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Bicoid functions without its TATA-binding protein-associated factor interaction domains

(Hunchback/morphogen/TAF_{II}/transcription)

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ABSTRACT **Four maternal systems are known to pattern the early *Drosophila* embryo. The key component of the anterior system is the homeodomain protein Bicoid (Bcd). Bcd needs the contribution of another anterior morphogen, Hunchback (Hb), to function properly: Bcd and Hb synergize to organize anterior development. A molecular mechanism for this synergy has been proposed to involve specific interactions of Bcd and Hb with TATA-binding protein-associated factors (TAF_{II}s) that are components of the general transcription machinery. Bcd contains three putative activation domains: a glutamine-rich region, which interacts *in vitro* with $TAF_{II}110$; an alanine-rich domain, which targets $TAF_{II}60$; and a C-terminal acidic region, which has an unknown role. We have generated flies carrying *bcd* transgenes lacking one or several of these domains to test their function *in vivo*. Surprisingly, a *bcd* transgene that lacks all three putative activation domains is able to rescue the bcd^{E1} null phenotype to viability. Moreover, the development of these embryos is not affected by the presence of dominant negative mutations in $TAF_{II}110$ or $TAF_{II}60$. This means that the interactions observed *in vitro* between Bcd and $TAF_{II}60$ or $TAF_{II}110$ aid transcriptional activation but are dispensable for normal development.**

In *Drosophila*, elaboration of the body plan is directed by maternally encoded information (1). Four maternal systems of genetic information are involved in setting up the pattern of the embryo. Patterning the anterior region requires input from both the terminal and anterior systems. The terminal system specifies the fate of the poles of the embryo by activating the uniformly distributed receptor tyrosine kinase Torso (Tor) at each end of the early embryo. Loss-of-function mutations in any of the genes implicated in the terminal system give rise to the loss of the anterior portion of the head and of all the structures posterior to and including the eighth abdominal segment (2–6).

The anterior region is organized by the *bicoid* (*bcd*) gene product, a homeodomain containing transcription factor (Bcd) (7, 8). *bcd* mRNA is synthesized during oogenesis and is transported into the egg, where it becomes localized at the anterior tip. This mRNA is translated after the egg is laid, and the protein diffuses away from the anterior tip in the syncytial environment of the early embryo, forming a concentration gradient (9). Bcd acts as a morphogen: it specifies distinct developmental fates by specifically activating zygotic target genes that respond to a series of Bcd concentration thresholds. In nuclei close to the anterior tip, high concentrations of Bcd activate head gap genes such as *orthodenticle* (10). At more posterior positions, lower concentrations of Bcd activate the thoracic gap gene *hunchback* (*hb*) (11–13). Finally, very low levels of Bcd are able to activate abdominal gap genes like *knirps* (14). In addition to its ability to transcriptionally activate a series of target genes, Bcd is also a repressor of posterior development by preventing translation of the ubiquitously distributed maternal *caudal* mRNA (15–17).

bcd mutant females produce embryos that lack all head and thoracic segments and some anterior abdominal segments and show duplication of the telson at the anterior end of the embryo (7). However, the remaining abdominal segments exhibit a normal anterior-posterior polarity that must be set up by other factors. Bcd is not the only morphogen active in the anterior region of the early fly embryo. A maternal gradient of the zinc-finger transcription factor Hb is also able to pattern the embryo (18–20). Therefore, not only does *hb* represent a zygotic gap gene, which is under the control of Bcd (11), but it also functions as a maternally provided morphogen. Translation of the homogeneously distributed maternal *hb* mRNA is blocked in the posterior of the embryo by *nanos* (21), thereby generating an anterior-posterior morphogenetic gradient of the Hb protein.

The two morphogens Bcd and Hb work together to organize proper anterior patterning of the fly embryo (22, 23). In the absence of both maternal and zygotic *hb* activities, *bcd* function is strongly affected. An artificial promoter that contains only strong Bcd binding sites is poorly activated in the anterior regions of the embryo. Addition of Hb sites dramatically increases the level of activation and enlarges the domain of reporter gene expression (23). Bcd and Hb synergize to fulfill their functions. The molecular mechanism underlying the synergy between Bcd and Hb has been elusive. Recently, biochemical studies of the interaction between Bcd, Hb, and the basal transcription factor complex TFIID led to a molecular model that could nicely explain how Bcd and Hb synergize in activating their target genes (24, 25).

Activation of transcription requires both the contribution of RNA polymerase II and the basal factors TFIIA, TFIIB, TFIIE, TFIIF, and TFIIH (reviewed in ref. 26). Moreover, the recruitment of the TFIID complex that contains the TATA-binding protein and the TATA-binding protein-associated factors (TAF_{II}s) is necessary for activated transcription. Most *Drosophila* TAF_{II}s have been cloned, among them $TAF_{II}60$ (27) and $TAF_{II}110$ (ref. 28; reviewed in ref. 29). It has been proposed that specific TAF_{II}s act as molecular bridges between specific activators and the general transcription machinery (30). However, different promoters might vary considerably in their requirement for specific TAF_{II}s for transcriptional activation (31–36).

Synergistic activation of transcription might result from two activators simultaneously contacting the TFIID complex

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Abbreviation: TAF_{II}, TATA-binding protein-associated factor.

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through different adapter molecules, thus stabilizing the binding of TFIID to the promoter and the association of the remaining basal transcription factors (reviewed in ref. 26). Sauer *et al.* (24, 25) showed that Bcd interacts with $TAF_{II}60$ and $TAF_{II}110$ through two distinct domains, whereas Hb interacts only with $TAF_{II}60$. They showed that transcriptional activation of a *bcd* target, the *hb* promoter, is synergistically enhanced *in vitro* by Bcd and Hb. However, this effect is observed only when both $TAF_{II}60$ and $TAF_{II}110$ are present. They suggested that the synergy observed *in vivo* is because of the corecruitment of $TAF_{II}110$ and $TAF_{II}60$ by Bcd and Hb, respectively.

To analyze the *in vivo* function of these interactions, we generated transgenic fly lines bearing deletion variants of *bcd* rescue constructs. Here, we show that the protein domains of Bcd that exhibit *in vitro* interaction with $TAF_{II}60$ and $TAF_{II}110$ are not essential *in vivo*. We also show that a *bcd* construct that lacks both domains is able to rescue *bcd* mutants to viability. Finally, even in this rescue situation, which should represent a highly sensitized system for detecting a function of TAF_{II}s, development of the embryo is not affected by mutations in $TAF_{II}110$ or $TAF_{II}60$. This suggests that the interactions observed *in vitro* between Bcd and $TAF_{II}60$ or $TAF_{II}110$ are not necessary for the function of *bcd* and its synergy with Hb *in vivo*.

MATERIALS AND METHODS

***bcd* Deletion Constructs.** To generate deletion constructs in the *bcd* coding region, we subcloned the 870-bp *Bsp*EI–*Nsi*I fragment from pKSgenomicBCD (37) into pSL1180 (Pharmacia), from which part of the polylinker had been removed by a *Hpa*I and *Sna*BI digest and religation. The fragment was then taken as a *Not*I–*Kpn*I fragment and cloned into pBluescriptKS (Stratagene), generating pKSbcdBsp-Nsi, which was used in the following cloning steps. pBcdΔQ is the result of a *Hin*cII and partial *Bst*UI digest and religation, thereby deleting amino acids 247–304. pBcdΔA and pBcdΔQA resulted from cloning the 550-bp *Nci*I (Klenow filled-in) to *Nsi*I fragment into a *Kas*I (Klenow filled-in)- and *Nsi*I- or *Hin*cII- and *Nsi*I-treated vector backbone, thereby deleting amino acids 331–344 or 247–344, respectively. pBcdΔAC and pBcdΔQAC were generated by a *Kas*I (Klenow filled-in) or *Hin*cII plus partial *Sca*I digest and religation, thereby deleting all amino acids C-terminal to amino acid 330 or 246, respectively. pBcdΔC and pBcdΔQC resulted from opening the vector pKSbcdBsp-Nsi with a *Bsp*EI and partial *Sca*I digest and cloning into it the *Bsp*EI to *Nci*I (Klenow filled-in) fragment from pKSbcdBsp-Nsi (320 bp) or pBcdΔQ (150 bp), thereby deleting all amino acids C-terminal to amino acid 344. The in-frame fusions were verified by sequencing. The *Bsp*EI–*Nsi*I fragments from pKSbcdBsp-Nsi and the deletion constructs were isolated and cloned back into pKSgenomicBCD, thereby replacing the wild-type BspEI–NsiI fragment. The complete genomic coding sequences were taken from these vectors and cloned as *Bam*HI fragments into the *Bgl*II site of pCaSpeRBcdBglII. These *bcd* rescue constructs were then used to generate different transgenic fly lines (37).

***In Vivo* Rescue.** Two BcdWT, two BcdΔA, four BcdΔQ, five BcdΔC, three BcdΔQA, three BcdΔQC, two BcdΔAC, and six BcdΔQAC transgenic lines on the first or second chromosome were crossed into the background to the amorphic bcd^{E1} allele, and rescue of the *bcd* phenotype was assessed by cuticular preparations (38). For each construct, the line with the strongest rescue ability was chosen for further analysis. The presence of the bcd^{E1} homozygous mutation in the mothers was assessed by the *ri* and *roe* markers that flank *bcd*. The nature of the transgene was verified by genomic PCR amplification by using oligonucleotides flanking the deleted regions in Bcd: 5′ oligonucleotide, GTCACATGCACATGCAGTAT; and 3′ oligonucleotide, ACTCCCAAATCTCATCGATC. The sizes of the obtained bands for BcdWT, BcdΔA, BcdΔQ, BcdΔC, BcdΔAC, BcdΔQC, BcdΔQA, and BcdΔQAC were 624, 578, 449, 353, 307, 178, 330, and 58 bp, respectively (data not shown).

Recombination of TAF_{II} Mutants onto *bcd* Mutant Chromosome. The $TAF_{II}60^{YY}$ dominant negative allele (39) was recombined onto the chromosome carrying the bcd^{E1} allele. We obtained the $TAF_{II}110^{\Delta C}$ dominant negative allele recombined on the bcd^{E1} chromosome from Gary Struhl (Columbia University, New York). The presence of the dominant alleles in the stocks was checked by using genomic PCR amplification, which confirmed the molecular nature of the two alleles used ($TAF_{II}110^{\Delta C}$ and $TAF_{II}60^{YY}$; G. Struhl, personal communication).

***In Situ* Hybridization.** Digoxigenin-labeled RNA probes for *in situ* hybridization were prepared as described (37). *In situ* hybridization on whole-mount embryos was performed as described originally (40), with an adaptation (M. Klingler, personal communication). Prehybridization and hybridization were performed at 70°C at pH 5. Embryos were mounted in methyl salicilate/Canada balsam (1:2) and photographed by using Nomarski optics.

RESULTS

***In Vivo* Function of the Putative Bcd Activation Domains.** Bcd contains three putative activation domains: a glutamine-rich (Q) domain, an alanine-rich (A) domain, and a C-terminal acidic region (C) (Fig. 1). The domains of interaction of Bcd with the TAF_{II}s have been mapped *in vitro*: $TAF_{II}110$ binds to the Q domain, whereas $TAF_{II}60$ binds to the A domain (25). The C-terminal region has been shown to be dispensable for *bcd* function *in vivo*, but it contributes to increase activation in yeast (41). Interestingly, the entire activation domain of Bcd can be functionally replaced by a generic activation domain (random acidic amphipathic helix B6) from *Escherichia coli* (37, 41–43).

Because the corecruitment of TAF_{II}s could provide a powerful explanation to the synergistic effect observed between Bcd and Hb during early development, we generated *bcd* rescue constructs encoding proteins lacking one or several domains of interaction with the TAF_{II}s (Fig. 1). These constructs contain DNA encoding the wild-type or deletion variants of the Bcd protein in the context of the genomic *bcd* locus. We examined their ability to rescue the bcd^{E1} mutant

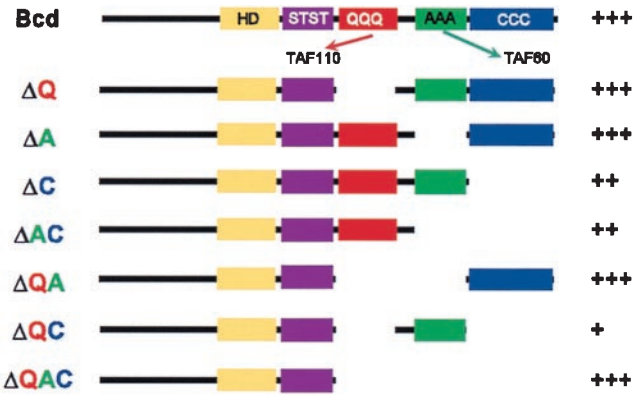

FIG. 1. Schematic representation of the Bcd deletion variants used in the *bcd* rescue experiment. For wild-type Bcd, the boxes represent identified domains in Bcd: HD, homeodomain; STST, S/T-rich region; QQQ, glutamine-rich domain that interacts with $TAF_{II}110$ *in vitro*; AAA, alanine-rich domain that interacts with $TAF_{II}60$ *in vitro*; CCC, C-terminal acidic region. Rescue of the bcd^{E1} phenotype by the different transgenes (assessed by cuticle preparations) is shown on the right. +++, rescue to viability with no cuticular defects; ++, thorax and most of the head structures are present; +, repression of the duplicated telson at the anterior end only.

phenotype. *bcd*^{E1} is a null allele that is truncated after the homeodomain and does not appear to produce a stable protein (12). Embryos from *bcd*^{E1} mothers lack all head and thorax segments and some abdominal segments. Instead, they have a second telson at the anterior end (Fig. 2*a*). A construct encoding a wild-type Bcd protein completely rescues the phenotype and behaves like the endogenous gene (Fig. 2*b*). Surprisingly, deletion of the A or Q (BcdΔA or BcdΔQ, respectively) domains or of both domains (BcdΔQA) does not abolish rescue activity (Fig. 2, *c*, *d*, and *f*). The BcdΔC transgene displays a hypomorphic *bcd* phenotype with only a very partial head but normal thorax and abdominal segments (Fig. 2*e*). The BcdΔAC construct shows an almost wild-type cuticular phenotype with only small head defects (Fig. 2*g*). The lines bearing the BcdΔQC deletion (Fig. 2*h*) exhibit very low rescue, with only the lack of a duplicated telson at the anterior. Although the lines lacking the C domain have a lower potential for rescue than the BcdΔQ, BcdΔA, and BcdΔQA lines, this is likely because of lower levels of expression rather than weaker activator proteins. Consistent with this interpretation, the domain of the *hb* staining is moved anteriorly in these lines (Fig. 3, *d*, *f*, and *g*). It has been argued that the position of the posterior border of *hb* indicates the amount of Bcd protein produced, whereas the intensity of staining within this domain reflects the activation potential of the Bcd deletion variant (41). In fact, two lines expressing a truncation construct lacking the C domain as well as both the Q and A domains (BcdΔQAC) have strong rescue activity: these lines exhibit rescue of the thorax and abdominal segments, loss of duplication of the telson at the anterior, and development of head structures (Fig. 2*i*). One of these two BcdΔQAC lines completely rescues the *bcd*^{E1} phenotype (Fig. 2*j*). Seventy-five percent of the embryos exhibit wild-type cuticles, and 30% of those are viable. This rescue is probably because of high-level expression of the transgene. Consistent with this, the posterior border of *bcd* target genes and the position of the cephalic furrow, both strong indicators of the slope of the Bcd protein gradient, are more posterior in this line than in wild-type embryos (Fig. 3*h* and data not shown). The ability of BcdΔQAC to activate transcription of target genes *in vivo* is consistent with Schneider cell cotransfection experiments in which the construct exhibits significant transcriptional activity (F.J., R. Sturny, F. Catala, and N. Dostatni, unpublished work). Because the phenotypic rescue of BcdΔQAC is not fully

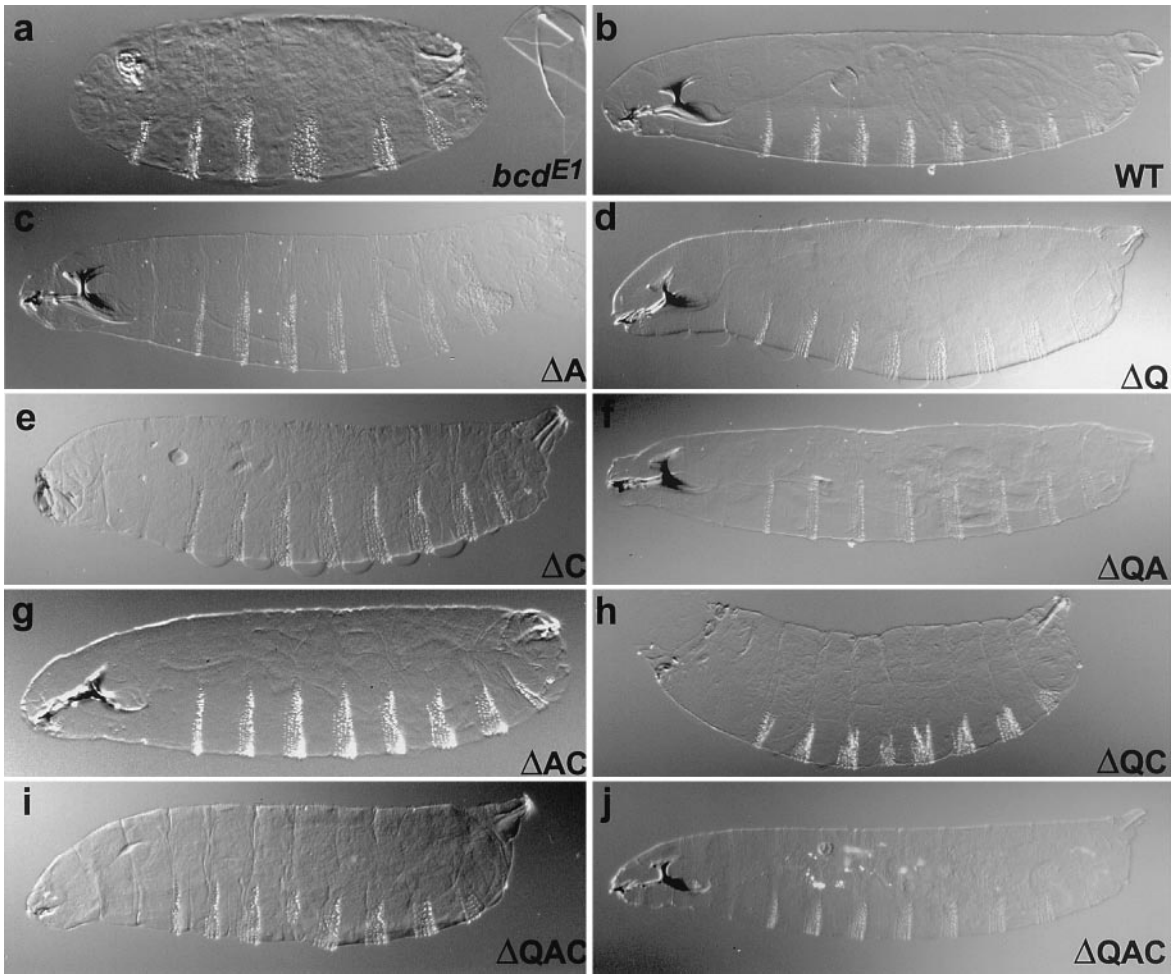

FIG. 2. Cuticle preparations of embryos from females homozygous for the strong *bcd*^{E1} allele. (*a*) Without rescuing construct. (*b*) Full rescue by two copies of a wild-type rescue construct. (*c–j*) Rescue by two copies of the Bcd deletion construct as indicated on each panel. Embryos from *bcd*^{E1} mothers lack head, thorax, and some abdominal segments. Instead, they have a second telson (anal plates, tuft, spiracles, and filzkörper) at the anterior end (*a*). A construct encoding a wild-type Bcd protein rescues completely the phenotype and behaves like the endogenous gene (*b*). Deletion of the A (*c*) or Q (*d*) domains or of both domains (*f*) does not abolish rescue activity. The BcdΔC (*e*) transgene displays only a very partial head but shows a normal thorax and abdominal segments. The BcdΔAC (*g*) construct shows an almost wild-type cuticular phenotype with only small head defects. The line bearing the BcdΔQC (*h*) deletion exhibits only the repression of the development of a telson at the anterior end. Two lines BcdΔQAC have strong rescue activity: these lines exhibit rescue of the thorax and abdominal segments, loss of duplication of the telson at the anterior, and development of head structures (*i*). One of these two BcdΔQAC lines completely rescues the *bcd*^{E1} phenotype (*j*). In all panels, anterior is to the left and dorsal is up.

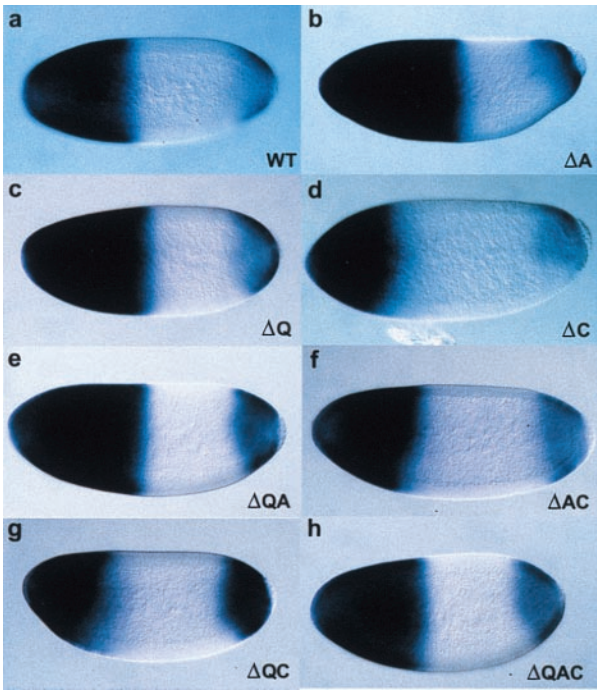

FIG. 3. *hb* RNA *in situ* hybridization of embryos from females homozygous for the strong bcd^{E1} allele and carrying: (*a*) two copies of a wild-type rescue construct; or (*b–h*) two copies of the Bcd deletion construct (representing the best rescuing lines), as indicated on each panel. *In situ* hybridization was performed with antisense probe for *hb* on whole-mount embryo preparations from the different transgenic lines containing Bcd deletion variants. The posterior boundary of the *hb* domain of expression is significantly moved anteriorly in embryos laid by females homozygous for the bcd^{E1} mutation and carrying two copies of the transgene: lines BcdΔC (*d*), BcdΔAC (*f*), and BcdΔQC (*g*). The BcdΔQ (*c*) and BcdΔQA (*e*) lines show normal amounts of *hb* expression within an enlarged domain. The BcdΔA line (*b*) exhibits a widely enlarged domain of *hb* transcription. The domain of expression mediated by the BcdΔQAC line that is able to rescue the bcd^{E1} phenotype to viability (*h*) appears slightly enlarged compared with wild-type Bcd.

penetrant, the Q, A, and C domains do provide some contribution to the activity of Bcd but are not essential.

Bcd-Dependent *hb* Activation. In a wild-type embryo (Fig. 3*a*), *hb* is transcribed in a broad anterior domain as well as in a more restricted posterior domain. Transcription of the anterior domain of *hb* is under the control of Bcd and, therefore, is lost in embryos from bcd^{E1} mutant mothers (13). Whenever Bcd activity is altered, *hb* expression is also changed (see above). The posterior border of the anterior *hb* expression domain is, like the cephalic furrow, positioned in response to the amount of Bcd, whereas the level of expression within this domain reflects the activation potential of the respective Bcd variant (41). In embryos laid by females homozygous for the bcd^{E1} mutation and carrying two copies of the transgene *BcdΔC*, *BcdΔAC*, or *BcdΔQC*, the posterior boundary of *hb* expression is significantly moved toward the anterior (Fig. 3, *d*, *f*, and *g*). This is consistent with the fact that these lines only show partial rescue of the bcd^{E1} phenotype, which appears to be because of a lower level of expression of the transgenes. However, within the *hb* domain, *hb* is transcribed at normal levels, which indicates normal activity of these Bcd deletions. The BcdΔQ (Fig. 3*c*) and BcdΔQA (Fig. 3*e*) lines that fully rescue the bcd^{E1} phenotype show normal amounts of *hb* expression within a slightly enlarged domain. One of the BcdΔA lines (Fig. 3*b*) exhibits a widely enlarged domain of *hb* transcription, likely because of the very high level of transgene expression observed by immunostaining (data not shown). Another BcdΔA line shows a normal rescue pattern and exhibits a normal position of the cephalic furrow (data not shown). Finally, the domain of expression mediated by the BcdΔQAC (Fig. 3*h*) line that is able to rescue the bcd^{E1} phenotype to viability appears slightly enlarged compared to wild-type Bcd, indicating a high level of transgene expression. Thus, Bcd is able to activate its target gene *hb*, even in the absence of the protein domains that have been shown to interact *in vitro* with $TAF_{II}60$ and $TAF_{II}110$.

Requirement of TAF_{II} Function for Bcd Activity. $TAF_{II}110$ and $TAF_{II}60$ have been shown to mediate transcriptional activation *in vitro* (24), and mutations in the respective genes have been isolated in a dominant modifier screen in *Drosophila* (39). Mutations in $TAF_{II}60$ and $TAF_{II}110$ are homozygous embryonic lethal and cell lethal during eye development and oogenesis. However, viable dominant negative alleles of these two genes have allowed Sauer *et al.* (39) to investigate their effects on transcription. These mutations do not appear to cause a general reduction of transcription, but instead, they affect the expression of only a subset of genes (33, 39). The $TAF_{II}60^{YY}$ allele encodes a protein that contains two tyrosines residues inserted at amino acid 207. The $TAF_{II}110^{\Delta C}$ allele lacks the C-terminal 126 amino acids of the protein. Although $TAF_{II}60$ and $TAF_{II}110$ have been shown to bind to both Bcd and $TAF_{II}250$, the mutant proteins are defective for their interaction with $TAF_{II}250$ but still retain their *in vitro* interaction with Bcd. Because these mutant proteins can still interact with the transcription activators but cannot mediate interaction with the transcription machinery, they can be considered dominant negative alleles.

Although the BcdΔQAC truncation construct lacks the identified $TAF_{II}60$ and $TAF_{II}110$ interaction domains (25), it is still able to strongly activate the *bcd* target genes. It is possible that the BcdΔQAC truncation does not affect Bcd function because it can still interact with Hb, its partner involved in synergistic activation. Hb might still recruit $TAF_{II}60$ to the promoter, even if Bcd cannot do it directly. If this is the case, the genetic rescue of bcd^{E1} by BcdΔQAC should be highly dependent on the interaction between Hb and $TAF_{II}60$. To test this possibility, we examined the ability of BcdΔQAC to rescue a *bcd* mutant phenotype in the presence of either the dominant negative $TAF_{II}60^{YY}$, which is expected to interfere with activation by BcdΔQAC, or $TAF_{II}110^{\Delta C}$, which should not interfere with the rescue. Typically, a single copy of the BcdΔQAC transgene is able to almost completely rescue the progeny of females homozygous mutant for bcd^{E1}(Fig. 4*a*). Neither of the $TAF_{II}60^{YY}$ nor $TAF_{II}110^{\Delta C}$ dominant negative mutations affect the rescue ability of the BcdΔQAC construct: the bcd^{E1} phenotype in the progeny of double mutant females $TAF_{II}60^{YY}$, bcd^{E1}/bcd^{E1} or $TAF_{II}110^{\Delta C}$, bcd^{E1}/bcd^{E1} is rescued by the BcdΔQAC as well as that of the single bcd^{E1}/bcd^{E1} mutant (Fig. 4, *b* and *c*). Rare rescue to wild type can be observed at the same frequency, independently of the presence (Fig. 4*d*) or absence of dominant negative TAF_{II} mutations (data not shown).

Because the mutations identified in the genes encoding $TAF_{II}110$ or $TAF_{II}60$ have no direct or specific effect on Bcd activity, even in a highly sensitized situation, this suggests that the synergy between Bcd and Hb is not based on interactions with TAF_{II}s and must, therefore, be mediated by another, still elusive mechanism.

DISCUSSION

Are the TAF_{II}s Dispensable for *in Vivo* Transcriptional Activation by Bcd? The mechanisms of transcriptional activation in eukaryotes have been studied extensively, and biochemical studies have established that *in vitro* mRNA synthesis by RNA polymerase II requires the regulated assembly of multiple protein complexes at promoters (45). Recent work points to the basal transcription factor complex TFIID as a key

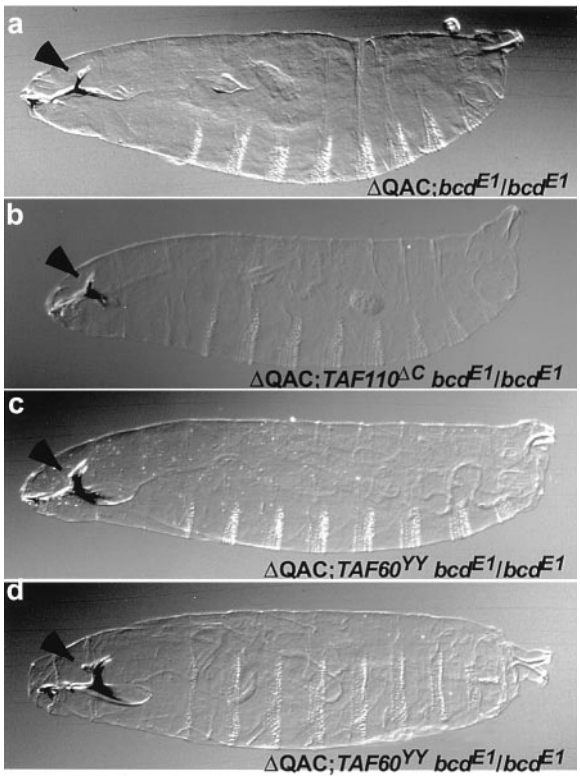

FIG. 4. Cuticle preparations of embryos from females homozygous for the strong bcd^{E1} allele and rescued by one copy of the BcdΔQAC construct (best rescuing line). Arrowhead, dorsal bridge. (*a*) Wild type for TAF_{II}s. (*b*) In the presence of dominant negative $TAF_{II}110^{\Delta C}$. (*c* and *d*) In the presence of dominant negative $TAF_{II}60^{YY}$. (*a*) A single copy of the BcdΔQAC transgene is able to almost completely rescue the amorphic mutant bcd^{E1}, with only the dorsal bridge reduced. In *b* and *c*, the same rescue is obtained as in *a*, whereas in *d*, a rare rescue to wild type is shown.

component in the response to transcription regulators (30, 46, 47). TFIID consists of the TATA binding protein and ~10 other TATA binding protein-associated factors (TAF_{II}s). In the yeast *Saccharomyces cerevisiae*, the TFIID complex shares functional and structural similarities with higher eukaryotic TFIID. In particular, most yeast TAF_{II}s are homologous to higher eukaryotic TAF_{II}s (29, 48–50). Earlier reports have indicated that several TAF_{II}s are dispensable in yeast for *in vivo* transcriptional activation, despite being essential for growth (51, 52). More recently, it has been shown that a mutation in $TAF_{II}250$ causes gene specific transcription defects (53). $TAF_{II}250$ is in direct contact with TATA binding protein and is thought to function as an important scaffold for the TFIID complex by interacting with other TAF_{II}s such as $TAF_{II}110$ and $TAF_{II}60$ (54). However, there is extensive controversy as to the role TAF_{II}s play *in vivo* (31–36).

Because TAF_{II}s are supplied maternally and because homozygous TAF_{II} mutants are cell lethal, it is, so far, impossible to create an early embryo that completely lacks TAF_{II} function. Therefore, to test the *in vivo* relevance of the interaction between the Bcd activation domains and $TAF_{II}60$ and $TAF_{II}110$, we used alleles of TAF_{II}s that are considered dominant-negative because they can still interact with Bcd but not with $TAF_{II}250$ (39). These mutations have been identified as suppressors of the rough eye phenotype induced by misexpression of an activated form of Ras1. This suppressor effect is likely because of the reduction of the *sevenless* promoter-mediated Ras1 expression by the TAF_{II}s mutations (39). Embryos derived from heterozygous mothers are loaded with both maternal wild type, and with the dominant negative mutant TAF_{II}s, we could show that the domains of interaction with the TAF_{II}s are dispensable for Bcd function *in vivo*, which suggests that Bcd does not use a specific interaction with $TAF_{II}60$ or $TAF_{II}110$ to activate its targets. Alternatively, it is possible that BcdΔQAC still contains cryptic TAF_{II} interaction domains that have not been identified in *in vitro* experiments. However, if this was the case, BcdΔQAC should only weakly interact with TAF_{II}s and should, therefore, critically depend on these TAF_{II}s to function. However, even in the highly sensitized situation of a rescue by BcdΔQAC, a dose of neither wild-type $TAF_{II}60$ nor wild-type $TAF_{II}110$ is required. This shows that the interaction domains identified *in vitro* are not absolutely necessary for Bcd function *in vivo*, and that the requirement for TAF_{II}s observed *in vitro* is not observed *in vivo*. The interaction with TAF_{II}s might have accessory character only and contribute to transcriptional activation in a quantitative way, without being crucially important for its biological function.

Comparison Between the Bcd Truncation Constructs and *bcd* Alleles. The extensive truncation of the BcdΔQAC protein shows that the putative activation domains are dispensable for proper development. Several *bcd* alleles have been characterized molecularly and have been placed into categories according to the extent of anterior pattern defects that they produce. Embryos from strong mutant alleles (bcd^{E1}, bcd^{E2}, and $bcd^{33\text{-}5}$) show a complete lack head and thorax (9, 12), deletions, and fusions of anterior abdominal segments as well as a duplicated telson at the anterior. Generally, these mutations truncate the Bcd protein right after the homeodomain, they are more extensive than BcdΔQAC, and they might not produce a stable protein. A less extensive C-terminal deletion of Bcd is encoded by the bcd^{E5} allele, which belongs to the weakest class of alleles (with bcd^{111} and $bcd^{2\text{-}13}$) (7). bcd^{E5} truncates the protein at amino acid 264 (12), which corresponds approximately to the BcdΔQAC deletion construct (truncation at amino acid 246). These weak alleles show only slight pattern defects in the anterior head region, lacking the labral derivatives (labrum and dorsal bridge), comparable to the defects produced by the weaker of the BcdΔQAC lines. It is likely that a slight increase of the amount of the bcd^{E5} protein would produce a near normal embryo.

Is the Tight Localization of the *bcd* mRNA Involved in the BcdΔQAC Rescue? The ability of BcdΔQAC to fully rescue the bcd^{E1} phenotype is surprising with respect to previous reports by using injection of mRNA encoding truncation constructs of *bcd* (41). mRNA injections of a truncated construct similar to BcdΔQAC showed a low rescue potential, even at high concentrations: the construct only suppressed the formation of posterior structures at the anterior. It induced with high frequency thoracic structures and, more rarely, structures of the gnathal region of the head (38). The difference between the results obtained by mRNA injection or by using transgenic flies could be because of the lack of anterior localization of the injected mRNA. To carry out its morphogenetic function, a gradient of Bcd activity is established by the tight localization of the *bcd* mRNA to the anterior pole of the egg. This localization depends on the maternal genes *exuperentia* (*exu*), *swallow* (*swa*), and *staufen* (*stau*). Embryos from females homozygous mutant for *exu*, *swa*, or *stau* (2, 55) show head defects similar to those of embryos from females mutant for weak *bcd* alleles. The labrum is absent and the pharyngeal head skeleton is reduced. Strikingly, the *swa*, *exu*, and *stau* mutants, the weak *bcd* alleles, and the embryos rescued by injection of the *bcd*(1–264) mRNA, are all reminiscent of the *torso* phenotype: lack of dorsal bridge and labrum. This suggests that that Tor has a enhancing effect on Bcd activity, as has been reported for *bcd* target gene expression (56–58). The tight localization of the *bcd* mRNA might, thus, be required for Tor to enhance the function of the Bcd protein before it migrates.

Tor-Dependent Phosphorylation of Bcd. It has been shown that Bcd is phosphorylated in response to Tor activity (44). The

strong remaining activity in the BcdΔQAC protein that presents such an extensive deletion might be because of the presence of the S/T rich region located between the homeodomain and the Q, A, and C activation domains (Fig. 1). The S/T-rich sequence is the target of phosphorylations induced by the activity of the terminal Tor pathway: in embryos lacking Tor activity, Bcd phosphorylation is greatly diminished (44). Activation of the Tor signal transduction pathway leads to activation of a mitogen-activated protein kinase, a nuclear kinase that has been implicated in the phosphorylation of transcription factors. Most mitogen-activated protein phosphorylation sites of Bcd have been mapped *in vitro* to the S/T rich region. Site-directed mutagenesis indicates that most of the Bcd phosphorylations occur on these sites *in vivo* (F.J., R. Sturny, F. Catala, and N. Dostatni, unpublished work). The ability of the Tor pathway to create negative charges in this region might allow the generation of an acidic activation domain that compensates for the loss of the other Q, A, and C activation domains.

It has been shown that the Bcd phosphorylations mediate part of the Tor enhancing effect on Bcd activity (F.J., R. Sturny, F. Catala, and N. Dostatni, unpublished work). Hence, the rescue by BcdΔQAC could be explained by the fact that the S/T rich region is phosphorylated *in vivo* by the Tor cascade, thus creating an acidic activation domain on Bcd before it forms its concentration gradient. As long as the homeodomain and the S/T rich region are intact, good rescue is achieved, especially if the transgene is expressed at high levels and mRNA translation occurs in the region of Tor activity.

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