Autonomous determination of anterior structures in the early *Drosophila* embryo by the *bicoid* morphogen

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Summary

A small number of maternal effect genes determine anterior–posterior pattern in the *Drosophila* embryo. Embryos from females mutant for the maternal gene *bicoid* lack head and thorax. *bed* mRNA becomes localized to the anterior tip of the egg during oogenesis and is the source for the morphogen gradient of *bed* protein.

Here we show that *in vitro* transcribed *bicoid* mRNA that has its own leader sequences substituted by the *Xenopus* β-globin 5′ untranslated sequences is translated more efficiently than *bicoid* mRNA with the natural 5′ mRNA leader when tested *in vitro* and in *Drosophila* Schneider cells. When injected into *bicoid* mutant embryos, only the *bed* mRNA with the β-globin leader sequence, substituted for the natural leader, is able to induce anterior development. We used P-transformation to show that sequences in the 5′ leader are neither necessary for localization of the transcript nor for the translational block of the *bed* mRNA during oogenesis.

For our injection experiments, we used only one of the identified splicing forms of *bed* mRNA. The bcd protein species derived from this mRNA is able to induce anterior development at any position along the anterior–posterior axis. Thus *bicoid* protein can induce development of head and thorax independent of any other specifically localized morphogenetic factor. Our findings further support the notion that the concentration gradient of *bed* protein, and not the existence of different forms of *bcd* protein, is responsible for specifying subregions of the embryo.

Key words: *bicoid*, morphogen, *Drosophila* embryogenesis, translational control, maternal gene.

Introduction

Development of the anterior half of the *Drosophila* embryo depends on the maternal gene *bicoid* (*bcd*) (Nüsslín-Volhard et al. 1987). Embryos from females that are homozygous mutant for strong *bcd* alleles lack head and thorax, and instead develop posterior terminal structures (the telson) at the anterior (Fig. 5) (Frohnhofer and Nüsslín-Volhard, 1986). Cytoplasmic transplantation experiments revealed the presence of an activity located at the anterior tip of embryos from wild-type but not from *bcd* mutant females. This activity, when transplanted to any position along the anterior–posterior axis of a recipient embryo, was able to induce anterior development and suppress the formation of posterior structures at the site of injection (Frohnhofer and Nüsslín-Volhard, 1986). However, these experiments could not distinguish whether *bcd* is the only anterior determinant in the *Drosophila* embryo, as other activities might have been cotransplanted.

The *bcd* gene is transcribed maternally and *bcd* mRNAs become localized at the anterior tip of the egg during oogenesis (Frigerio et al. 1986; Berleth et al. 1988). At least four distinct phases of *bcd* mRNA localization can be distinguished during oogenesis (St. Johnston et al. 1989). Between stages 6 and 9 of oogenesis (staging according to King, 1970), *bcd* mRNA accumulates in a ring at the anterior end of the oocyte. In stage 9–10a follicles, *bcd* mRNA also localizes to the apical regions of the nurse cells, but as the nurse cells contract during stage 10b–11, all the *bcd* mRNA becomes localized to the cortex at the anterior end of the oocyte. Finally, between stage 12 of oogenesis and egg deposition, *bcd* mRNA becomes localized to a spherical region of the egg that occupies a slightly dorsal position at the anterior pole. The gene products of the maternal genes *exuperantia* (Schüpbach and Wieschaus, 1986), *swallow* (Gans et al. 1975), and *staufen* (Schüpbach and Wieschaus, 1986) are involved in the process of *bcd* mRNA localization (Berleth et al. 1988; St. Johnston et al. 1989). Macdonald and Struhl (1988) demonstrated that sequences in the 3′ untranslated region of the *bcd* mRNA are responsible for a
Materials and methods

Plasmids and in vitro transcription

bcd mRNAs were transcribed from three different transcription vectors; the additional vector sequences at the 5' end are: pGem 1 vector (Promega) 58 bases, pGem 3 vector (Promega) 62 bases and Bluscript vector (Stratagene) 73 bases. The constructs are described by Berleth et al. (1988) and Driever and Nüsslein-Volhard (1989). Transcripts from all three vectors had similar translation rates (data not shown). bcd TN3 transcripts were generated using a pSP64 vector (Melton et al. 1984), and include additional 12 bases of vector sequences. The construct bcdTN3 was generated by inserting the NdeI (end repaired with Klenow polymerase)– EcoRV fragment from pARbcdNB and the EcoRV– EcoRI fragment from c5.46.6c (both described in Driever and Nüsslein-Volhard, 1989) into the Ncol (blunt ended with mung bean nuclease)– EcoRI digested pSP64 vector, a pSP64 derivative containing a slightly modified Xenopus ß-globin mRNA leader fragment (Siegel and Walter, 1988). The pSP64 vector is based on the pSP64T vector (D.A. Melton, Harvard University).

In vitro transcription was performed according to a protocol slightly modified from D.A. Melton et al. (1984). Template plasmids were linearized with EcoRI, extracted with phenol, precipitated and dissolved at 0.5 Ìg/ìl in TE (10 mM Tris–HCl pH 7.5; 2 mM EDTA). Setup of the transcription reaction: 5.5 Ìl diethyl pyrocarbonate (DEPC) treated distilled water; 6 Ìl 5X salts (200 mM Tris–HCl pH 7.5, 30 mM MgCl2, 10 mM spermidine); 3 Ìl 0.1 M diethoethiol; 2 Ìl human placental ribonuclease inhibitor (Promega 10–20 units ìl−1); 6 Ìl capped NTP mix 5X (2.5 mM each ATP, UTP, CTP, 2.5 mM G(5')ppp(5')G, 0.5 mM GTP); 6 Ìl linearized DNA in TE 0.5 Ìg ìl−1; 1.5 Ìl Sp6 RNA Polymerase (10–20 units ìl−1). Transcription was performed for 60 min at 40°C, then 1.5 Ìl 10 mM GTP were added and incubation continued for 15 min. The RNA was extracted with phenol and chloroform, precipitated with ethanol in the presence of 0.3 M NaOAc, the pellet washed with cold 70 % ethanol, dried and dissolved in 20 Ìl DEPC treated water (typical yield 1.5 Ìg ìl−1).

In vitro translation and expression in Drosophila Schneider cells

The mRNAs were translated in vitro using reticulocyte lysate or wheat germ extract (Amersham) according to the manufacturers' protocol. The relative efficiencies of in vitro translation were determined by cutting out the bcd protein bands from SDS–PAGE like the one shown in Fig. 2 and measuring the incorporated radioactivity by liquid scintillation counting. Transient expression in Drosophila Schneider cells, the preparation of extracts and the immunoblot analysis were as described (Driever and Nüsslein-Volhard, 1989). We constructed the plasmids pMetbcDNA3 and pMetbcDN3 by inserting the HindIII Fragment from pbcDN3 and, respectively, the EcoRI Fragment from c5.46.6c, into the pRhmA-3 metallothionein promoter expression vector (Bunch et al. 1988).

Injection into embryos

The RNA was injected into pre-polecell-stage embryos at various dilutions (using DEPC-treated water) according to standard procedures (Frohnhöfer and Nüsslein-Volhard, 1986). The biological activity of the injected mRNA was scored by analyzing the cuticular phenotypes of the injected animals after 48 h of development at 18°C. Photography was
performed using dark-field or phase-contrast optics.

P-Transformation and immunohistochemical analysis
The HindIll-EcoRI fragment with the globin leader, the bcd ORF and the 3' untranslated region of the bcd transcript was cloned into the P-vector pCaSpeR/BcdBglII, which was kindly provided by David Stein, Tubingen. This vector, a pCaSpeR derivative (Pitrotta, 1988) carries the 2 kb maternal bcd promoter fragment from the BamHI site to the PstI site at position 1244 and the genomic fragment from 4292 to the EcoRI site at 5.9 kb (pPbcdTN3): numbering of the genomic sequence according to Berleth et al. 1988. P transformation was as previously described (Driever et al., 1989a). Immunohistochemistry using monoclonal anti-bcd protein antibody (Driever and Nusslein-Volhard, 1988a) was performed using the Vectastain ABC elite kit peroxidase (Vector Laboratories). Ovaries were dissected in BSS (Chan and Gehring, 1971), frozen in liquid nitrogen and melted again while adding twofold concentrated SDS/mercaptoethanol sample buffer with 8 M urea and sonicating. Extracts from embryos were prepared using the same procedure.

Results
Injection of bcd mRNA into embryos from bcdE1 mutant females
Injection of poly(A)+ mRNA isolated from young wild-type embryos into early embryos from mutant females was shown to rescue the mutant phenotype of several maternal effect genes that regulate pattern along the dorsoventral axis (Anderson and Nusslein-Volhard, 1984). This approach has not been successful for rescue of the bcd mutant phenotype (Berleth, 1989). Similar to experiments performed with the maternal gene easier (Chasan and Anderson, 1989), we tried to substitute the bcd activity by injection of in vitro synthesized bcd mRNA into the anterior tip of embryos from females homozygous for the strong bcdE1 allele. We were not able to induce the formation of any anterior wild-type cuticular structures upon injection of the mRNA at a wide range of concentrations (0.1 to 5 μg μl⁻¹) (Berleth, 1989; Table 1A and data not shown). The bcd cDNA of the most abundant splice type a (Fig. 1A) that we used

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<th>Table 1. Frequency of the induction of anterior structures by the injection of bcd mRNAs</th>
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<tr>
<td><strong>(A) Injection into the anterior of embryos from bcdE1 females</strong></td>
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<tr>
<td>RNA concentration (μg μl⁻¹)</td>
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<tr>
<td>Number of embryos</td>
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<tr>
<td>Phenotype</td>
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<tr>
<td>- anterior not developed</td>
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<tr>
<td>- bcd⁺ mutant (Filzkörper present at the anterior)</td>
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<td>- thoracic and gnathal structures induced</td>
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<td>- head and thorax completely rescued</td>
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<th><strong>(B) Injection into the posterior of embryos from wild-type females</strong></th>
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<td>RNA concentration (μg μl⁻¹)</td>
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<tr>
<td>Number of embryos</td>
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<tr>
<td>Phenotype (in the posterior half of the embryo)</td>
</tr>
<tr>
<td>- telson (Filzkörper present, most often abdominal segmentation distorted)</td>
</tr>
<tr>
<td>- no telson and no anterior structures formed</td>
</tr>
<tr>
<td>- thoracic structures and parts of the telson formed</td>
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<tr>
<td>- thoracic and cephalic structures formed (abdomen reduced)</td>
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<td>- complete head duplicated</td>
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<th><strong>(C) Injection into the middle of embryos from bcdE1 mutant females</strong></th>
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<td>RNA concentration (μg μl⁻¹)</td>
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<td>Number of embryos</td>
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<tr>
<td>Phenotype</td>
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<tr>
<td>- bcd⁻ mutant</td>
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<tr>
<td>- abdominal development distorted</td>
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<tr>
<td>- thoracic structures formed in the middle of the embryo</td>
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<tr>
<td>- thoracic and cephalic structures formed in the middle of the embryo</td>
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Embryos were injected with in vitro transcribed mRNA at the indicated concentrations. The number of embryos indicates the total number of embryos injected in brackets (in sets of 100) and the number of those that developed cuticle and were scored for the phenotype. For details see Materials and methods section.
Fig. 1. Structure of bed mRNAs. (A) Splicing patterns of the primary bed transcript according to Berleth et al. 1988. AUG is the beginning and UAG the end of the largest open reading frame; PRD is the paired repeat, a histidine- and proline-rich sequence; HD is the bed homeo domain. (B) Structure of the 5' ends of bed mRNA and bcdTN3 mRNA transcripts (globin 5'=Xenopus \( \beta \)-globin 5' untranslated sequences). The figure also indicates the translation initiation sequences from the bed mRNA and the heterologous construct in comparison with the translation initiation consensus sequences for eukaryotes and for Drosophila (Kozak, 1986; Cavener, 1987). In addition to the sequences shown, the in vitro transcripts include vector sequences (see Materials and methods).

Fig. 2. Sequences in the 5' non-translated leader regulate the efficiency of bed mRNA translation. In vitro transcribed bed mRNA (lanes 1) and bcdTN3 mRNA with the bed 5' untranslated region exchanged with the one from the Xenopus \( \beta \)-globin (lanes 2), were translated in vitro in a wheat germ extract (WG) and a rabbit reticulocyte lysate (RL) in the presence of \( \text{[35S]} \)methionine. The products were displayed by SDS-PAGE and visualized by autoradiography. For the products of the bed mRNA translations, ten times more material was loaded on the gel than for those of bcdTN3 mRNA. Both types of transcripts were also transiently expressed from the metallothionein promoter in Drosophila Schneider cells (DSC); cells were lysed and extracts analysed by immuno blotting: The lane designated 0 shows extracts from control cells. Relative molecular masses are indicated at the left and right side.

As a template for the transcription begins a few bases downstream of a TATA box, contains a polyadenylation signal and is at both ends only a few bases longer than a number of other cDNAs (Berleth et al. 1988). Thus we believe that our template is a full-length cDNA, though the transcription start site has not been mapped. All missense mutations in the cDNA with respect to the genomic sequence have been corrected (Driever and Nüsslein-Volhard, 1989).

**Translation efficiency of bed mRNA**

We tested the mRNAs by in vitro translation in a wheat germ and a reticulocyte lysate system and found that they were translated very inefficiently (Fig. 2, lanes WG 1 and RL 1). In addition, expression of the same cDNA in Drosophila Schneider cells from the metallothionein promoter resulted in low levels of bed protein (Fig. 2, lane DSC 1). In an attempt to circumvent putative translational control mechanisms that would reduce the efficiency of translation, we exchanged the 5'-non-translated sequence of the abundant splice type a of bed mRNA (Fig. 1) for that of the Xenopus \( \beta \)-globin mRNA (bcdTN3 expression construct). This sequence was chosen because it has neither upstream AUGs nor a strong secondary structure. Tests in all three translation systems showed that bcdTN3 mRNA with the globin leader is translated about 50 times more efficiently than the native bed mRNAs (Fig. 2). From wheat germ translations, we recovered 41 (±3) times more activity for bcdTN3 mRNA than for bed mRNA templates. Using reticulocyte lysates, the factor was 51 (±4; average of three determinations each).

**Rescue of the bed mutant phenotype by the injection of bcdTN3 mRNA**

The development of anterior structures can be induced by the injection of bcdTN3 mRNA at appropriate concentrations into the anterior tip of embryos from bed\(^{21}\) mutant females (Table 1A, Fig. 4 and 5B; see also Driever et al. 1989b). By autoradiography and by in situ hybridization, we find that injected mRNAs maintain a high point at the site of injection for more than one hour (V.S. and D. Ferrandon, unpublished). When we analyse the distribution of bed protein 90 min after
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In injection, using a monoclonal antibody that detects only the full-length bed protein derived from the injected mRNA in whole-mount immunostain reactions (Fig. 3 A and B), we detect the formation of a protein concentration gradient similar to the one in embryos from wild-type females.

Injection of bcdTN3 mRNA at a concentration of 0.4 \( \mu g/l \) suppressed the formation of telson structures at the anterior in nearly all of the injected embryos, and induced the development of all the anterior structures present in wild-type larvae (acron, head and thorax) in about 30% of these embryos (Table 1 A). About 10% of the larvae hatched, and some developed into adults. Embryos that failed to hatch frequently exhibited fused abdominal segments (A3, A4 and A5 were affected in most cases). Since similar phenotypes can be observed in embryos from females that carry 6 or 8 functional copies of the bed gene in their genome (Berleth, 1989; our unpublished data), we interpret these defects as being caused by the injection of excessive amounts of bcdTN3 mRNA into the embryo. We propose that the defects in abdominal segmentation we obtain are an indirect effect: increased levels of bed protein lead to a posteriorward extension of the \( hb \) protein gradient and thereby might inhibit knirps expression, which is necessary for abdomen formation (Hülskamp et al. 1989; Irish et al. 1989; Struhl, 1989).

Our findings support the model in which bed protein concentration directly determines position; an elevated level of mRNA and thus protein results in an enlargement of anterior and a compression of posterior pattern, giving rise to defects in abdominal segmentation. Indeed, during gastrulation in some cases we observed that the cephalic furrow was shifted back as far as 40% egg length (the normal position of the cephalic furrow is at 65% egg length; 0% is at the posterior pole; data not shown).

Induction of anterior development at ectopic positions
Confirming and extending the results from cytoplasmic transplantations (Frohnhöfer and Nüsslein-Volhard, 1986), we found that \textit{in vitro} transcribed bcdTN3 mRNA can induce anterior development at any ectopic position along the anterior-posterior axis (Fig. 5). Injection into the posterior pole of embryos from bcd~ (not shown) or wild-type females (Table 1B and Fig. 5E) suppresses posterior development and induces the formation of head and thoracic structures at the posterior end. Injection of bcdTN3 mRNA at 1.7 \( \mu g/l \) into the posterior pole in one case resulted in the development of a complete second head and thorax (Fig. 6). Fig. 3 demonstrates that bed protein gradients similar to the one in the anterior of wild-type embryos are formed upon the injection of the RNA. The requirement for a 4 to 5 times greater amount of bed mRNA for the induction of a head at the posterior as opposed to the anterior end of the embryo may be due to the negative effect of the activity of posterior group genes on bed mRNA stability (Frohnhöfer and Nüsslein-Volhard, 1986; Wharton and Struhl, 1989).
formation of duplicated abdominal segments with mirror-image symmetry (A1 in Fig. 5E) appears to be an indirect organizing influence of bcd on abdominal pattern formation, as bcd on its own is not able to induce abdominal structures (Frohnhöfer and Nüsslein-Volhard, 1986; Lehmann and Frohnhöfer, 1989).

Injection into the middle of embryos from bcd− females results in the formation of gnathal and thoracic structures at the site of injection (Table 1C, Fig. 5 C and D, results summarized in Fig. 4): we were able to identify pro-, meso- and metathoracic structures, antennal and maxillary sense organs, mouth hooks, cirri and other sclerotic structures that could not be assigned unambiguously. The structures usually appear pairwise and are arranged in a bilateral symmetric way transverse to the anterior–posterior axis of the embryo. None of the involuted parts of a wild-type head or acronal structures were identified. This result is consistent with data obtained from the analysis of maternal genes that affect terminal development in the embryo. The formation of acronal structures requires, in addition to bcd, the torso gene group, whose activity is restricted to the termini of the embryo (Nüsslein-Volhard et al. 1987; Klingler et al. 1988).

Is the bcd mRNA leader involved in translational control during oogenesis?

To investigate the influence of the 5' sequences on translational control, we used a P-transformation vector that allows the expression of bcdTN3 mRNA during oogenesis. The CaSpeR-derived plasmid pPbcdTN3 carries a 2 kb bcd promoter fragment as well as bcd 3' sequences and has the bcdTN3 cDNA sequences inserted at the PstI site just a few basepairs downstream from the putative start site of transcription. We generated 12 transgenic lines in a w1 stock, 4 of which carried insertions on the second chromosome. Those were crossed into bcdE1/TM3 flies. Females homozygous for the strong allele bcdE1 that carry one pPbcdTN3 insertion give rise to progeny with a phenotype similar to that of weak bcd alleles (Fig. 7A; Frohnhöfer and Nüsslein-Volhard, 1986). The analysis of the expression pattern of even-skipped (Frasch et al. 1987) reveals that the gnathal and thoracic region is expanded and shifted slightly more anteriorwards than in embryos from mothers heterozygote for a deficiency (Fig. 7B; Frohnhöfer and Nüsslein-Volhard, 1987). Two copies of pPbcdTN3 in the germ line rescue the bcdE1 mutant phenotype completely and give rise to fertile adults. We conclude that our construct provides slightly less bcd⁺ activity to the embryo than a wild-type copy of the gene does. For the in vivo function of the gene, in our assay the 169 bp leader sequence seems not to contain any essential elements.

To test whether the observed translational control of bcd mRNA during oogenesis is due to sequence elements in the leader, we analysed ovaries from females carrying two pPbcdTN3 insertions for the presence of bcd protein. In whole mounts of ovaries, immunohisto- logically stained for bcd protein with a monoclonal antibody, we were not able to detect any specific, bcd-dependent nuclear staining in the nurse cells where the mRNA is synthesized and present at distinct apical patches during early stages of development (data not shown). Neither could we detect any specific staining in the oocyte. Though we used the same method of immunodetection that gives strong signals for bcd protein during syncytial blastoderm stages, we might have failed to detect very low levels of expression during oogenesis. Therefore, we prepared extracts for immunoblot analysis from ovaries of females homozygous for bcdE1 and pPbcdTN3 (Fig. 8). As a control, we analysed on the same Western blot extracts of blastoderm stage embryos from females of the genotypes pPbcdTN3/pPbcdTN3; bcdE1/bcdE1, pPbcdTN3+/+; bcdE1/bcdE1 and from a strain carrying 8 functional copies of the bcd gene, for which we also prepared ovarian extracts. We estimate that stages of
oogenesis where \textit{bcd} mRNA is detectable contribute to more than 80% of the ovary of a well-fed female. In none of the ovarian extracts were we able to detect \textit{bcd} protein, although we obtained strong signals for \textit{bcd} protein from the embryonic extracts. Thus we conclude that, like wild-type \textit{bcd} mRNA, \textit{bcdTN3} mRNA is not translated during oogenesis. The \textit{bcd} mRNA leader sequence seems not to be required for translational control during oogenesis.

Fig. 5. Cuticular phenotypes of embryos injected at various positions with \textit{in vitro} transcribed mRNAs coding for \textit{bcd} protein. (A) Uninjected control embryo from females homozygous mutant for the strong \textit{bcd}\textsuperscript{E1} allele. Instead of head and thorax, a duplication of posterior terminal structures is formed at the anterior. (B) Embryo from a \textit{bcd}\textsuperscript{E1} mutant female injected at the anterior tip with \textit{bcdTN3} mRNA (0.4 \mu g/\mu l\textsuperscript{-1}). All the structures present in wild-type head and thorax are completely rescued. (C) Embryo from a \textit{bcd}\textsuperscript{E1} mutant female injected at the middle of the embryo with \textit{bcdTN3} mRNA (1.5 \mu g/\mu l\textsuperscript{-1}). While posterior terminal structures still form at both termini, head structures are formed at the site of injection, surrounded by thoracic dentical belts and sense organs. (D) Close-up of the induced head region from the embryo shown in C. The bilateral symmetry of the induced head structures is typical for the arrangement of structures in such animals (see also Frohnhöfer and Nüsslein-Volhard, 1986). (E) Wild-type embryo injected at the posterior pole with \textit{bcdTN3} mRNA (1.7 \mu g/\mu l\textsuperscript{-1}). Head structures and thorax are induced in the posterior half of the embryo. All embryos were injected during early cleavages (stage 2, before pole cell formation). Pictures were taken using dark-field optics (except D, phase contrast optics). Anterior is always to the left, dorsal at top. A=Abdomen, A1=first abdominal segment, A8=eighth abdominal segment, ap=anal plate, aso=antennal sense organ, ci=cirri, fk=filzkörper, mh=mouth hooks, mso=maxillary sense organs, T1 to T3=first through third thoracic segment. The structures of the head are described by Jürgens et al. 1986.

Fig. 6. Experimental induction of dicephalic embryos. A wild-type embryo, injected with \textit{bcdTN3} mRNA at the posterior pole, developed an exceptionally perfect mirror-image duplication of head and thorax. The only hint to the posterior origin of the right head is the size reduction of the labrum, which is hardly visible underneath the epistomal sclerite (eps) in (A). A1=first abdominal segment, T1 to T3=first through third thoracic segment, ci=cirri, m=mouth hooks, pv=proventriculus. Anterior is left. Phase-contrast pictures: (A) focus at central plane, (B) focus on ventral cuticle.
Fig. 7. Rescue of anterior structures in embryos from homozygous mutant bcdEl females carrying a single pPbcdTN3 insertion. (A) Anti-bed protein immunostain of a syncytial blastoderm embryo visualizing the formation of the bcd protein gradient translated from maternally transcribed bcdTN3 mRNA. (B) Anti-eve protein immunostain of a cellular blastoderm embryo. The segmented anlagen of head and thorax are restored but shifted further anterior and more expanded than in embryos from females carrying Def (3R) tin, including bed. (C) Dark-field micrograph of the cuticle pattern. The development of gnathal and thoracic derivatives is completely rescued when compared to the bcdEl mutant phenotype, a strong bed allele. Fragments of the cephalopharyngeal skeleton, but no labral sclerites were found.

Discussion

Translational control of bcd mRNA

Previous investigations have suggested that bcd mRNA is subject to translational control. Though the bcd mRNA is already present in late previtellogenic follicles (Frigerio et al. 1986; Berleth et al. 1988), bcd protein has not been detected during any stage of oogenesis (Driever and Nüsslein-Volhard, 1988a). Thus bcd mRNA appears not to be efficiently translated before egg deposition. Further, cytoplasm from mature stage 14 oocytes but not from earlier stages of oogenesis, when the bcd mRNA is already detectable, has the ability to rescue the bcd mutant phenotype when transplanted into embryos from bcdEl mutant females (Sander and Lehmann, 1988).

Selective translational repression of maternal transcripts during oogenesis has been reported in several systems, and different transcripts seem to be regulated by different mechanisms. For example, in Xenopus oocytes some transcripts are bound by specific proteins and are thus inaccessible to the translational machinery, a phenomenon known as maternal masking (Raff, 1980; Richter and Smith, 1984). In other cases, the translational machinery itself (e.g., eIF-4F) is altered, either in activity or availability (Audet et al. 1987; Huang et al. 1987). Finally it has been shown that polyadenylation of mRNAs can affect their translatability (Vassalli et al. 1989; Fox et al. 1989).

When we analysed the translation efficiency of bcd mRNA and bcdTN3 mRNA in test systems from plant, vertebrate and insect we obtained similar results. Thus we suspected that the primary structure of the bcd mRNA is responsible for the low efficiency of translation. A small open reading frame of 14 codons (including one additional in frame AUG) is located at the 5' end of the mRNA. Several alternative structures can be proposed that have the GC-rich region from nucleotide 149...
to 155 (sometimes extending till nucleotide 161) base paired with other parts of the molecule (e.g. the region from nucleotide 172 to 185; the AUG starts at nucleotide 170). We think it unlikely that the higher translation rate of the bcd TN3 mRNA may simply be caused by a better translation initiation context, as the natural bcd mRNA has the greater homology to the Drosophila translation initiation consensus (Cavener, 1987).

Replacement of the bcd upstream leader sequence with that of β-globin did not relieve its translational repression during oogenesis. This finding argues against an important role of the leader for the translational block. Which part of the bcd mRNA confers translational control still remains to be elucidated. It is possible that the translational block is closely linked to the different phases of bcd mRNA localization during oogenesis and early embryogenesis (St. Johnston et al. 1989).

**bcd protein determines anterior development independent of any other localized, anterior specific morphogenetic activity**

Though a large body of information on the function of bcd during early embryogenesis has accumulated (Frohnhofer and Nüsslein-Volhard, 1986; Driever and Nüsslein-Volhard, 1988a,b; Driever et al. 1989a,b; Struhl et al. 1989), some major questions concerning the function of bcd as the morphogen for anterior development had remained. First, it is unclear whether the presence of different spliced forms of the bcd transcript is functionally significant. For example, the different transcripts could code for functionally distinct proteins which would then be responsible for subdividing the embryo into domains. Our data argue against this, since they demonstrate that a single bcd protein species derived from one splice form of bcd mRNA (Type a in Fig. 1) can exert all morphogenetic functions of the bcd gene. The minor splice variants may result from aberrant processing of the mRNA and may not fulfill a specific function during embryonic development.

Second, we could not previously exclude the presence of other, so far unidentified localized anterior activities in the egg, which might cooperate with, or modify bcd activity, possibly in a region-specific manner. The transplantation experiments performed by Frohnhofer and Nüsslein-Volhard (1986) demonstrate that the localized bcd activity is necessary for the induction of anterior development, but they can not prove that it is sufficient, as other anterior activities might have been cotransplanted.

The induction of anterior structures by the injections of in vitro synthesized bcd mRNAs at ectopic positions reveals that bcd is the only localized morphogenetic maternal factor specific for anterior development. Independent of any other localized activity, bcd can induce the formation of gnathal structures and thorax at any position along the anterior–posterior axis. These findings rule out that bcd protein might be subject to region-specific (anterior) modifications in order to generate differentially active bcd protein species. We were able to induce acronal structures at the termini of the embryo only, consistent with previous data (Nüsslein-Volhard et al. 1987; Klingler et al. 1988) demonstrating that terminal structures depend on the activity of the torso group of maternal effect genes. The presence of bcd activity in the terminal region results in the development of anterior terminal structures (the acron) instead of posterior terminal structures (the telson).

Our analysis strongly supports the idea that bcd acts as a morphogen, specifying subregions of the embryo in a concentration-dependent manner (Driever and Nüsslein-Volhard, 1988b). There is no evidence indicating the existence of other, anterior-specific maternal morphogens; indeed all the identified maternal genes affecting anterior development appear to act by modifying bcd mRNA localization or stability and thus bcd protein distribution (exuperantia, swallow, and staufen: Berleth et al. 1988; Driever and Nüsslein-Volhard, 1988b; St Johnston et al. 1989; bicaudal: Wharton and Struhl, 1989), rather than presenting a parallel maternal input into anterior pattern formation.

We thank F. Sprenger for help with the RNA secondary structure analysis, G. Thoma for excellent technical assistance, W.B. Hansen for the pSPB4 vector and D. Stein for the pCasPerbedBgllII vector. We also thank H. Doyle, D. Ferrandon, R. Schnabel, D. Stein and S. Roth for critically reading earlier versions of the manuscript. V.S. is a fellow of the Jane Coffin Childs Memorial Fund for Medical Research. The work was supported by the DFG (Leipniz Programm) and a grant from the Jane Coffin Childs Memorial Fund for Medical Research.

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(14 May 1990)