudal*. The zinc finger transcription factor Hunchback is sufficient to promote the central domain of *Krüppel* (Hülskamp et al., 1990) and the homeodomain protein Caudal is sufficient to maintain the posterior expression domains of *knirps* and *giant* (Rivera-Pomar and Jäckle, 1996; Rivera-Pomar et al., 1995).

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Global polarity of the gap gene scaffold is provided by the Bicoid gradient, which, in addition to activating gap gene transcription, also represses the translation of the ubiquitous maternal *caudal* transcript at the anterior pole (Cho et al., 2005; Niessing et al., 1999; Rivera-Pomar et al., 1996). Polarity is additionally provided by Nanos, which is enriched in the posterior embryo (Wang et al., 1994; Wang and Lehmann, 1991). Nanos represses translation of ubiquitous maternal *hunchback* transcript and thereby allows for the activation of *knirps* and *giant* (Hülskamp et al., 1989; Irish et al., 1989; Kraut and Levine, 1991; Sonoda and Wharton, 1999; Struhl, 1989; Wharton and Struhl, 1991). In the absence of *bicoid* and maternal *hunchback* activity, global AP polarity is lost, *knirps*, *giant* and Torso-dependent domains are expressed symmetrically, and anterior and central gap gene domains are absent (Gavis and Lehmann, 1992; Hülskamp et al., 1990).

As part of our efforts to trace the evolution of the maternal initiators of embryonic pattern formation in dipteran insects, we used the scaffold of gap gene domains to assess the initiation of embryonic pattern formation in the hoverfly *Episyrphus balteatus* (Syrphidae). *Episyrphus* belongs to the sister taxon of higher cyclorrhaphan flies (Schizophora, including *Drosophila*) (Grimaldi and Engel, 2005; Lemke and Schmidt-Ott, 2009; Yeates and Wiegmann, 2005) and is currently one of the most 'basal' dipteran species amenable to functional genetic studies in early embryos (Lemke and Schmidt-Ott, 2009; Rafiqi et al., 2008). Like *Drosophila*, *Episyrphus* specifies segments simultaneously prior to gastrulation (long-germ development) (Bullock et al., 2004). In a previous study, we have shown that AP patterning in *Episyrphus* relies more heavily on *caudal* than embryonic development in *Drosophila*. *Episyrphus caudal* (*Eba-cad*) RNAi disrupts or deletes post-oral segments (Lemke and Schmidt-Ott, 2009), whereas *caudal*-deficient *Drosophila* embryos show segmentation in the head, thorax and even parts of the abdomen (Macdonald et al., 1986; Rivera-Pomar and Jäckle, 1996; Olesnicky et al., 2006). Furthermore, we reported that ectopic expression of *Episyrphus nanos* at the anterior pole suppresses the development of the head, thorax and five abdominal segments (Lemke and Schmidt-Ott, 2009). Here, we provide a comprehensive analysis of gap gene activation in *Episyrphus* and show that, although the basic scaffold of gap gene expression has remained conserved, maternal regulatory input of all coordinate genes has shifted between *Episyrphus* and *Drosophila*.

MATERIALS AND METHODS

Cloning procedures

Fragments of *Episyrphus* orthologs were obtained by PCR on cDNA using pairs of degenerate primers. A single primer pair was used to isolate *Episyrphus* orthologs of *knirps* and *knirps-related* (*Eba-kni* and *Eba-knrl*; 5′-CCGGCRGCNGGNTTYCAYTTYGG and 5′-ARRCARTGRATY-TTRAACCARTTNGA), *huckebein* (*Eba-hkb*; 5′-CARACVTAYTCR-CGNYTNTTCCG and 5′-CGYTCYWKNGGCAWRTGMGTYTT) and *tailless* (*Eba-tll*) (Schröder et al., 2000). Two primer pairs were used in a nested PCR for the isolation of *Episyrphus* orthologs of *Krüppel* (*Eba-Kr*) (Rafiqi et al., 2008), *giant* [*Eba-gt*; 5′-TTCAARGCNTWYCCN-MRNGAYCC and 5′-GCCCKRATNGCNATYTCRTCYTCYT; nested 5′-GARMGNMGNMGNAARAAYAA (Bucher and Klingler, 2004) and 5′-GCCCKRATNGCNATYTCRTCYTCYT] and *torso* [*Eba-tor*; 5′-GTNCAYMGNGAYYTNGCNGC (Schoppmeier and Schröder, 2005) and 5′-TKCCNCCKAGNGTKNNKATCTC; nested 5′-TNYCMGAYTTY-GGNCTNAGTCGNGA and 5′-ARNAYNCCRAANSWCCANACRTC (Schoppmeier and Schröder, 2005)]. A homolog of *bicoid* (*Eba-bcd*) was identified in 454 transcriptome sequences of normalized cDNA from 0- to 4-hour-old *Episyrphus* embryos. A detailed description of the transcriptome data will be published elsewhere. The 3′ untranslated region (UTR) of *Eba-bcd* (0.7 kb including the putative polyadenylation signal), as well as larger cDNA fragments of *Eba-kni*, *Eba-knrl*, *Eba-Kr*, *Eba-gt*, *Eba-tll* and *Eba-tor*, were isolated by rapid amplification of cDNA ends (RACE; see Table S1 in the supplementary material).

Double-stranded RNA (dsRNA) of *Eba-bcd* spanned nucleotides 1 to 837 of the *Eba-bcd* open reading frame (ORF; 1 is the first nucleotide of the ORF). *Eba-tor* dsRNA spanned nucleotides –1237 to –604 (1 is the first nucleotide of the stop codon) in all described analyses. In addition, *Eba-cad* expression was analyzed after knockdown of *Eba-tor* by an *Eba-tor* dsRNA fragment spanning nucleotides –414 to –230, and *Eba-otd* expression was analyzed after knockdown of *Eba-tor* by an *Eba-tor* dsRNA fragment spanning nucleotides –414 to 276 (1 is the first nucleotide of the stop codon). dsRNA for *Episyrphus caudal*, *Episyrphus hunchback* and *Megaselia bicoid* was generated as described (Lemke and Schmidt-Ott, 2009; Lemke et al., 2008). The template for capped *Eba-bcd* mRNA was amplified by PCR from cDNA with primer pair 5′-CATGCCATGGCGGAAGAA-CCATGTGTGAC and 5′-ACGCGTCGACTAAACAATTTCTAAAG-TATTTGCGTGTTCCG, which introduced an *Nco*I site at the 5′ end and a *Sal*I site at the 3′ end of the ORF. The PCR product was digested with *Nco*I and *Sal*I and cloned into pSP35, and capped mRNA was synthesized as described (Lemke and Schmidt-Ott, 2009). Injection and fixation of embryos was carried out as described (Lemke and Schmidt-Ott, 2009; Rafiqi et al., 2008).

In situ hybridization

RNA probes were labeled with digoxigenin, fluorescein or biotin, and wholemount in situ hybridization was performed essentially as described (Kosman et al., 2004; Tautz and Pfeifle, 1989). The *Eba-bcd* probe comprised nucleotides 1 to 837 of the ORF (1 is the first nucleotide of the ORF), the *Eba-kni* probe comprised 418 nucleotides of 5′UTR and adjacent nucleotides 1 to 192 of the ORF, the *Eba-knrl* probe comprised nucleotides 116 to 339 of the presumably truncated ORF plus 630 adjacent nucleotides of potentially intronic sequence, the *Eba-Kr* probe comprised nucleotides 845 to 1518 of the ORF plus 54 adjacent nucleotides of 3′UTR, the *Eba-gt* probe comprised 153 nucleotides of 5′UTR and adjacent nucleotides 1 to 1042 of the ORF, the *Eba-tll* probe comprised nucleotides 8 to 1258 of the ORF and the *Eba-tor* probe comprised nucleotides –414 to 1 of the ORF (1 is the first nucleotide of the stop codon) plus 276 adjacent nucleotides of 3′UTR. The *Eba-hkb* probe comprised the 677 nucleotides of the ORF that were amplified by PCR using the indicated degenerate primer pair. Probes for *Eba-otd* and *Eba-hb* were prepared as described (Lemke and Schmidt-Ott, 2009). First-instar cuticles were prepared as described (Stern and Sucena, 2000) with a 2:1 mixture of Hoyer's medium and lactic acid.

RESULTS

A diverged *bicoid* homolog controls global AP polarity of the *Episyrphus* embryo

A *bicoid* homolog of *Episyrphus* (*Eba-bcd*) was isolated from 454-transcriptome sequences of 0- to 4-hour-old *Episyrphus* embryos by sequence similarity to *bicoid* (Fig. 1A). The open reading frame (ORF) of *Eba-bcd* encodes a homeodomain (with the canonical lysine at position 50) and several conserved Bicoid motifs, including the SLVMRR peptide motif immediately upstream of the homeodomain, the NSEXXEPLTP peptide motif at the N-terminus of the acidic domain and the TPXPLTPXSTP peptide motif close to the C-terminus of the PEST domain, which has been shown to contribute to Bicoid-dependent repression of *caudal* mRNA translation (Niessing et al., 1999). *Eba-bcd* does not display sequence conservation in the N-terminal self-inhibiting domain (SID) (Zhao et al., 2002) or in the C-terminal portion of the acidic domain, which influences the ability of Bicoid to function as a transcriptional activator (Schaeffer et al., 1999). *Eba-bcd* also lacks sequence similarity in the d4EHP binding domain, although two amino acids of the motif might be conserved (Y66 and L73). In the *Drosophila* protein, these residues are essential for the

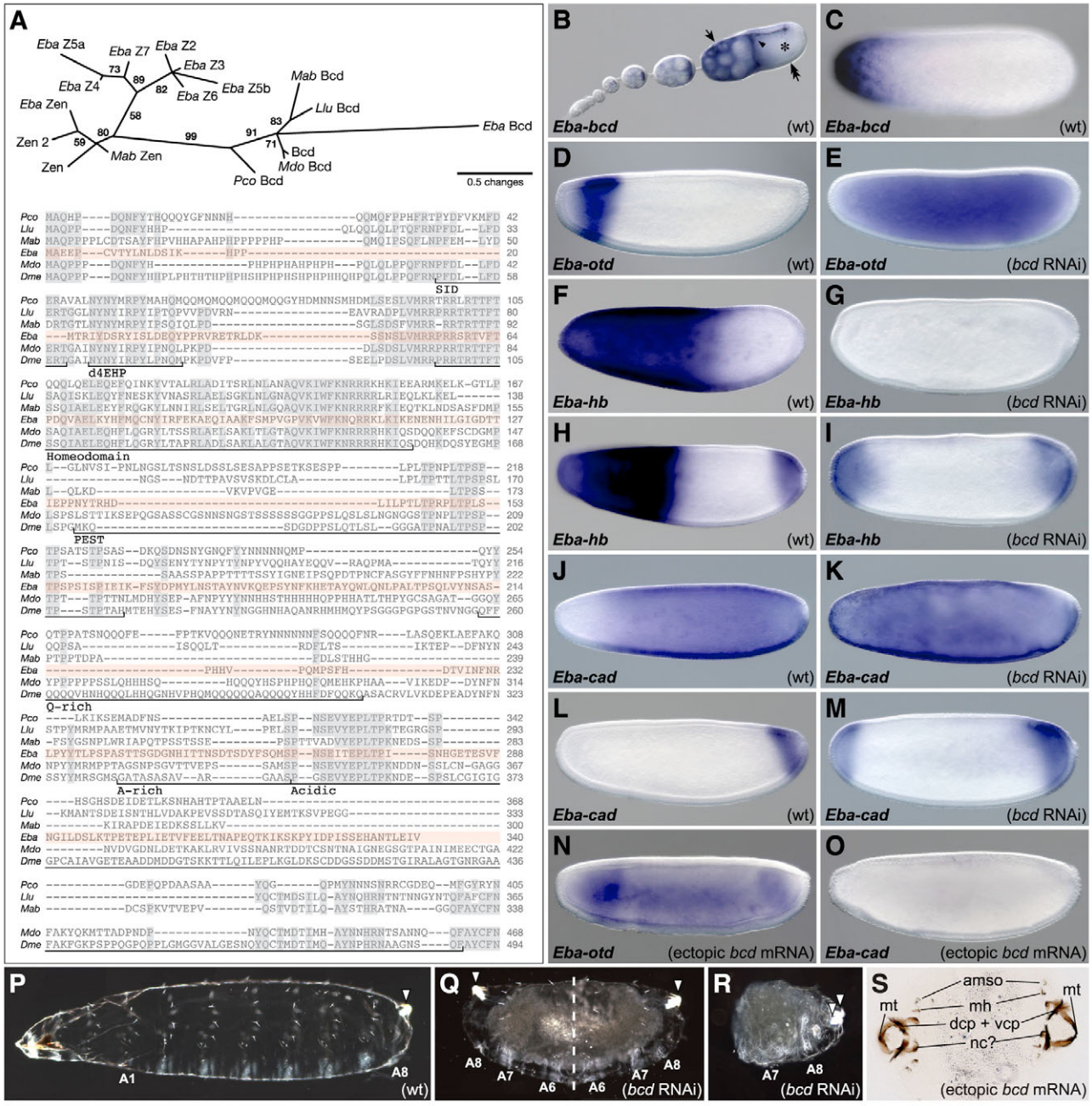

Fig. 1. Sequence, expression and function of *Eba-bcd*. (**A**) Homeodomain tree and alignment of predicted full-length Bicoid homologs. Phylogenetic distances of cyclorrhaphan Bicoid (Bcd) and Zerknüllt (Zen) homeodomains were calculated using the quartet maximum-likelihood algorithm in TREE-PUZZLE (Schmidt et al., 2002). Numbers refer to reliability values in percent. In the protein alignment, motifs and domains of the *Drosophila* sequence are underlined. The self-inhibition domain (SID), the d4EHP-binding domain, the homeodomain, the PEST-domain, as well as Q-rich, A-rich and acidic portions of *Drosophila* Bicoid are underlined. Sequences are from *Drosophila melanogaster* (Bcd, X07870; Zen, NM_057445; Zen2, NM_057446), *Episyrphus balteatus* (Eba Bcd, HM044914; Eba Zen, DQ323932; Eba Zen2 and Eba Zen3, EU999029; Eba Zen4-7, EU999030), *Lonchoptera lutea* (Llu Bcd, EU589575), *Megaselia abdita* (Mab Bcd, AJ133024), *Musca domestica* (Mdo Bcd, AJ297854) and *Platypeza consobrina* (Pco Bcd, EU589580). (**B**,**C**) *Eba-bcd* expression in ovarian follicles (B) and the syncytial blastoderm (C). Note *Eba-bcd* transcript at the anterior and dorsal periphery of the oocyte (asterisks, the position of the nucleus is indicated by an arrowhead) in the nurse cells (arrow), but not in the follicle cells (double arrow). (**D**,**E**) Wild-type (D) and *Eba-bcd* RNAi (E) blastoderm embryos probed for *Eba-otd* expression. There was an absence of expression throughout the blastoderm; however, ubiquitous staining of the yolk was observed regardless of the stage and might be a staining artifact. (**F-M**) Wild-type (F,H,J,L) and *Eba-bcd* RNAi (G,I,K,M) embryos at two consecutive blastoderm stages hybridized against *Eba-hb* (F-I) or *Eba-cad* (J-M). (**N**,**O**) *Eba-otd* (N) or *Eba-cad* (O) expression in blastoderm embryos following injection of *Eba-bcd* mRNA at the posterior pole. (**P-S**) Cuticle preparations of a wild-type first-instar larva (P), a strongly affected *Eba-bcd* RNAi embryo (Q), a putative hypomorphic *Eba-bcd* RNAi embryo (R) and an embryo following ectopic posterior *Eba-bcd* mRNA injection (S). Abdominal segments (A1-A8), the filzkörper (triangle), the antennomaxillary sense organs (amso), the median tooth (mt), the dorsal and ventral cephalopharyngeal plates (dcp, vcp), the mouth hooks (mh) and putative neck clasps (nc) of the cephalopharyngeal skeleton are indicated. Embryos and cuticles are shown in lateral (B-R) or dorsal view (S). Anterior is left (A-P) or undetermined (Q,S).

repression of *caudal* translation (Cho et al., 2005). Taken together, the sequence data suggest that *Eba-bcd* is a diverged ortholog of *bicoid*.

To test whether *Eba-bcd* functions as maternal anterior determinant during embryogenesis, we analyzed its expression by wholemount in situ hybridization and its function by RNA interference (RNAi) and ectopic mRNA injection experiments. During oogenesis, *Eba-bcd* transcript was detected in the nurse cells and in anterior and dorsal portions of the oocyte (Fig. 1B). In early embryos, *Eba-bcd* transcript was localized at the anterior pole (Fig. 1C). *Eba-bcd* transcripts disappeared prior to the onset of cellularization and zygotic expression was not observed (data not shown).

To assess the function of *Eba-bcd*, we used blastoderm gap gene expression domains of *Episyrphus orthodenticle* (*Eba-otd*), *Episyrphus hunchback* (*Eba-hb*) and *Episyrphus caudal* (*Eba-cad*) as molecular markers of early *Episyrphus* segmentation (Lemke and Schmidt-Ott, 2009). The *Eba-otd* domain spans the anterior quarter of the syncytial blastoderm and retracts from the anterior pole by the onset of cellularization. In *Eba-bcd* RNAi embryos, this domain was absent (17/17; Fig. 1D,E). *Eba-hb* is expressed in a broad anterior domain, which appears prior to cellularization, and in a narrow posterior domain, which appears at the onset of cellularization. In early *Eba-bcd* RNAi embryos, i.e. prior to the penultimate nuclear division cycle of the blastoderm, *Eba-hb* expression was absent (8/13; Fig. 1F,G) or strongly reduced (4/13); one embryo showed wild-type expression. In older *Eba-bcd* RNAi embryos, i.e. at or after the onset of cellularization, *Eba-hb* was expressed in a narrow domain at the posterior pole (2/10) or symmetrically at both poles (8/10; Fig. 1H,I). Zygotic *Eba-cad* is activated throughout the posterior three quarters of the early blastoderm and becomes confined to a narrow posterior cap at later blastoderm stages. In *Eba-bcd* RNAi embryos at early blastoderm stages, *Eba-cad* was expressed ubiquitously (7/7; Fig. 1J,K). At later blastoderm stages it was expressed in roughly symmetrical narrow caps at both ends (9/9; Fig. 1L,M). Conversely, injection of capped *Eba-bcd* mRNA into the posterior pole resulted in ubiquitous expression of *Eba-otd* with higher levels at both poles (Fig. 1N) and in the repression of *Eba-cad* (Fig. 1O). Cuticles of *Eba-bcd* RNAi embryos lacked the head, the thorax and three to five abdominal segments. In most of these cuticles, the missing anterior structures were replaced by a mirror-image duplication of the posterior abdomen (21/26; Fig. 1P,Q). However, some cuticles (5/26) lacked posterior structures at the anterior pole and were similar to the strongest phenotypes that we previously obtained following injection of *Eba-nos* mRNA at the anterior pole (Fig. 1R) (Lemke and Schmidt-Ott, 2009). In *Eba-bcd* RNAi embryos, this phenotype might reflect the incomplete knockdown, whereas in embryos expressing anterior Nanos, it might reflect incomplete translational suppression through an atypical Nanos response element (see Fig. S1 in the supplementary material). Cuticles of embryos that had been injected with *Eba-bcd* mRNA at the posterior pole lacked abdominal as well as thoracic segments, and exhibited a mirror-image duplication of a reduced head-skeleton (47/85; Fig. 1S); in a few of these cuticles, remnants of thoracic structures could be discerned between the two head-skeletons (6/47; data not shown). In the remaining cuticles, head structures were only partially duplicated at the assumed posterior pole (34/85; data not shown), and four cuticles were wild type. Taken together, the results suggest that *Eba-bcd* is necessary and sufficient to determine global AP polarity in *Episyrphus* embryos.

Eba-bcd and *Eba-cad* control non-overlapping sets of gap gene expression domains

In *Episyrphus*, the development of the abdomen, the thorax and parts of the gnathocephalon depends on *Eba-cad* (Lemke and Schmidt-Ott, 2009). In comparison with the strong RNAi phenotypes of *Eba-bcd*, these data indicate that at least five abdominal segments, the entire thorax and parts of the gnathocephalon depend on *Eba-cad* as well as on *Eba-bcd*. To test whether the overlapping cuticular phenotypes reflect overlapping deletion patterns in the expression domains of gap genes, we cloned *Episyrphus* homologs of *Krüppel* (*Eba-Kr*), *knirps* (*Eba-kni*) and *knirps-related* (*Eba-knrl*) (see Fig. S2 in the supplementary material), as well as *giant* (*Eba-gt*), and examined their expression in wild-type embryos, *Eba-bcd* RNAi embryos and *Eba-cad* RNAi embryos.

Like *Eba-hb*, none of the newly identified genes showed detectable levels of maternal expression in wild-type embryos (data not shown). *Eba-Kr* expression was initiated in a broad central domain from 40-70% egg length (EL; 0% is the anterior pole; Fig. 2A). At the onset of cellularization, *Eba-Kr* was activated in a second domain from 0-15% EL (Fig. 2B), which resolved during cellularization into a dorsal horseshoe-like stripe at about 15% EL (Fig. 2C,C′). During gastrulation, the central *Eba-Kr* domain split into two narrower stripes and new expression domains appeared at the posterior pole around the prospective proctodeal invagination, in the prospective serosa and in each segment (Fig. 2D,E′). Older embryos expressed *Eba-Kr* in the ventral nerve cord, the amnion and parts of the head (Fig. 2F). *Eba-kni* was activated in a broad posterior domain spanning 55-85% EL (Fig. 2G). A second domain appeared shortly thereafter in the ventral blastoderm at 0-30% EL. By the onset of cellularization, the posterior boundary of the posterior domain had shifted to 75% EL and the anterior domain had expanded in a narrow transverse stripe, which marked the prospective anterior rim of the cephalic furrow (Fig. 2H). At the onset of gastrulation, the posterior domain had disappeared (Fig. 2I). The anterior domain persisted during germ band extension (Fig. 2J). Expression of *Eba-knrl* was not observed above background levels in blastoderm embryos (data not shown). *Eba-gt* expression was initiated in an anterior domain from 5% to 40% EL, and in a posterior stripe spanning 85-90% EL (Fig. 2K). By the onset of cellularization, the posterior domain had expanded, spanning 75-95% EL (Fig. 2L). The anterior domain fragmented during cellularization, first into two and then into three stripes (Fig. 2L,M). During gastrulation, the anterior expression domains were further resolved, while the posterior domain became narrow and faint (Fig. 2N). Following the onset of germ band extension, *Eba-gt* was also expressed in the serosa (Fig. 2O,P).

To test whether *Eba-bcd* and *Eba-cad* control in part the same gap gene expression domains, we examined the expression patterns of gap genes in *Eba-bcd* and *Eba-cad* RNAi embryos. Following the knockdown of *Eba-bcd*, *Eba-Kr* expression was lost in both the anterior and the central domains (22/36; Fig. 2Q) or only in the central domain (9/36; data not shown); a few embryos were indistinguishable from wild type (5/36). *Eba-kni* was expressed broadly in *Eba-bcd* RNAi embryos, with a clearance at both poles (26/39; Fig. 2R) or only at the anterior pole (4/39), or it was expressed ubiquitously (9/39). *Eba-gt* was expressed in *Eba-bcd* RNAi embryos nearly ubiquitously with clearings at the poles (15/40; Fig. 2S); in the remaining embryos the expression pattern of *Eba-gt* was highly variable (data not shown).

In *Eba-cad* RNAi embryos, the anterior and central expression domains of *Eba-otd* (Lemke and Schmidt-Ott, 2009), *Eba-hb* (see next section) and *Eba-Kr* (18/18; Fig. 2T) could not be distinguished

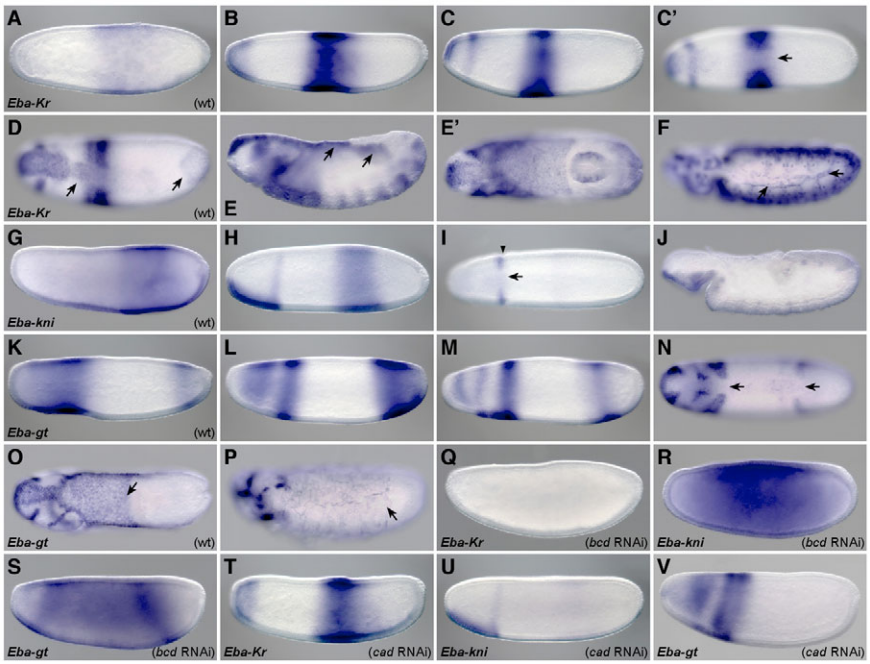

Fig. 2. Expression of *Eba-Kr*, *Eba-kni* and *Eba-gt*, and their regulation by *Eba-bcd* and *Eba-cad*. (**A-F**) *Eba-Kr* expression is shown at three consecutive blastoderm stages (A-C′), at the onset of gastrulation (D) and during germ band extension (E,E′,F). Arrows in D and E demarcate *Eba-Kr* expression in the presumptive posterior hindgut and the extraembryonic serosa. Arrows in F demarcate *Eba-Kr* expression in the amnion (note that serosal tissue of this embryo has been removed). (**G-J**) *Eba-kni* expression at two consecutive blastoderm stages (G,H), at the onset of gastrulation (I) and during germ band extension (J). The position of the cephalic furrow is indicated by an arrowhead. (**K-P**) *Eba-gt* expression at three consecutive blastoderm stages (K-M), at the onset of gastrulation (N) and during germ band extension (O,P). Arrows in O and P point to *Eba-gt* expression in the developing serosa. (**Q-S**) *Eba-bcd* RNAi embryos at blastoderm stage probed for *Eba-Kr* (Q), *Eba-kni* (R) and *Eba-gt* (S). (**T-V**) *Eba-cad* RNAi embryos at blastoderm stage probed for *Eba-Kr* (T), *Eba-kni* (U) and *Eba-gt* (V). Note clearance along the dorsal midline of all gap genes at, or prior to, the onset of gastrulation (arrows in C′,I,N). Embryos are shown with anterior towards the left in lateral view, except in C′,D,E′,I,N,O, which are dorsal views.

from the respective wild-type domains. However, the posterior *Eba-kni* domain was strongly reduced (2/15) or missing (13/15; Fig. 2U). Similarly, the posterior *Eba-gt* domain was strongly reduced (16/17) or completely missing (1/17; Fig. 2V). In addition, *Eba-cad* RNAi affected the refinement of the anterior *Eba-gt* domain. We conclude from this analysis that *Eba-cad* is necessary for the activation of posterior trunk gap genes, and that *Eba-bcd* and *Eba-cad* are essential for distinct, non-overlapping domains of gap gene expression.

Eba-cad activates terminal gap genes and *Eba-hb* at the posterior pole

To test whether *Eba-cad* also controls the posterior expression of terminal gap genes, we isolated *Episyrphus* homologs of *tailless* (*Eba-tll*) and *huckebein* (*Eba-hkb*). *Eba-tll* was activated at both poles of the syncytial blastoderm, spanning 0-20% EL and 80-100% EL, respectively (Fig. 3A). At this stage, we also observed several embryos with weaker, exclusively posterior staining (data not shown), suggesting that *Eba-tll* activation at the posterior pole might precede *Eba-tll* activation at the anterior pole. During cellularization, the posterior domain narrowed to a slim cap and the anterior domain retracted from anterior and ventral portions of the blastoderm (Fig. 3B); shortly before the onset of gastrulation, the anterior domain was cleared along the dorsal midline (Fig. 3C). *Eba-hkb* was activated at both poles of the syncytial blastoderm in comparatively narrow domains (Fig. 3D). During cellularization, expression in the anterior domain appeared weaker than in the posterior domain and shifted ventrally (Fig. 3E). By the onset of gastrulation, *Eba-hkb* was additionally expressed in two small patches on either side in the presumptive prognathal head (Fig. 3F).

The posterior *Eba-tll* domain was missing in *Eba-cad* RNAi embryos at the blastoderm stage (13/13; Fig. 3G); in older embryos, *Eba-tll* expression at the posterior pole was strongly reduced (data not shown). The posterior domain of *Eba-hkb* was strongly reduced (41/42); in one embryo it was missing completely (Fig. 3H). In addition, we noticed the absence of the posterior domain of *Eba-hb* in all but one embryo (12/13; Fig. 3I). Thus, *Eba-cad* is an essential activator of terminal gap genes at the posterior pole.

Eba-tor shifts positional information in the anterior blastoderm

To assess the regulatory input from the terminal system on *Episyrphus* gap gene regulation, we isolated a homolog of *torso* (*Eba-tor*). *Eba-tor* transcript was detected in the nurse cells and anterior oocyte of ovarian follicles (Fig. 4A) and throughout early embryos but was absent in the posterior pole plasm (Fig. 4B). After the onset of blastoderm cellularization, *Eba-tor* transcript was no longer detected (data not shown). Cuticles of *Eba-tor* RNAi embryos all lacked filzkörper, the abdominal segment A8 was lost and A7 was either reduced or absent, whereas in the anterior we distinguished three main classes of head patterning defects (compare Fig. 4C-F with 4G-N). The most severely affected embryos developed an anterodorsal hole in the cuticle and only rudiments of the cephalopharyngeal skeleton could be identified (4/30;

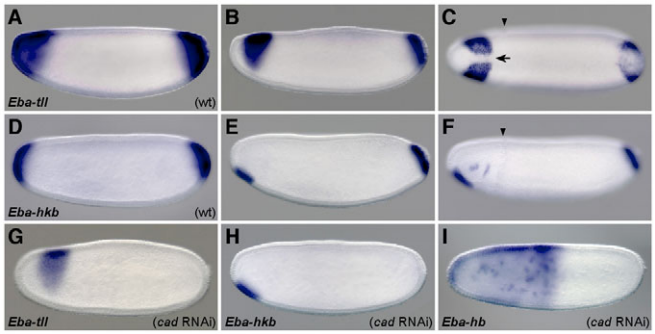

Fig. 3. Expression of *Eba-tll* and *Eba-hkb*, and the regulation of posterior gap gene expression by *Eba-cad*. (**A-C**) *Eba-tll* expression at two consecutive blastoderm stages (A,B) and at the onset of gastrulation (C). Note clearance along the dorsal midline (arrow in C). (**D-F**) *Eba-hkb* expression at two consecutive blastoderm stages (D,E) and at the onset of gastrulation (F). The position of the cephalic furrow is indicated by an arrowhead (C,F). (**G-I**) *Eba-cad* RNAi embryos at blastoderm stage probed for *Eba-tll* (G), *Eba-hkb* (H) and *Eba-hb* (I). Embryos are shown with anterior towards the left in lateral view, except in C, which is a dorsal view.

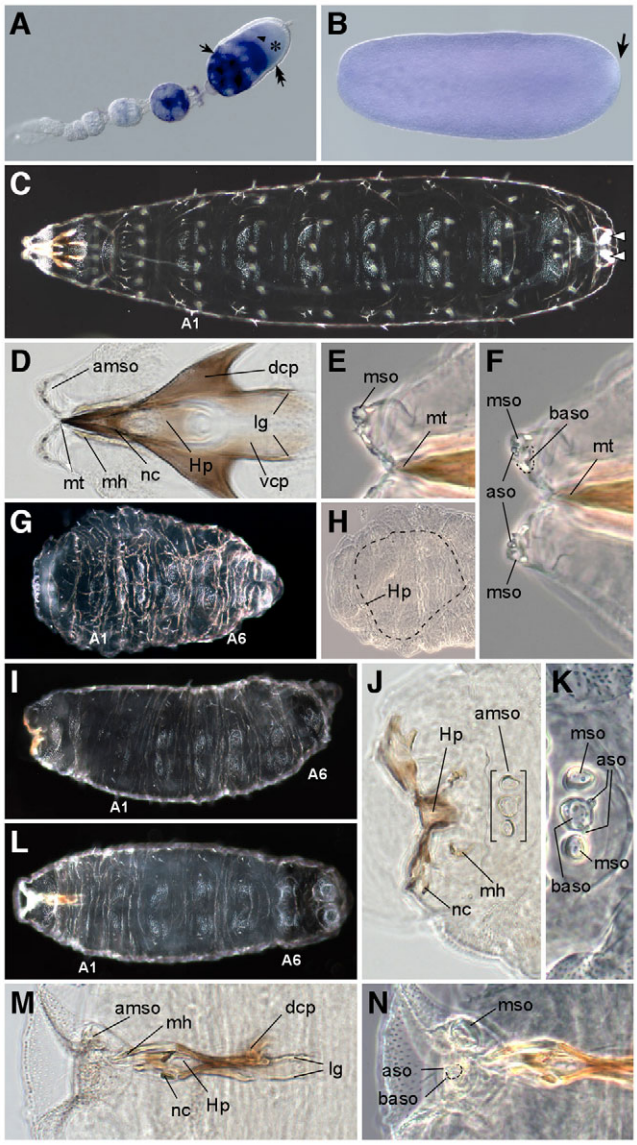

Fig. 4. Expression and cuticular RNAi phenotype of *Eba-tor*. (**A**,**B**) *Eba-tor* expression in ovarian follicles (A) and a pre-blastoderm embryo (B). *Eba-tor* transcript is detected in the oocyte (asterisk, the position of the nucleus is indicated by an arrowhead) and the nurse cells (arrow) but not in the somatic follicle cells (double arrow). In pre-blastoderm embryos (lateral view), the ubiquitous transcripts are cleared from the pole plasm (arrow in B). (**C-F**) Wild-type cuticle of a first-instar larva and magnified head-skeleton. The first abdominal segment (A1), filzkörper (white triangles), antennomaxillary sense organ (amso), median tooth (mt), mouth hooks (mh), dorsal and ventral cephalopharyngeal plates (dcp, vcp), H-piece (Hp), neck clasps (nc), Lateralgräten (lg) and the base of an antennal sense organ (baso; dashed line in F) are indicated. (**G-N**) Strong (G,H), intermediate (I-K) and weak (L-N) cuticular phenotypes of *Eba-tor* RNAi larvae. Note the hole in the anterodorsal cuticle of a strong phenotype (H, dashed line demarcates boundary of dorsal hole) and the medially fused base of the antennal sense organs in the intermediate phenotype (J,K). Cuticles are shown in ventral view. Anterior is towards the left.

Fig. 4G,H). Less-severely affected embryos developed a complete anterior cuticle but lacked the median tooth and the cephalopharyngeal plates, and the base of the antennal sense organ was fused (3/30; Fig. 4I-K). The majority of cuticles displayed the least severe head phenotype, in which the median tooth was absent and the cephalopharyngeal plates were reduced (23/30; Fig. 4L,M). This weaker phenotype is most similar to the *torso* phenotype in *Drosophila* (Schüpbach and Wieschaus, 1986).

To examine the role of *Eba-tor* in blastoderm patterning, we analyzed the expression of the terminal gap genes in *Eba-tor* RNAi embryos. In most *Eba-tor* RNAi embryos, posterior *Eba-tll* expression was lost, whereas a reduced expression domain could still be observed at the anterior pole (18/28; Fig. 5A), suggesting that *Eba-bcd* might promote the activation of *Eba-tll* at the anterior pole. In the remaining embryos, anterior expression of *Eba-tll* expression was reduced even further (6/28) or lost (4/28; Fig. 5B). As in the case of *Eba-tll*, remnants of the anterior *Eba-hkb* domain were observed in several *Eba-tor* RNAi embryos (6/24; Fig. 5C), which suggests that *Eba-bcd* also promotes the expression of *Eba-hkb*. However, in most *Eba-tor* RNAi embryos, *Eba-hkb* expression was absent entirely (18/24; Fig. 5D).

Eba-cad expression prior to the onset of cellularization was expanded (25/65; Fig. 1J; Fig. 5E), ubiquitous (11/65; Fig. 5F) or indistinguishable from wild type (29/65) in *Eba-tor* RNAi embryos. A similar result, albeit with lower penetrance, was obtained with a non-overlapping smaller fragment of *Eba-tor* dsRNA (see Materials and Methods). In embryos injected with the smaller dsRNA fragment, most embryos showed wild-type *Eba-cad* expression (17/23), some embryos displayed anteriorly expanded expression (5/23) and a single embryo displayed ubiquitous *Eba-cad* expression. Anterior expansion or ubiquitous *caudal* expression was not observed in wild-type embryos or embryos injected with *Megaselia bicoid* dsRNA (Lemke et al., 2008) stained in parallel with *Eba-tor* RNAi embryos (data not shown). The narrow posterior expression domain of *Eba-cad* during late cellularization (Fig. 1L) was typically lost (9/15) or reduced (4/15; data not shown) following *Eba-tor* RNAi, whereas the two remaining embryos showed wild-type expression. Thus, the activity of *Eba-tor* transiently promotes the repression of *Eba-cad* at the anterior pole and is required to maintain late blastoderm expression of *Eba-cad* at the posterior pole.

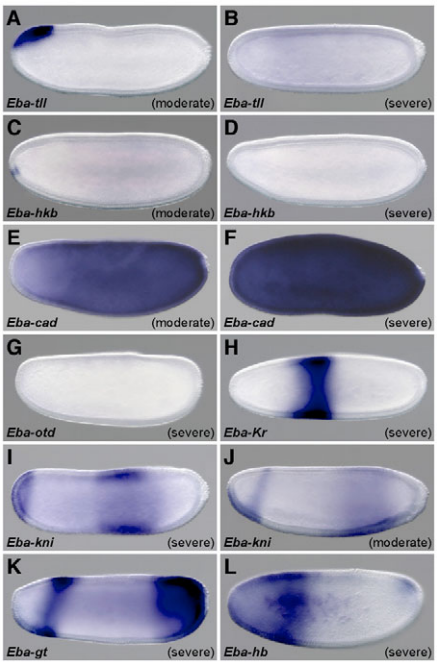

Fig. 5. Gap gene regulation by *Eba-tor*. *Eba-tor* RNAi phenotypes visualized by in situ hybridization of blastoderm embryos using probes against *Eba-tll* (**A**,**B**), *Eba-hkb* (**C**,**D**), *Eba-cad* (**E**,**F**), *Eba-otd* (**G**), *Eba-Kr* (**H**), *Eba-kni* (**I**,**J**), *Eba-gt* (**K**) and *Eba-hb* (**L**). Embryos are shown at the blastoderm stage. Anterior is left and dorsal up.

Expression of the remaining head and trunk gap genes was affected mostly at the termini in *Eba-tor* RNAi embryos. *Eba-otd* was strongly reduced (25/33) or absent (8/33; Fig. 5G), which we confirmed using a non-overlapping fragment of *Eba-tor* dsRNA (see Materials and Methods). In embryos injected with the alternative dsRNA fragment, *Eba-otd* expression was reduced (29/56), absent (2/56) or indistinguishable from wild-type (25/56). The anterior domain of *Eba-Kr* was missing (14/16) or strongly reduced (2/16) in *Eba-tor* RNAi embryos, whereas the central domain of *Eba-Kr* was present, although slightly shifted towards the anterior pole (Fig. 5H). The anterior domain of *Eba-kni* was moderately (7/37) or strongly (26/37) compressed and shifted towards the anterior pole (Fig. 5I,J), whereas in the remaining embryos, anterior expression was indistinguishable from wild type. The posterior *Eba-kni* domain was present, although slightly expanded, and its boundaries appeared less well-defined. Like the anterior domain of *Eba-kni*, the anterior domain of *Eba-gt* was typically shifted towards the anterior pole and lacked the two anterior-most head stripes (13/14), whereas the posterior domain failed to fully retract from the posterior pole (Fig. 5K). In embryos at the onset of gastrulation, this anterior shift of *Eba-kni* and *Eba-gt* expression in the presumptive head region coincided with a parallel shift of the cephalic furrow (data not shown). Finally, the posterior *hunchback* domain was strongly reduced (36/39; Fig. 5L), absent (1/39) or indistinguishable from wild type (2/39); the early anterior expression domain of *Eba-hb* appeared to be unaffected (9/9; data not shown). Taken together, our analysis of head and trunk gap gene expression domains in *Eba-tor* RNAi embryos suggests that the knockdown of *Eba-tor* shifts positional information in the anterior blastoderm by about 10-20% EL towards the anterior pole.

DISCUSSION

Eba-bcd substitutes for *bicoid* and maternal *hunchback*

Two independent protein gradients contribute to global AP polarity in *Drosophila* – Bicoid and maternal Hunchback (Tautz, 1988). Although maternal *hunchback* is not required to establish global AP polarity (Lehmann and Nüsslein-Volhard, 1987), embryos from *bicoid*-deficient mothers are asymmetric as they duplicate only the 'telson' (i.e. filzkörper and anal plates) (Frohnhöfer and Nüsslein-Volhard, 1986). The Nanos-dependent gradient of maternal *hunchback* activity ensures that *Krüppel*, *knirps* and *giant* are still expressed in distinct, albeit anteriorly shifted, domains (Fig. 6A) (Eldon and Pirrotta, 1991; Hülskamp et al., 1990; Kraut and Levine, 1991). Embryos without *bicoid* and maternal *hunchback* activity lack the central *Krüppel* domain, exhibit symmetrical *knirps* and *giant* expression and develop a mirror-image duplication of the posterior abdomen with a symmetry plane in the sixth abdominal segment (Hülskamp et al., 1990). Here, we have shown that global AP polarity of the *Episyrphus* embryo depends entirely on *Eba-bcd*. Early *Eba-bcd* RNAi embryos lacked *Eba-hb* and *Eba-otd* expression, and *Eba-cad* was derepressed at the anterior pole (Fig. 1E,G,K). *Eba-bcd* RNAi embryos also lacked the central domain of *Eba-Kr* (Fig. 2Q; Fig. 6A), exhibited symmetrical *Eba-kni* and *Eba-gt* expression (Fig. 2R,S; Fig. 6A) and developed a mirror-image duplication of the posterior abdomen with a symmetry plane in the sixth abdominal segment (Fig. 1Q). Thus, in contrast to our previous model (which postulated distinct regulators for anterior *Eba-cad* repression and anterior gap gene activation) (Lemke and Schmidt-Ott, 2009), head-to-tail polarity of the *Episyrphus* embryo appears to rely on a single gene, *Eba-bcd*.

Although apparently absent in *Episyrphus*, maternal *hunchback* expression has been observed in a wide range of dipterans, including higher (*Drosophila*, *Musca*), lower (*Megaselia*) and non-cyclorrhaphan flies (*Clogmia*) (Rohr et al., 1999; Sommer and Tautz, 1991; Stauber et al., 2000). As the maternal expression and posterior localization of the *nanos* transcript is also widely conserved in early dipteran embryos (Calvo et al., 2005; Curtis et al., 1995; Goltsev et al., 2004; Lemke and Schmidt-Ott, 2009), and as *nanos* homologs from cyclorrhaphan and non-cyclorrhaphan dipterans exhibit rescue activity in *nanos*-deficient *Drosophila* embryos (Curtis et al., 1995), the absence of maternal *hunchback* input as a second source of AP polarity in *Episyrphus* might simply reflect plasticity in early dipteran development [for other examples see Schetelig et al. (Schetelig et al., 2008), Stauber et al. (Stauber et al., 2008)]. However, direct functional evidence for a conserved role of *nanos* and maternal *hunchback* expression in providing head-to-tail polarity in dipteran embryos is currently limited to higher cyclorrhaphan flies. *Musca bicoid* RNAi embryos display head defects as well as deletions in the abdomen similar to those seen in hypomorphic *bicoid* mutants but the AP polarity of cuticles is not disrupted (Shaw et al., 2001). This observation is consistent with the activity of a second source of AP polarity that might be provided by a *nanos*-dependent maternal Hunchback gradient. Yet in *Megaselia*, a cyclorrhaphan outgroup of the *Episyrphus–Musca–Drosophila* clade, strong *bicoid* RNAi induces the formation of a symmetrical double abdomen (Lemke et al., 2008; Stauber et al., 2000), and *nanos* RNAi does not cause a segmentation phenotype (S.L. and U.S.-O., unpublished). Hence, in *Megaselia*, maternal *hunchback* and *nanos* are not sufficient for generating global AP polarity of the segmented body plan, just like in *Episyrphus*. Accordingly, this contribution has been either lost independently in the lineages leading to *Megaselia* and *Episyrphus*, or a morphologically sizable input of maternal *hunchback* and *nanos* activities in specifying AP

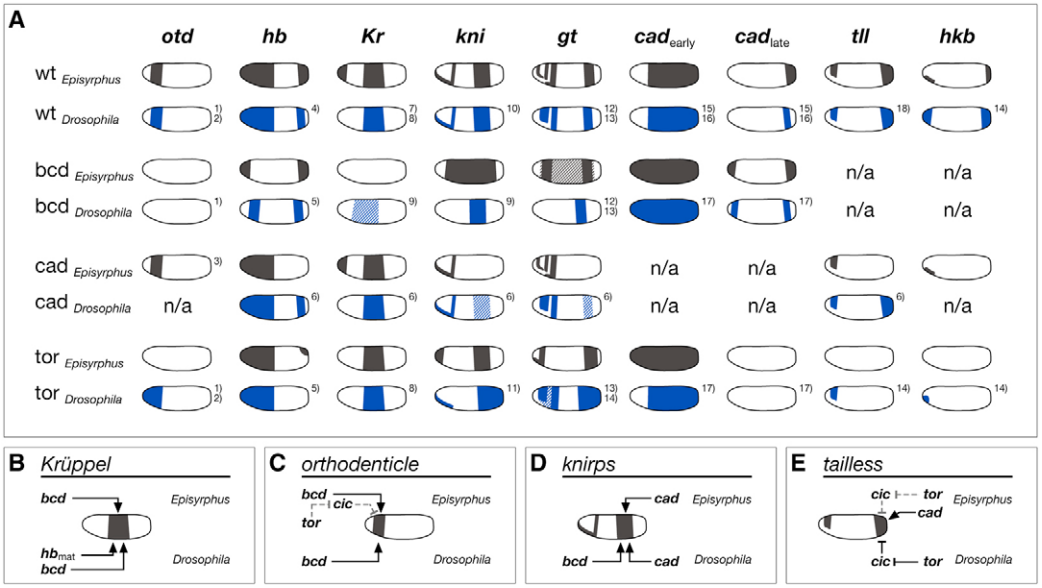

Fig. 6. Maternal regulation of gap genes in *Episyrphus* and *Drosophila*. (**A**) Schematic representation of gap gene expression patterns in wild-type and RNAi mutant blastoderm embryos of *Episyrphus* and *Drosophila*. (**B-E**) Schematic representations comparing the activating maternal input between *Episyrphus* and *Drosophila* on the regulation of *Krüppel* (B), *orthodenticle* (C), *knirps* (D) and *tailless* (E). Dashed grey lines indicate presumed interactions. References: 1 (Finkelstein and Perrimon, 1990); 2 (Gao et al., 1996); 3 (Lemke and Schmidt-Ott, 2009); 4 (Tautz et al., 1987); 5 (Tautz, 1988); 6 (Olesnicky et al., 2006); 7 (Knipple et al., 1985); 8 (Gaul and Jäckle, 1987); 9 (Hülskamp et al., 1990); 10 (Rothe et al., 1989); 11 (Rothe et al., 1994), note that *knrl* is expressed similarly to *kni* but weaker in its posterior domain, and that posterior *knrl* expression remains unchanged in Torso-deficient embryos; 12 (Eldon and Pirrotta, 1991); 13 (Kraut and Levine, 1991); 14 (Brönner and Jäckle, 1991); 15 (Macdonald and Struhl, 1986); 16 (Mlodzik and Gehring, 1987a); 17 (Mlodzik and Gehring, 1987b); 18 (Pignoni et al., 1990).

polarity is characteristic of higher cyclorrhaphan flies and generally absent in lower dipterans. Functional studies in lower dipterans will be necessary to distinguish between these possibilities.

The terminal system is required for Bicoid-dependent gene regulation in *Episyrphus*

We found that *Eba-tor*, in addition to regulating its canonical targets *Eba-tll* and *Eba-hkb*, transiently regulates anterior repression of *Eba-cad* and contributes to the activation of *Eba-otd* (Fig. 5A-G; Fig. 6A,C). This contribution of *Eba-tor* to anterior patterning differs from *Drosophila*, where *bicoid* is the only known repressor of *caudal*, and where *torso* contributes to *orthodenticle* regulation mainly by repressing it at the anterior tip of late blastoderm embryos (Finkelstein and Perrimon, 1990; Gao et al., 1996). Consistent with a stronger *torso* input on blastoderm patterning in *Episyrphus*, we observed more-severe head defects in cuticles of *Eba-tor* RNAi embryos than have been reported for *torso*-mutant *Drosophila* embryos (Schüpbach and Wieschaus, 1986).

Regulation of *Eba-cad* by *Eba-tor* appears to be independent of *Eba-otd* because *Eba-otd* RNAi does not cause an expansion of the *Eba-cad* domain (Lemke and Schmidt-Ott, 2009). Repression of *Eba-cad* is also likely to be independent of the canonical *torso* targets *tailless* and *huckebein*, which are co-expressed with *Eba-cad* at the posterior pole. It is possible, however, that *Eba-tor* interacts directly with *Eba-bcd* to provide anterior repression of *Eba-cad*. In *Drosophila*, Bicoid is believed to be phosphorylated in a Torso-dependent manner, possibly indicating a direct modification of Bcd activity by the Torso pathway (Ronchi et al., 1993). The functional significance of these modifications on *Drosophila* AP patterning is not clear, but, in *Episyrphus*, similar post-translational modifications of Bicoid might be required to repress zygotic transcription of *Eba-cad* at the anterior pole. Alternatively, an unidentified repressor, jointly regulated by *Eba-tor* and *Eba-bcd*, might account for the repression of early zygotic *Eba-cad* expression in the anterior quarter of the *Episyrphus* embryo.

Regulation of *Eba-otd* by *Eba-tor* could be caused by ectopic *Eba-cad* expression at the anterior pole. However, we found that *Eba-cad* was eventually repressed at the anterior pole in *Eba-tor* RNAi embryos but we did not observe a corresponding delayed onset of *Eba-otd* expression, which suggests that ectopic *Eba-cad* expression is not the cause of *Eba-otd* suppression. Instead, Torso activity might be required for *Eba-otd* expression by suppressing the activity of a ubiquitous repressor. This mechanism would be comparable with Torso-dependent *huckebein* and *tailless* expression at the posterior pole of *Drosophila* embryos, where *torso* suppresses the activity of two ubiquitous repressors, Capicua and Groucho (Jiménez et al., 2000; Paroush et al., 1997). A similar mechanism might also contribute to the regulation of *Eba-kni* and *Eba-gt*, which, unlike in *Drosophila*, are also strongly affected by the loss of *torso* activity (Fig. 5I-K; Fig. 6A). Although this particular model might be difficult to test if Capicua and Groucho are provided as maternal proteins (in preliminary experiments we did not observe derepression of *tailless*, *huckebein* or *orthodenticle* in *Episyrphus capicua* RNAi embryos) (S.L. and U.S.-O., unpublished), our results suggest that *Eba-tor* is crucial for anterior gap gene expression in general. Thus, despite being the sole anterior determinant, *Eba-bcd* might not have the same activation potential as *bicoid*, which in *Drosophila* can overcome the repressing input of *capicua* or *groucho* in the absence of *torso* activity (Schaeffer et al., 2000). Consistent with a weak activation potential of *Eba-bcd*, we found that in *Episyrphus* posterior gap gene expression domains depend on the activating input of *caudal*.

Independent regulation of gap and pair-rule genes by *caudal*

In *Drosophila*, gap genes are required for regulating the expression of pair-rule segmentation genes in partially overlapping sets of seven transverse stripes (Pankratz and Jäckle, 1993). Independently of the gap genes, *caudal* appears to contribute directly to the activation of pair-rule genes (Li et al., 2008; Schroeder et al., 2004). In Caudal-deficient embryos, up to four pair-rule stripes are disrupted or missing (Macdonald and Struhl, 1986; Olesnicky et al., 2006) and cuticles of Caudal-deficient *Drosophila* embryos tend to show pair-rule segmentation defects in the abdomen and thorax (Macdonald and Struhl, 1986). Reduced posterior domains of *knirps* and *giant* in *caudal*-deficient *Drosophila* embryos can only partly account for this phenotype (Olesnicky et al., 2006; Rivera-Pomar and Jäckle, 1996; Rivera-Pomar et al., 1995). In other insects, it has been more difficult to distinguish the role of *caudal* in the regulation of gap and pair-rule genes, as the expression patterns of both groups of genes are strongly affected by *caudal* RNAi. In *Nasonia*, *caudal* RNAi embryos retain only one or two anterior stripes of *even-skipped*, but they also lack the anterior domain of *knirps*, the central domain of *Krüppel* and all posterior gap gene domains (Olesnicky et al., 2006). Similarly, *caudal* RNAi suppresses all stripes of *even-skipped* in the cricket *Gryllus bimaculatus*, but it also suppresses *hunchback* and *Krüppel* (other gap genes have not been analyzed) (Shinmyo et al., 2005).

In *Episyrphus*, knockdown of *caudal* suppresses all but the first stripe of *even-skipped* (Lemke and Schmidt-Ott, 2009) but only the posterior gap gene domains of *knirps*, *giant* and *tailless* (Fig. 2U,V; Fig. 3G-I; Fig. 6A,D,E). Compared with *Drosophila*, the *Episyrphus* data reveal an expanded role of *caudal* in the activation of gap genes similar to, but not as strong as, in *Nasonia* and *Gryllus*. However, the presence of the central domain of *Krüppel* and the anterior domain of *hunchback* in *Eba-cad* RNAi embryos suggest that the loss of gap gene domains does not fully account for missing pair-rule stripes. Taken together with the *Drosophila* data, our observations therefore suggest that, independent of its role in gap gene activation, *caudal* is required for pair-rule gene expression in *Episyrphus*. This gap-gene-independent regulation of pair-rule genes by *caudal* might be an ancient heritage of insects.

Conclusions

Episyrphus and other lower cyclorrhaphan flies establish global AP polarity only through *bicoid* and lack sizable input of *nanos*, although endogenous *nanos* activity in these species might stabilize the AP axis by repressing anterior development. Despite the absence of a redundant maternal system to generate global AP polarity, *Eba-bcd* appears to be a less potent transcriptional activator than Bicoid. In contrast to *Drosophila*, gap gene activation at the anterior pole of the *Episyrphus* embryo requires a strong contribution of the terminal system, whereas the posterior domains of *knirps* and *giant* are strictly dependent on *caudal* and do not appear to receive a significant activating input by *Eba-bcd*. Thus, rather than a strong activation potential, the exclusive control of the central *Eba-Kr* domain by *Eba-bcd* appears to be the crucial difference to *Drosophila*, which renders AP polarity in the *Episyrphus* embryo entirely dependent on *bicoid*.

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Competing interests statement

The authors declare no competing financial interests.

Supplementary material

Supplementary material for this article is available at
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References

Berleth, T., Burri, M., Thoma, G., Bopp, D., Richstein, S., Frigerio, G., Noll, M. and Nüsslein-Volhard, C. (1988). The role of localization of *bicoid* RNA in organizing the anterior pattern of the *Drosophila* embryo. *EMBO J.* **7**, 1749-1756.

Brent, A., Yucel, G., Small, S. and Desplan, C. (2007). Permissive and instructive anterior patterning rely on mRNA localization in the wasp embryo. *Science* **315**, 1841-1843.

Brönner, G. and Jäckle, H. (1991). Control and function of terminal gap gene activity in the posterior pole region of the *Drosophila* embryo. *Mech. Dev.* **35**, 205-211.

Bucher, G. and Klingler, M. (2004). Divergent segmentation mechanism in the short germ insect *Tribolium* revealed by *giant* expression and function. *Development* **131**, 1729-1740.

Bullock, S., Stauber, M., Prell, A., Hughes, J., Ish-Horowicz, D. and Schmidt-Ott, U. (2004). Differential cytoplasmic mRNA localisation adjusts pair-rule transcription factor activity to cytoarchitecture in dipteran evolution. *Development* **131**, 4251-4261.

Calvo, E., Walter, M., Adelman, Z., Jimenez, A., Onal, S., Marinotti, O. and James, A. (2005). Nanos (*nos*) genes of the vector mosquitoes, *Anopheles gambiae*, *Anopheles stephensi* and *Aedes aegypti*. *Insect Biochem. Mol. Biol.* **35**, 789-798.

Casanova, J. and Struhl, G. (1993). The *torso* receptor localizes as well as transduces the spatial signal specifying terminal body pattern in *Drosophila*. *Nature* **362**, 152-155.

Cerny, A., Grossmann, D., Bucher, G. and Klingler, M. (2008). The *Tribolium* ortholog of *knirps* and *knirps-related* is crucial for head segmentation but plays a minor role during abdominal patterning. *Dev. Biol.* **321**, 284-294.

Cho, P., Poulin, F., Cho-Park, Y., Cho-Park, I., Chicoine, J., Lasko, P. and Sonenberg, N. (2005). A new paradigm for translational control: inhibition via 5′-3′ mRNA tethering by Bicoid and the eIF4E cognate 4EHP. *Cell* **121**, 411-423.

Curtis, D., Apfeld, J. and Lehmann, R. (1995). *nanos* is an evolutionarily conserved organizer of anterior-posterior polarity. *Development* **121**, 1899-1910.

Davis, G. and Patel, N. (2002). Short, long, and beyond: molecular and embryological approaches to insect segmentation. *Annu. Rev. Entomol.* **47**, 669-699.

Driever, W. and Nüsslein-Volhard, C. (1988a). The *bicoid* protein determines position in the *Drosophila* embryo in a concentration-dependent manner. *Cell* **54**, 95-104.

Driever, W. and Nüsslein-Volhard, C. (1988b). A gradient of *bicoid* protein in *Drosophila* embryos. *Cell* **54**, 83-93.

Driever, W., Thoma, G. and Nüsslein-Volhard, C. (1989). Determination of spatial domains of zygotic gene expression in the *Drosophila* embryo by the affinity of binding sites for the *bicoid* morphogen. *Nature* **340**, 363-367.

Eldon, E. and Pirrotta, V. (1991). Interactions of the *Drosophila* gap gene *giant* with maternal and zygotic pattern-forming genes. *Development* **111**, 367-378.

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

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

Furriols, M. and Casanova, J. (2003). In and out of Torso RTK signalling. *EMBO J.* **22**, 1947-1952.

Gao, Q., Wang, Y. and Finkelstein, R. (1996). Orthodenticle regulation during embryonic head development in *Drosophila*. *Mech. Dev.* **56**, 3-15.

Gaul, U. and Jäckle, H. (1987). Pole region-dependent repression of the *Drosophila* gap gene *Krüppel* by maternal gene products. *Cell* **51**, 549-555.

Gavis, E. and Lehmann, R. (1992). Localization of *nanos* RNA controls embryonic polarity. *Cell* **71**, 301-313.

Goltsev, Y., Hsiong, W., Lanzaro, G. and Levine, M. (2004). Different combinations of gap repressors for common stripes in *Anopheles* and *Drosophila* embryos. *Dev. Biol.* **275**, 435-446.

Gregor, T., Tank, D., Wieschaus, E. and Bialek, W. (2007a). Probing the limits to positional information. *Cell* **130**, 153-164.

Gregor, T., Wieschaus, E., McGregor, A., Bialek, W. and Tank, D. (2007b). Stability and nuclear dynamics of the *bicoid* morphogen gradient. *Cell* **130**, 141-152.

Grimaldi, D. and Engel, M. (2005). *Evolution of the Insects.* New York: Cambridge University Press.

Hülskamp, M., Schröder, C., Pfeifle, C., Jäckle, H. and Tautz, D. (1989). Posterior segmentation of the *Drosophila* embryo in the absence of a maternal posterior organizer gene. *Nature* **338**, 629-632.
Hülskamp, M., Pfeifle, C. and Tautz, D. (1990). A morphogenetic gradient of *hunchback* protein organizes the expression of the gap genes *Krüppel* and *knirps* in the early *Drosophila* embryo. *Nature* **346**, 577-580.
Irish, V., Lehmann, R. and Akam, M. (1989). The *Drosophila* posterior-group gene *nanos* functions by repressing *hunchback* activity. *Nature* **338**, 646-648.
Jiménez, G., Guichet, A., Ephrussi, A. and Casanova, J. (2000). Relief of gene repression by *torso* RTK signaling: role of *capicua* in *Drosophila* terminal and dorsoventral patterning. *Genes Dev.* **14**, 224-231.
Knipple, D., Seifert, E., Rosenberg, U., Preiss, A. and Jäckle, H. (1985). Spatial and temporal patterns of *Krüppel* gene expression in early *Drosophila* embryos. *Nature* **317**, 40-44.
Kosman, D., Mizutani, C., Lemons, D., Cox, W., McGinnis, W. and Bier, E. (2004). Multiplex detection of RNA expression in *Drosophila* embryos. *Science* **305**, 846.
Kraut, R. and Levine, M. (1991). Spatial regulation of the gap gene *giant* during *Drosophila* development. *Development* **111**, 601-609.
Lehmann, R. and Nüsslein-Volhard, C. (1987). *hunchback*, a gene required for segmentation of an anterior and posterior region of the *Drosophila* embryo. *Dev. Biol.* **119**, 402-417.
Lemke, S. and Schmidt-Ott, U. (2009). Evidence for a composite anterior determinant in the hover fly *Episyrphus balteatus* (Syrphidae), a cyclorrhaphan fly with an anterodorsal serosa anlage. *Development* **136**, 117-127.
Lemke, S., Stauber, M., Shaw, P., Rafiqi, A., Prell, A. and Schmidt-Ott, U. (2008). Bicoid occurrence and Bicoid-dependent *hunchback* regulation in lower cyclorrhaphan flies. *Evol. Dev.* **10**, 413-420.
Li, X., MacArthur, S., Bourgon, R., Nix, D., Pollard, D., Iyer, V., Hechmer, A., Simirenko, L., Stapleton, M., Luengo Hendriks, C. et al. (2008). Transcription factors bind thousands of active and inactive regions in the *Drosophila* blastoderm. *PLoS Biol.* **6**, e27.
Liaw, G. and Lengyel, J. (1993). Control of *tailless* expression by *bicoid*, *dorsal* and synergistically interacting terminal system regulatory elements. *Mech. Dev.* **40**, 47-61.
Liu, P. and Patel, N. (2010). *giant* is a bona fide gap gene in the intermediate germband insect, Oncopeltus fasciatus. *Development* **137**, 835-844.
Lynch, J., Brent, A., Leaf, D., Pultz, M. and Desplan, C. (2006a). Localized maternal *orthodenticle* patterns anterior and posterior in the long germ wasp *Nasonia*. *Nature* **439**, 728-732.
Lynch, J., Olesnicky, E. and Desplan, C. (2006b). Regulation and function of *tailless* in the long germ wasp *Nasonia vitripennis*. *Dev. Genes Evol.* **216**, 493-498.
Macdonald, P. and Struhl, G. (1986). A molecular gradient in early *Drosophila* embryos and its role in specifying the body pattern. *Nature* **324**, 537-545.
Macdonald, P., Ingham, P. and Struhl, G. (1986). Isolation, structure, and expression of *even-skipped*: a second pair-rule gene of *Drosophila* containing a homeo box. *Cell* **47**, 721-734.
Marques-Souza, H., Aranda, M. and Tautz, D. (2008). Delimiting the conserved features of *hunchback* function for the trunk organization of insects. *Development* **135**, 881-888.
Mlodzik, M. and Gehring, W. (1987a). Expression of the *caudal* gene in the germ line of *Drosophila*: formation of an RNA and protein gradient during early embryogenesis. *Cell* **48**, 465-478.
Mlodzik, M. and Gehring, W. (1987b). Hierarchy of the genetic interactions that specify the anteroposterior segmentation pattern of the *Drosophila* embryo as monitored by *caudal* protein expression. *Development* **101**, 421-435.
Niessing, D., Dostatni, N., Jäckle, H. and Rivera-Pomar, R. (1999). Sequence interval within the PEST motif of Bicoid is important for translational repression of *caudal* mRNA in the anterior region of the *Drosophila* embryo. *EMBO J.* **18**, 1966-1973.
Ochoa-Espinosa, A., Yu, D., Tsirigos, A., Struffi, P. and Small, S. (2009). Anterior-posterior positional information in the absence of a strong Bicoid gradient. *Proc. Natl. Acad. Sci. USA* **106**, 3823-3828.
Olesnicky, E., Brent, A., Tonnes, L., Walker, M., Pultz, M., Leaf, D. and Desplan, C. (2006). A *caudal* mRNA gradient controls posterior development in the wasp *Nasonia*. *Development* **133**, 3973-3982.
Pankratz, M. and Jäckle, H. (1993). Blastoderm segmentation. In *The Development of Drosophila melanogaster*, vol. 1 (ed. M. Bate and A. Martinez-Arias), pp. 467-516. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.
Paroush, Z., Wainwright, S. and Ish-Horowicz, D. (1997). Torso signalling regulates terminal patterning in *Drosophila* by antagonising Groucho-mediated repression. *Development* **124**, 3827-3834.
Pignoni, F., Baldarelli, R., Steingrímsson, E., Diaz, R., Patapoutian, A., Merriam, J. and Lengyel, J. (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. (1992). *bicoid* and the terminal system activate *tailless* expression in the early *Drosophila* embryo. *Development* **115**, 239-251.
Pultz, M., Westendorf, L., Gale, S., Hawkins, K., Lynch, J., Pitt, J., Reeves, N., Yao, J., Small, S., Desplan, C. et al. (2005). A major role for zygotic *hunchback* in patterning the *Nasonia* embryo. *Development* **132**, 3705-3715.
Rafiqi, A., Lemke, S., Ferguson, S., Stauber, M. and Schmidt-Ott, U. (2008). Evolutionary origin of the amnioserosa in cyclorrhaphan flies correlates with spatial and temporal expression changes of *zen*. *Proc. Natl. Acad. Sci. USA* **105**, 234-239.
Rivera-Pomar, R. and Jäckle, H. (1996). From gradients to stripes in *Drosophila* embryogenesis: filling in the gaps. *Trends Genet.* **12**, 478-483.
Rivera-Pomar, R., Lu, X., Perrimon, N., Taubert, H. and Jäckle, H. (1995). Activation of posterior gap gene expression in the *Drosophila* blastoderm. *Nature* **376**, 253-256.
Rivera-Pomar, R., Niessing, D., Schmidt-Ott, U., Gehring, W. and Jäckle, H. (1996). RNA binding and translational suppression by *bicoid*. *Nature* **379**, 746-749.
Rohr, K., Tautz, D. and Sander, K. (1999). Segmentation gene expression in the mothmidge *Clogmia albipunctata* (Diptera, psychodidae) and other primitive dipterans. *Dev. Genes Evol.* **209**, 145-154.
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.
Rothe, M., Nauber, U. and Jäckle, H. (1989). Three hormone receptor-like *Drosophila* genes encode an identical DNA-binding finger. *EMBO J.* **8**, 3087-3094.
Rothe, M., Wimmer, E., Pankratz, M., González-Gaitán, M. and Jäckle, H. (1994). Identical transacting factor requirement for *knirps* and *knirps-related* gene expression in the anterior but not in the posterior region of the *Drosophila* embryo. *Mech. Dev.* **46**, 169-181.
Schaeffer, V., Janody, F., Loss, C., Desplan, C. and Wimmer, E. (1999). Bicoid functions without its TATA-binding protein-associated factor interaction domains. *Proc. Natl. Acad. Sci. USA* **96**, 4461-4466.
Schaeffer, V., Killian, D., Desplan, C. and Wimmer, E. (2000). High *bicoid* levels render the terminal system dispensable for *Drosophila* head development. *Development* **127**, 3993-3999.
Schetelig, M., Schmid, B., Zimowska, G. and Wimmer, E. (2008). Plasticity in mRNA expression and localization of *orthodenticle* within higher Diptera. *Evol. Dev.* **10**, 700-704.
Schmidt, H., Strimmer, K., Vingron, M. and von Haeseler, A. (2002). TREE-PUZZLE: maximum likelihood phylogenetic analysis using quartets and parallel computing. *Bioinformatics* **18**, 502-504.
Schoppmeier, M. and Schröder, R. (2005). Maternal *torso* signaling controls body axis elongation in a short germ insect. *Curr. Biol.* **15**, 2131-2136.
Schroeder, M., Pearce, M., Fak, J., Fan, H., Unnerstall, U., Emberly, E., Rajewsky, N., Siggia, E. and Gaul, U. (2004). Transcriptional control in the segmentation gene network of *Drosophila*. *PLoS Biol.* **2**, E271.
Schröder, R. (2003). The genes *orthodenticle* and *hunchback* substitute for *bicoid* in the beetle *Tribolium*. *Nature* **422**, 621-625.
Schröder, R., Eckert, C., Wolff, C. and Tautz, D. (2000). Conserved and divergent aspects of terminal patterning in the beetle *Tribolium castaneum*. *Proc. Natl. Acad. Sci. USA* **97**, 6591-6596.
Schüpbach, T. and Wieschaus, E. (1986). Maternal-effect mutations altering the anterior-posterior pattern of the *Drosophila* embryo. *Roux's Arch. Dev. Biol.* **195**, 302-317.
Shaw, P., Salameh, A., McGregor, A., Bala, S. and Dover, G. (2001). Divergent structure and function of the *bicoid* gene in Muscoidea fly species. *Evol. Dev.* **3**, 251-262.
Shinmyo, Y., Mito, T., Matsushita, T., Sarashina, I., Miyawaki, K., Ohuchi, H. and Noji, S. (2005). *caudal* is required for gnathal and thoracic patterning and for posterior elongation in the intermediate-germband cricket *Gryllus* bimaculatus. *Mech. Dev.* **122**, 231-239.
Sommer, R. and Tautz, D. (1991). Segmentation gene expression in the housefly *Musca domestica*. *Development* **113**, 419-430.
Sonoda, J. and Wharton, R. (1999). Recruitment of Nanos to *hunchback* mRNA by Pumilio. *Genes Dev.* **13**, 2704-2712.
Spirov, A., Fahmy, K., Schneider, M., Frei, E., Noll, M. and Baumgartner, S. (2009). Formation of the *bicoid* morphogen gradient: an mRNA gradient dictates the protein gradient. *Development* **136**, 605-614.
Sprenger, F. and Nüsslein-Volhard, C. (1992). Torso receptor activity is regulated by a diffusible ligand produced at the extracellular terminal regions of the *Drosophila* egg. *Cell* **71**, 987-1001.
St Johnston, D. and Nüsslein-Volhard, C. (1992). The origin of pattern and polarity in the *Drosophila* embryo. *Cell* **68**, 201-219.
Stauber, M., Taubert, H. and Schmidt-Ott, U. (2000). Function of *bicoid* and *hunchback* homologs in the basal cyclorrhaphan fly *Megaselia* (Phoridae). *Proc. Natl. Acad. Sci. USA* **97**, 10844-10849.
Stauber, M., Prell, A. and Schmidt-Ott, U. (2002). A single Hox3 gene with composite *bicoid* and *zerknüllt* expression characteristics in non-Cyclorrhaphan flies. *Proc. Natl. Acad. Sci. USA* **99**, 274-279.
Stauber, M., Lemke, S. and Schmidt-Ott, U. (2008). Expression and regulation of *caudal* in the lower cyclorrhaphan fly *Megaselia*. *Dev. Genes Evol.* **218**, 81-87.

Stern, D. and Sucena, E. (2000). Preparation of larval and adult cuticles for light microscopy. In *Drosophila Protocols* (ed. W. Sullivan, M. Ashburner and R. Hawley), pp. 601-615. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
Struhl, G. (1989). Differing strategies for organizing anterior and posterior body pattern in *Drosophila* embryos. *Nature* **338**, 741-744.
Surkova, S., Kosman, D., Kozlov, K., Manu, Myasnikova, E., Samsonova, A. A., Spirov, A., Vanario-Alonso, C. E., Samsonova, M. and Reinitz, J. (2008). Characterization of the *Drosophila* segment determination morphome. *Dev. Biol.* **313**, 844-862.
Tautz, D. (1988). Regulation of the *Drosophila* segmentation gene *hunchback* by two maternal morphogenetic centres. *Nature* **332**, 281-284.
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.
Tautz, D., Lehmann, R., Schnürch, H., Schuh, R., Seifert, E., Kienlin, A., Jones, K. and Jäckle, H. (1987). Finger protein of novel structure encoded by *hunchback*, a second member of the gap class of *Drosophila* segmentation genes. *Nature* **327**, 383-389.
Walldorf, U. and Gehring, W. (1992). Empty spiracles, a gap gene containing a homeobox involved in *Drosophila* head development. *EMBO J.* **11**, 2247-2259.
Wang, C. and Lehmann, R. (1991). Nanos is the localized posterior determinant in *Drosophila*. *Cell* **66**, 637-647.
Wang, C., Dickinson, L. and Lehmann, R. (1994). Genetics of *nanos* localization in *Drosophila*. *Dev. Dyn.* **199**, 103-115.
Wharton, R. and Struhl, G. (1991). RNA regulatory elements mediate control of *Drosophila* body pattern by the posterior morphogen *nanos*. *Cell* **67**, 955-967.
Wimmer, E., Simpson-Brose, M., Cohen, S., Desplan, C. and Jäckle, H. (1995). Trans- and cis-acting requirements for blastodermal expression of the head gap gene buttonhead. *Mech. Dev.* **53**, 235-245.
Wolff, C., Sommer, R., Schröder, R., Glaser, G. and Tautz, D. (1995). Conserved and divergent expression aspects of the *Drosophila* segmentation gene *hunchback* in the short germ band embryo of the flour beetle *Tribolium*. *Development* **121**, 4227-4236.
Yeates, D. and Wiegmann, B. (2005). Phylogeny and evolution of Diptera: recent insights and new perspectives. In *The Evolutionary Biology of Flies* (ed. D. Yeates and B. Wiegmann), pp. 14-44. New York: Columbia University Press.
Zhao, C., York, A., Yang, F., Forsthoefel, D., Dave, V., Fu, D., Zhang, D., Corado, M., Small, S., Seeger, M. et al. (2002). The activity of the *Drosophila* morphogenetic protein Bicoid is inhibited by a domain located outside its homeodomain. *Development* **129**, 1669-1680.