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Pattern formation in the limbs of *Drosophila*: *bric à brac* is expressed in both a gradient and a wave-like pattern and is required for specification and proper segmentation of the tarsus

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SUMMARY

We have identified the gene *bric à brac* and show that it is required for pattern formation along the proximal-distal axis of the leg and antenna of *Drosophila*. In *bric à brac* mutant legs, the bristle pattern of the three central tarsal segments is transformed towards the pattern of the most proximal tarsal segment. In addition, *bric à brac* mutant legs and antennae have segmentation defects. *bric à brac* encodes a nuclear protein that shares a highly conserved domain with two transcription factors from *Drosophila*. *bric à brac* function is dosage dependent and is required in a graded manner for the specification of tarsal segments. The graded requirement for *bric à brac* correlates with its graded expression pattern, suggesting that the concentration of BRIC À BRAC protein specifies segment identity in the tarsus.

Key words: *Drosophila*, *bric à brac*, limb development, imaginal discs, homeotic transformation, BTB domain

INTRODUCTION

Drosophila melanogaster has been used extensively as a model system for the study of development and pattern formation. Both the anterior-posterior and dorsal-ventral axis of the embryo are established by the formation of concentration gradients of transcription factors which form by protein diffusion in the syncytial blastoderm of the embryo (for review see StJohnston and Nüsslein-Volhard 1992). In contrast, pattern formation in many other developmental systems occurs in a growing field that is cellularized. The mechanisms by which postional values along an axis are defined in a growing tissue is not well understood. One developmental system in which this problem can be examined is the proximal-distal axis of the limbs of *Drosophila*.

The legs of insects are composed of segmental units, which are, from proximal to distal, the coxa, trochanter, femur, tibia and tarsus. The tarsus of flies is further subdivided into five tarsal segments. The limbs of *Drosophila* develop from imaginal discs, small epithelial cell groups that invaginate from the epidermis of the embryo (Cohen, 1990; Bate and Martinez-Arias, 1991; Cohen et al., 1991), grow during the larval stages and give rise to the adult body structures during metamorphosis. In imaginal discs, the anlagen of the adult leg structures are concentrically arranged, with the anlage for the tarsus in the center and the anlagen for the more proximal structures toward the periphery (Schubiger, 1968). Morphologically distinct primordia of limb segments appear during the third larval instar, when the monolayered epithelium of the disc, while growing (for review see Bryant, 1978), forms concentric folds that represent the primordia of the segments, with the folds of the tarsal segments appearing last (Fristrom and Fristrom, 1975). Only 20-45 cells are initially incorporated into a leg imaginal disc (Madhavan and Schneiderman, 1977), a number that is too small to have already defined all the pattern elements of the adult leg. It has been suggested, therefore, that the early limb disc is provided with only a few positional cues that become more elaborated during subsequent growth of the disc (Schubiger, 1974; for review see Bryant, 1978; Cohen and Jürgens, 1989a).

A number of genes have been identified in *Drosophila* that are involved in pattern formation of the proximal-distal axis of the limb. These genes can be divided into two classes. The first class is characterized by an expression pattern in the form of a wedge-like sector or a longitudinal stripe in the leg disc, and by the fact that mutations in these genes cause loss of distal structures of the limb (for review see Bryant, 1993). Genes that belong to this class include the TGF-β homolog *decapentaplegic* (Spencer et al., 1982; Bryant, 1988; for review see Gelbart, 1989) and the segment-polarity genes *hedgehog* (Mohler, 1988) and *wingless* (*wg*; Baker, 1988a; Couso et al., 1993). *wg* has been shown to be required for dorsal-ventral organization in imaginal discs by expression studies and clonal analysis (Couso et al., 1993) and has been suggested to define a concentration-dependent ventralizing signal based on experi-

ments of ectopic *wg* expression in the leg disc (Struhl and Basler, 1993). The analysis of this class of genes suggests that the proximal-distal axis depends on the formation of positional values that are oriented circumferentially around this axis (anterior-posterior and dorsal-ventral values). A second class of genes, which are expressed in circumferential stripes (rings) in the leg imaginal disc, appears to be required specifically for formation of positional values along the proximal-distal axis (for review see Bryant, 1993). One member of this class is *Distal-less* (*Dll*, also called *Brista;* Sunkel and Whittle, 1987; Cohen and Jürgens, 1989a), a homeobox gene (Cohen et al., 1989) that is required for the development of all leg segments except the most proximal one (Cohen and Jürgens, 1989b). The requirement for *Dll* is graded within the developing leg, with the tarsus being the structure most sensitive to a loss of *Dll* activity. A global pattern-organizing function has been suggested for *Dll* along the proximal-distal axis (Cohen and Jürgens, 1989a; Cohen et al., 1989).

The gene *bric à brac* (*bab*), which we describe here, belongs to the second class of genes and is specifically involved in the development of the tarsus. Mutations in *bab* cause homeotic transformation of the bristle pattern of tarsal segments as well as defects in or loss of intersegmental joints in the tarsus. The distribution of the *bab* gene product develops from a rather uniform pattern into a wave-like pattern with the peaks of expression level corresponding to the segments and the valleys corresponding to the intersegmental joints during the last larval stage, a period in which the tarsal primordium more than doubles its size. The peaks of expression level form a gradient from distal to proximal in the tarsal primordium. This gradient will be discussed in relation to the graded requirement for *bab* function in tarsal segment specification along the proximal-distal axis.

MATERIALS AND METHODS

Drosophila stocks

The mutant *bab* alleles bab^{E1}, bab^{P}, bab^{PRDS}, bab^{PR11}, bab^{PR23}, bab^{PR24}, bab^{PR72} and the deficiency $Df(3L)bab^{PG}$ were isolated during this study. The mutant chromosomes carried recessive genetic markers and were maintained as *TM3, Sb* ry^{rRK} or *TM6B, Tb e ca* balanced stocks. Balancer chromosomes and genetic markers are described in Lindsley and Zimm (1992). Enhancer trap line P[*lacZ,* ry^{+}]A30 is described in O'Kane and Gehring (1987), and P[lArB]A128.1F3 in Bellen et al. (1989). The translocation *T(3;Y)A114* was isolated by Lindsley et al. (1972). Oregon R was used as a wild-type stock.

Mutagenesis

(1) P-element mediated mutagenesis. The enhancer trap line bab^{P} was isolated as a recessive female semisterile mutation in a P-element mediated mutagenesis, using P[*lacZ,* ry^{+}] as an enhancer detector (O'Kane and Gehring, 1987) and P[ry^{+}(Δ2-3)]99B as a transposase source (Laski et al., 1986; Robertson et al., 1988) in a ry^{506} background.

(2) P-element mediated reversion of bab^{P}. To ascertain that the mutant phenotype of bab^{P} is caused by the P[*lacZ,* ry^{+}] insertion, the P-element was remobilized by transposase. Males of the genotype bab^{P} *ry* e^{s} *ca/ Ki* p^{p} P[ry^{+}(Δ2-3)]99B were crossed individually to ry^{506} females. Progeny were screened for reversion of the ry^{+} phenotype. About 200 independent male revertants were tested for complementation of the bab^{P} mutant ovary phenotype. 31 revertant lines failed to complement bab^{P}, among which, 24 showed a stronger mutant phenotype than bab^{P}. The new *bab* alleles are designated as bab^{PR}. Some of the remaining lines that complemented bab^{P} were analyzed and found to be phenotypically wild type; demonstrating that the *bab* mutant phenotype is caused by the P-element insertion.

(3) $Df(3L)bab^{PG}$ was isolated in a gamma-ray mutagenesis. 0-48 hours old homozygous bab^{P} *ry* e^{s} *ca* males were irradiated by 4000 rad gamma-rays and immediately crossed to ry^{506} females. F_1 males were screened for reversion of the ry^{+} marker. Revertant chromosomes were checked for complementation against bab^{P}. From 7000 irradiated chromosomes, one deletion, $Df(3L)bab^{PG}$ [61D3-E1;61F5-8], was isolated.

(4) The bab^{E1} mutation was isolated as an EMS allele based on its dominant leg phenotype. The EMS mutagenesis was performed according to Lewis and Bacher (1968). *ri e* males were mutagenized and crossed to wild-type females. The progeny were raised at the non-permissive temperature (30°C) for the dominant phenotype. F_1 males were screened for ectopic sex combs on the prothoracic legs.

Temperature-shift experiments

The temperature-sensitive dominant phenotype of the bab^{PRDS} allele was used for temperature-shift experiments. Wild-type females were crossed to homozygous bab^{PRDS} males and were allowed to lay eggs in vials at 25°C for 4 hours. The eggs were kept at 25°C for 24 hours and the 1st instar larvae transferred to either 18°C or 30°C (±0.5°C). A temperature shift was performed at different developmental stages (Fig. 2), and the flies were kept at the shifted temperature until they hatched. The heterozygous male progeny were screened for ectopic sex combs on the prothoracic legs under a dissecting microscope at 20-32× magnification. Both legs were screened because the phenotypic strength of two legs of a fly differ in the same manner as do legs from different flies at a given temperature. The penetrance of ectopic sex combs as well as the number of sex comb bristles per ectopic sex comb were counted.

Analysis of the leg bristle pattern

Wild-type and mutant flies were raised at 25°C. Large numbers of flies per genotype were screened for ectopic sex combs and fusion of segments under a dissecting microscope. For a detailed analysis of the bristle pattern of legs and antennae were mounted in Hoyer's medium (Van der Meer, 1977). The cuticle preparations of legs and antennae of 15-20 males and females, respectively, per genotype were viewed with a Zeiss Axiophot microscope.

Collection of larvae and pupae

Eggs were collected on apple juice agar plates (Wieschaus and Nüsslein-Volhard, 1986) in 2 hours intervals, and the hatched larvae were fed yeast while growing on the plates at 25°C. Staging of larvae and prepupae was based on the descriptions of Ashburner (1989).

Molecular analysis

The P-*lacZ* insertion of the bab^{P} allele was used as a tag for the cloning of sequences adjacent to the insert by the inverse PCR method (Ochman et al., 1990). The inverse PCR product was sequenced to determine the insertion site of the P-*lacZ* construct and was used as a probe to screen a lambda genomic library (Tamkun et al., 1992) using standard methods (Sambrook et al., 1989). The genomic 4.2 kb *Eco*RI restriction enzyme fragment E199, into which the P-*lacZ* element inserted, was used to screen a number of cDNA libraries. The cDNA clones were characterized by restriction enzyme mapping and DNA sequencing (Sanger et al., 1977). The DNA sequence was analyzed using DNA Strider

(written by Christian Marck, Department de Biologie, Institut de Recherche, Commissariat à L'Energie Atomique, France) and the University of Wisconsin software package (Devereux et al., 1984). The 1.7 kb open reading frame was compared with sequences in the GenBank and EMBL data bases using the FASTA program (Pearson and Lipman, 1988).

In situ hybridization

A biotin-labeled DNA probe, using a genomic DNA fragment adjacent to the bab^P insertion site, was used for chromosome in situ hybridization (Ashburner, 1989). In situ hybridizations to imaginal discs were performed with the 4.2 kb genomic *Eco*RI fragment E199 and the 1.2 kb subclone 504 (Fig. 6A). The procedure followed a modified version of the protocol of Tautz and Pfeifle (1989) using the Genius Kit of Boehringer-Mannheim. Dissection and fixation were done as described for antibody staining and Proteinase K treatment for 5 minutes. Hybridization with digoxigenin-labelled DNA probe (about 50 ng DNA/100 µl hybridization solution) was performed at 50°C for 16 hours and followed by washing in hybridization solution, 1:1 mixture of hybridization solution and PBT, and PBT at 50°C each for 1 hour. The hybridized imaginal discs were dehydrated and mounted as described for antibody-stained discs.

Production of polyclonal anti-BAB antibodies

The *Bam*HI fragment from the subclone 504 (Fig. 6A) that encodes a 24×10^3 M_r protein was subcloned into the pGEX-1N (Amrad) expression vector. Expression of the glutathione S-transferase fusion protein and purification of the soluble 50×10^3 M_r fusion protein were performed according to Smith and Johnson (1988). The fusion protein (1 mg/ml 50 mM Tris-HCl pH 7.5) was sent to Pocono Rabbit Farm and Laboratories (PO Box 240, Dutch Hill Road, Canadensis PA 18325) for immunization of rats. The sera were provided with 0.01% Timerosal and tested by tissue antibody staining.

Antibody staining

Imaginal discs were dissected from 3rd instar larvae and prepupae in PBS and kept on ice until fixation in 5% formaldehyde (Polysciences), 50 mM EGTA in PBS for 20 minutes. The tissue was incubated in methanol for 3-5 minutes and washed in PBT (PBS, O.1% Tween-20) for 30 minutes, followed by a 30 minutes incubation in PBTBS (PBT, 0.1% BSA (Sigma, globulin-free), 2% goat serum (Gibco)). Incubation with the first antibody, anti-β-gal (Cappel, polyclonal IgG) in a 1:3000 dilution in PBTBS or the polyclonal rat anti-BAB r2 antibody in a 1:5000 dilution in PBTBS, which was preabsorbed on fixed embryos for 1-2 hours, was done at 4°C overnight. The discs were washed in PBT for 4×15 minutes, incubated in PBTBS for 1 hour and subsequently in the secondary biotinylated antibody, anti-rabbit (Vector Laboratories) or anti-rat (Jackson Laboratories) in a 1:500 dilution for 2 hours at room temperature. After washing in PBT for 4×15 minutes the discs were treated with biotinylated horse radish peroxidase (HRP)-avidin complex (Vector Laboratories, ABC Kit) in a 1:50 dilution in PBT for 1 hour and washed again 3×15 minutes in PBT. HRP reaction was performed in staining solution containing 0.2 mg DAB (Sigma) and 0.01% H_2O_2/ ml PBT. The discs were dehydrated and mounted in a 1:4 mixture of Canada balsam (Sigma) and methyl salicylate (Sigma) for whole-mount views or were embedded in an Epon/Araldite mixture (Polysciences, Ted Pella) for histological sections. A complete series of semi-thin sections (5 µm) of 8-10 imaginal discs were prepared for each chosen developmental larval or prepupal stage.

Measurement of antibody staining in sections

Sections of anti-β-gal-stained bab^{A128}/+ imaginal discs were viewed using bright-field illumination on a Zeiss Axiophot microscope equipped with a image processing system (Hamamatsu Argus-10). The intensity of staining was linearly processed into different colors. For each cell, a value betweeen 0 and 10 was defined by reading the color directly from the monitor. 1-2 longitudinal sections from 10 different leg imaginal discs were examined.

RESULTS

Genetic characterization of *bab*

The first *bab* allele, bab^P, was isolated from an enhancer trap screen as a female-semisterile mutation. The French name *bric à brac* refers to the disorganized structure of the *bab* mutant ovaries. Twenty-fouradditional *bab* alleles that have a stronger female-sterile phenotype were isolated by remobilization of the P-element insert in the bab^P line. Examination of these alleles showed that they also cause defects in the leg and the antenna of the fly. An additional mutation, the EMS-induced bab^{E1} mutation was identified as a *bab* allele on the basis of its mutant phenotype and because it fails to complement other *bab* alleles. A deficiency, $Df(3L)bab^{PG}$ (61D3-E1; 61F5-8), was isolated that uncovers the *bab* locus which was mapped to the cytological position 61F1-2 (Fig. 6C). Two more P-element insertions, P[*lacZ*, ry^+]A30 and P[IArB]A128.1F3 (the latter designated as bab^{A128}) map to the same chromosomal position (O'Kane & Gehring, 1987; Bellen et al., 1989) and express the *lacZ* reporter gene in a pattern similar to bab^P but do not cause a mutant phenotype in homozygous flies. The isolated *bab* mutations were classified as having either a strong, intermediate or weak mutant leg phenotype according to the severity of the leg defects. The homozygous mutant phenotype of strong *bab* alleles is slightly weaker than the transheterozygous phenotype of these alleles over $Df(3L)bab^{PG}$ suggesting that these *bab* alleles are strongly hypomorphic. The *bab* alleles that were chosen for phenotypic analysis are listed in Table 1.

Table 1. Loss-of-function *bab* alleles and *bab* mutant leg phenotype

Allele	Mutagen	Cytology	Recessive phenotype	Dominant phenotype
bab^P	P-element	P-insert at 61F1/2	weak†	none
bab^{PRDS}	P-reversion		strong	yes (79%)‡
bab^{PR10}	P-reversion		strong	yes (66%)‡
bab^{PR24}	P-reversion		strong	yes (67%)‡
bab^{PR72}	P-reversion		strong	yes (82%)‡
bab^{PR11}	P-reversion		weak	yes (4%)‡
bab^{PR30}	P-reversion		weak	yes
bab^{PR23}	P-reversion		intermediate	yes (43%)†
bab^{E1}	EMS	normal	intermediate	yes
$Df(3L)bab^{PG}$	g-ray	61D3-E1; 61F5-8	lethal	yes
*T(3;Y)A114**	X-ray	61A;61F	lethal	yes

*Lindsley et al., 1972

†A mutant phenotype is only seen in flies raised at 29°C.

‡The prothoracic legs of 50 male flies heterozygous for the respective *bab* allele over a wild-type chromosome were examined. Flies were grown at 25°C.

bab mutations cause homeotic transformation of the bristle pattern of tarsal segments

Homozygotes and transheterozygotes of strong *bab* mutations show a change of the bristle pattern of tarsal segments (TS) TS2, TS3 and TS4 towards the most proximal tarsal segment, TS1. TS1, also called the basitarsus, can be distinguished from the other tarsal segments by specific bristle markers. The most prominent marker of TS1 is the sex comb on the prothoracic (front) legs of males. The sex comb is a longitudinal row of about eleven blunt, black sex comb bristles (SCB), which is located in the distal region of TS1 (Hannah-Alava, 1958; Fig. 1A,C). In addition to the normal sex comb on TS1, males homozygous for a strong *bab* mutation exhibit ectopic sex combs on TS2, TS3 and occasionally TS4 of the prothoracic legs (Fig. 1B,E). The ectopic sex combs were defined as such based on the color, shape, orientation and position of the bristles. The ectopic sex comb on TS2 usually contains 5-6 SCB, half as many as is present in the normal sex comb on TS1. The ectopic sex comb on TS3 is even smaller and usually contains 1-2 SCB, the one on TS4 not more than one SCB (Fig. 1B,E). The appearance of ectopic sex combs in distal tarsal segments indicates a homeotic transformation of the bristle pattern of TS2, TS3 and TS4 towards TS1.

This conclusion is supported by the observation that the homeotic transformation is not limited to the SCB but includes other characteristics of TS1, and that the transformation is neither sex nor leg specific. In wild-type flies, the ventral bristles of the prothoracic TS1 are arranged in tightly packed transverse rows (Hannah-Alava, 1958; Fig. 1G). In contrast, the distal tarsal segments of the prothoracic legs have separated longitudinal columns of single bristles (Fig. 1G). In strong *bab* mutant flies, both male and female, the normal bristles on the ventral side of TS2 and TS3 are replaced by a larger number of bristles that are arranged in such transverse rows (Fig. 1H). The analysis of the bristle pattern of TS4 was difficult because of its small size and because it is fused to TS5 in strong *bab* mutants; however, the bristle pattern of TS4 is clearly affected by *bab* mutations as indicated by the occasional appearance of a SCB. In contrast, no alterations in the bristle pattern could be detected in the size-reduced TS5, which suggests that the identity of TS5 is not affected. Similar to the prothoracic legs, in metathoracic legs (third leg pair) the bristle pattern of TS1 is repeated in the distal tarsal segments TS2-TS4 (data not shown). The bristle pattern in the mesothoracic tarsal segments is indistinguishable and therefore not accessible to an analysis.

The size of the basitarsus in wild-type and *bab* mutant legs is much larger than that of the other tarsal segments (Fig. 1A). Although the bristle pattern is changed in TS2-TS4 of *bab* mutant flies, the size of these segments is not enlarged to the size of TS1, suggesting that the specification of the tarsal segments has changed upon a segment primordia of normal size. The analysis of the phenotype of strong loss-of-function *bab* alleles demonstrates that the bristle pattern of the distal tarsal segments TS2, TS3 and TS4 is transformed towards the bristle pattern of the basitarsus, indicating that the *bab* gene is required for the specification of the three central tarsal segments.

Fig. 1. Phenotypic traits in the *bab* mutant leg and antenna. Panels A-E and G-H show homeotic transformation of the bristle pattern of TS2, TS3 and TS4 towards TS1. (A) Whole leg and (C) tarsus of a wild-type fly. The sex comb (arrow) is a distinct marker of TS1 in the male prothoracic legs. The claw organ marks the distal tip of the leg. Tarsal segment size decreases from proximal to distal. (B) Whole leg and (E) tarsus of a $bab^{PR24}/Df(3L)bab^{PG}$ mutant male that expresses a strong *bab* mutant phenotype. The leg displays a normal sex comb on TS1 (arrow) and ectopic sex combs on TS2, TS3 and TS4 (arrowheads). The number of SCB decreases form proximal to distal. The joint between TS4 and TS5 is missing, resulting in fusion of these segments. (D) Tarsus of a *bab* hemizygote ($Df(3L)bab^{PG}/+$) that exhibits a dominant haploinsufficient phenotype. Note the ectopic sex comb on TS2 (arrowhead) of a male that was grown at 30°C. (G) Wild-type bristle pattern on the ventral side of TS1 and TS2. Only the ventral bristles of TS1 are organized in tightly packed transverse rows (each dot denotes one transverse row). (H) In a $bab^{PR24}/Df(3L)bab^{PG}$ transheterozygote, the ventral bristles of TS2 are organized in transverse rows (dots) as in TS1. TS2 has also an ectopic sex comb (arrowhead) and more ventral bristles than wild type (G). The tarsal segments are shorter and thicker in a *bab* mutant than in wild type. Panels F and I-L show segmentation defects in the tarsus of the leg and the basal cylinder of the antenna. (F) A $bab^{EI}/Df(3L)bab^{PG}$ mutant tarsus, which has a kink in TS3 (arrow) in addition to ectopic sex combs (arrowheads). (I) Segmental joints (arrows) in a wild-type and (J) in a $bab^{PR24}/Df(3L)bab^{PG}$ tarsus. There is a complete fusion of TS4 to TS5 and partial fusion of TS4 to TS3, and TS3 to TS2 in this strong *bab* mutant. On the dorsal side, only slight indentations (dots) are left of the joints. (K) Wild-type and (L) bab^{PR72}/bab^{PR72} antenna. The basal cylinder of the antenna is shown at higher magnification in the insets. The basal cylinder consists of two small segments in wild type (arrows). In a strong *bab* mutant antenna, these segments are fused together and to the arista. The light spots in the fused segments are remnants of the joint material. Cuticle preparations of legs and antennae were photographed using bright-field (A-H, K, L) or Nomarski optics (I, J). Abbreviations are: numbers 1-5 designate the tarsal segments; 5^4 indicates fusion of TS4 and TS5; T, tibia; F, femur; A, arista; 3.S, third antennal segment. Distal is always to the left.

Dominant phenotype and phenotypic series of loss of function *bab* mutations

In addition to its recessive phenotype, strong *bab* mutations cause a dominant mutant phenotype. Heterozygous males have an ectopic sex comb on TS2, indicating a homeotic transformation of TS2 towards TS1. The same dominant effect is also detected in hemizygous flies that carry a wild-type chromosome over the deficiency $Df(3L)bab^{PG}$ (Fig. 1D) or over the terminal deletion of the translocation *T(3;Y)A114* (61A1; 61F). This indicates that the dominant phenotype is caused by a reduction of the gene dosage of *bab*, and defines *bab* as a haploinsufficient gene. In contrast to the recessive phenotype, only the TS2 bristle pattern has changed, and on TS2 itself the ectopic sex comb is the only clear indication of a homeotic transformation. This shows that the dominant phenotype of strong *bab* mutations is similar to but weaker than the recessive phenotype of strong *bab* alleles. A comparison between the recessive and dominant phenotype of strong *bab* mutations indicates that TS2 is more sensitive to transformation towards TS1 than either TS3 or TS4. This effect can be interpreted as a graded requirement for *bab* along the proximal-distal axis of the tarsus.

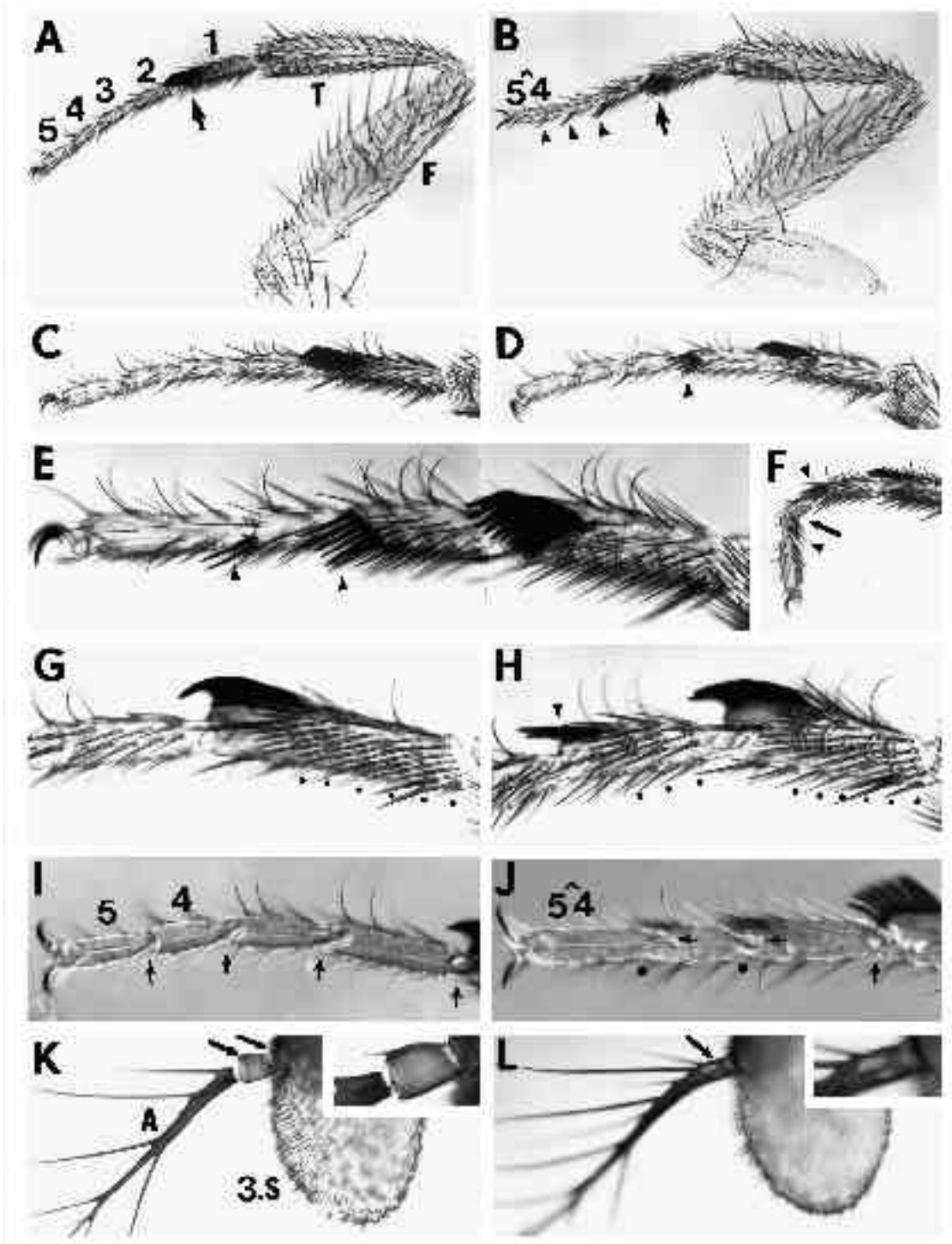

Fig. 1

The graded requirement for *bab* activity is supported by the analysis of weaker *bab* alleles. Flies homozygous for bab^{PR11} or bab^{PR30} have small ectopic sex combs (2-3 SCB) on TS2. Therefore, the recessive phenotype of these *bab* alleles is comparable to the dominant phenotype of strong *bab* alleles. A stronger transformation of distal tarsal segments was observed with the alleles bab^{E1} and bab^{PR23}. On the prothoracic legs of flies homozygous for these alleles, TS2 exhibits a sex comb with usually 4 SCB as well as transverse bristle rows. On TS3, however, neither a sex comb nor transverse bristle rows are observed. In the metathoracic legs of wild-type flies, TS1 contains transverse bristle rows with more than 7 bristles per row, TS2 contains somewhat irregular transverse rows containing 3 and 4 bristles, whereas TS3 and TS4 lack transverse row bristles. The larger number of 4 and 5 bristles in the transverse rows

on TS2 of bab^{E1} and bab^{PR23} metathoracic legs suggests a transformation of TS2 towards TS1 as described previously for the prothoracic leg. The additional appearance of transverse rows on TS3 (2 and 3 bristles per row) indicates that TS3 also has taken on the identity of a more proximal tarsal segment. This observation might suggest that TS3 of metathoracic legs is more sensitive than TS3 of the prothoracic legs to transformation towards TS1. Alternatively, it might suggest that TS3 is transformed towards TS2, a transformation that would not be identifiable on the prothoracic legs because of a lack of distinguishing markers between TS2 and TS3. The analysis of *bab* mutants of different phenotypic strength indicates a gradual transformation of the bristle pattern of the tarsal segments.

bab mutations cause segmentation defects in the tarsus

Strong *bab* alleles produce a complete fusion of tarsal segments TS5 and TS4 in all six legs as homozygotes (Fig. 1B,J). The segmental joint is missing between TS5 and TS4, and the fused double segment is shorter and thicker than that of TS4 and TS5 together in wild-type legs. In flies carrying a strong *bab* allele over $Df(3L)bab^{PG}$, TS4 and TS3, and TS3 and TS2 are partially fused as well (Fig. 1J). In weaker *bab* mutants, TS5 and TS4 are only partially fused and this occurs with incomplete penetrance.

The analysis of different *bab* alleles shows that the extent of tarsal fusion and homeotic transformation are related. These two phenotypic traits, however, overlap spatially only in the strong *bab* mutant phenotype. In weaker *bab* mutants, the transformation is only seen in the proximal region of the tarsus and the segmental fusion only in the distal region. This separation suggests that these defects are not dependent of one another.

In addition to the segmentation defects that are associated with loss of tarsal joints, *bab* mutant flies occasionally produce legs with a kink in TS3 (Fig. 1F) or legs where structures of the tarsus that lie distal to the position where the kinks occur are completely missing. The defects might be caused by the cell death, which can be detected in the imaginal primordium of TS3 (unpublished observations). They are observed more frequently in the metathoracic legs than in other legs and usually only in one leg of a fly. They appear rarely (<1%) in the case of the described strong *bab* mutations, which derived from the bab^{P} mutation, but occur frequently in flies of the genotype $bab^{E1}/Df(3R)bab^{PG}$ (19% of 130 flies).

bab mutations affect homologous structures in the leg and the antenna

Defects in the legs of *bab* mutants are restricted to a specific subdistal domain of the tarsus (Fig. 1B). Structures proximal to this domain as well as the most distal structure of the leg, the claw organ, are not affected. In addition to the leg, *bab* mutations affect the morphology of the antenna. The antenna of *Drosophila* is a structure homologous to the leg and similarly subdivided into different segments. The basal cylinder of the antenna, which consists of two small segments (Fig. 1K), has been shown to be homologous to TS2-TS4 (Postlethwait and Schneiderman, 1971). Strong *bab* mutations cause defects in the basal cylinder of the antenna suggesting that *bab* is required in a homologous region of the leg and antenna. Defects at the arista, the distalmost structure of the antenna, and at structures lying proximal to the basal cylinder were not observed. The two segments of the basal cylinder are fused to a variable degree to each other and to the arista in strong *bab* mutants (Fig. 1L). This segmentation defect is accompanied by loss of the segmental joints. Based on available morphological markers, no homeotic transformation of the basal cylinder was detected.

Temperature-sensitive period of the dominant *bab* phenotype

The penetrance and strength of the dominant leg phenotype is temperature sensitive. Flies heterozygous for a strong *bab* allele develop legs with a stronger homeotic defect at 30°C than at lower temperatures. The temperature sensitivity is not allele specific and can be seen in hemizygous $Df(3L)bab^{PG}/+$ flies as well. The strong allele bab^{PRDS} was used to perform temperature-shift experiments for which the penetrance of ectopic sex combs was chosen as the phenotypic parameter (Fig. 2). Along with its penetrance, the size of an ectopic sex comb changes gradually as summarized in Fig. 2A and B. The temperature-sensitive period of the dominant homeotic *bab* phenotype centers on the prepupal stage (Fig. 2C) and defines a critical period for tarsal segment specification. Because the temperature sensitivity is not an allele-specific effect, we cannot be certain that it is caused by the BAB protein. However, *bab* is expressed in the leg imaginal disc at the corresponding stage (see below), which suggests that the phenocritical period is the likely time of *bab* requirement.

bab is expressed in a concentric domain of leg and antenna imaginal discs

The bab^{P} enhancer trap line expresses β-galactosidase (β-gal) in the leg and antenna imaginal discs. Flies of the line bab^{A128}, which have a similar β-gal expression pattern but are phenotypic wild type as homozygotes, were chosen for a detailed analysis of the β-gal expression pattern to avoid any effects that might be caused by haploinsufficiency associated with the bab^{P} mutation. Imaginal discs from the early third instar larva until the mid prepupa [6 hours post puparium formation (PP)] were studied. The distribution of the *bab* transcript was also examined by tissue in situ hybridization to imaginal discs from mid and late third larval instar using *bab*-specific DNA probes (see description below and Fig. 6A). In addition, a polyclonal antibody (anti-BAB r2), which is directed to a protein that corresponds to part of the defined open reading frame (see Fig. 6A and Materials and methods), was used to study the *bab* expression pattern in imaginal discs in the third larval instar and throughout the prepupal stage. The patterns of expression of β-gal in the enhancer trap lines and of the *bab* transcript and the BAB protein were found to be equivalent in leg and antenna imaginal discs (Figs 3 and 5).

From mid third larval instar onward the *bab* product is present in a concentric domain around the center of the imaginal discs, a region that corresponds to parts of the tarsal primordium of the legs (Fig. 3A,C), and the subdistal structures of the antenna (Fig. 3E,F). No staining is detected

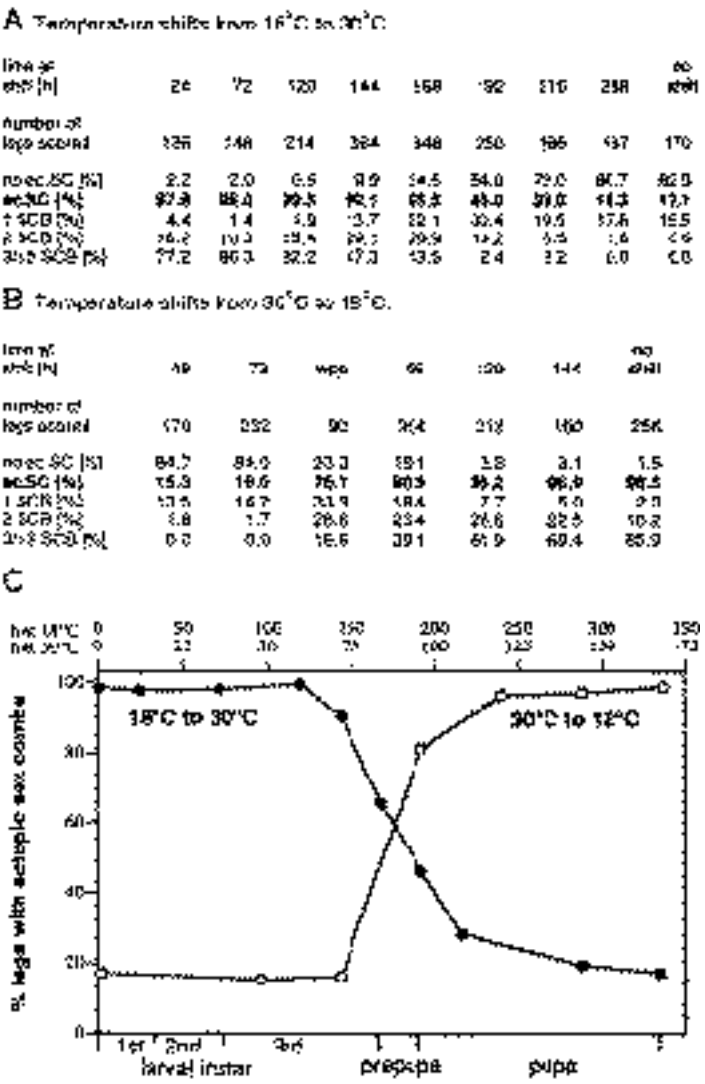

Fig. 2. Temperature-sensitive period of the dominant *bab* mutant phenotype. At 30°C, an ectopic sex comb on TS2 of a fly heterozygous for a strong *bab* mutation appears at a frequency of 98% and usually contains 3-5 SCB. At 18°C, the frequency is reduced to 17% and the size of the sex comb is 1-2 SCB. This effect was used to measure the temperature-sensitive period of the haploinsufficient phenotype. Flies heterozygous for a strong *bab* allele (bab^{PRDS}) were shifted (A) from 18°C to 30°C and (B) from 30°C to 18°C at different time points of development ([h] denotes hours after hatching of first instar larvae). Listed are the total number of legs that were scored, the percentages of legs with and without an ectopic sex comb, and, in addition, the percentages of legs with 1, 2 and 3 or more ectopic SCB. No shift denotes that flies were grown constantly at 18°C and 30°C, respectively. wpp designates that flies were selectively shifted as white prepupa. The bold data were used for the graph in panel C. (C) The graph shows the percentage of legs with an ectopic sex comb on TS2 for the different time points at which the temperature shift occurred. The corresponding developmental stages are shown.

in the center of the imaginal discs, which is the primordium of TS5 of the leg and the arista of the antenna (Figs 3, 4). At mid third larval instar, β-gal is weakly expressed in the furrow between the two central folds of the leg imaginal disc (Fig. 4A). The staining level becomes stronger during late third larval instar, when the domain where *bab* is expressed in the leg imaginal disc gives rise to three additional folds, the primordia of TS2 to TS4. In addition to the region of TS2 to TS4, *bab* product is also found in the distal margin of TS1 (Fig. 4B). In anti-BAB r2 stainings and RNA in situ hybridizations, we were not able to detect a signal prior to this stage. During evagination in the prepupal stage, when the tarsal segments have expanded, *bab* is expressed in the region from distal TS1 through TS4 (Figs 3B,D and 5A,B). The exact boundaries of the expression domain are difficult to localize because the staining drops strongly towards the edges of the domain. Differences in the distribution of the *bab* product in the imaginal discs for different leg pairs are not observed. The oldest antibody-stained discs that were examined were from 6 hours old prepupae, when the evagination is completed. To correlate the *bab* expression domain to the future segments is more difficult in the antenna than in the leg imaginal disc, however, strong β-gal activity is detectable in the two segments of the basal cylinder in the adult antenna of bab^{A128} flies (data not shown), suggesting that the two rings of strong anti-β-gal staining in the antenna disc (Fig. 3E) correspond to the primordia of these structures. The analysis of the distribution of the *bab* transcript and protein in leg and antenna imaginal discs shows that *bab* expression is restricted to those regions of the antenna and leg discs that are affected by *bab* mutations.

The analysis of *bab* expression with the anti-BAB r2 antibody reveals that the BAB protein is localized to the nuclei of cells (Figs 3D and 5B). Although the nucleus appears to be stained as a whole, the antibody detects spots of especially high antigen concentration inside the nucleus (Fig. 5B).

The graded distribution of the *bab* gene product in leg imaginal discs

In the *bab* expression domain that extends from distal TS1 through TS4, the *bab* product is found in all cells but the amount differs from cell to cell. At mid third instar, the *bab* expression domain comprises less than 20 cell diameters along the proximal-distal axis and shows a higher level of staining distally than proximally (Fig. 4A). During the late third larval instar, when the tarsal primordium grows by cell division (Madhavan and Schneiderman, 1977) and becomes folded, *bab* expression becomes stronger and more differentiated. The staining level is highest in TS4 and TS3, lower in TS2 and lowest in the distal margin of TS1. Instead of a simple distal-proximal gradient, however, cells within each of the folds show different staining intensity (Fig. 4B).

At puparium formation when the tarsal folds are established and the evagination process starts, the *bab* expression pattern appears to be fully developed and remains stable throughout the early prepupal stage. In the expanding tarsal primordium, the *bab* expression domain comprises about 40 cell diameters along the proximal-distal axis. The complex distribution of the *bab* product has two characteristics. First, a wave-like pattern is observed in the expression domain. Each tarsal fold in the *bab* domain shows a bell-shaped distribution of staining intensity. The cells at the ridges of the segmental folds show a much higher staining level than the

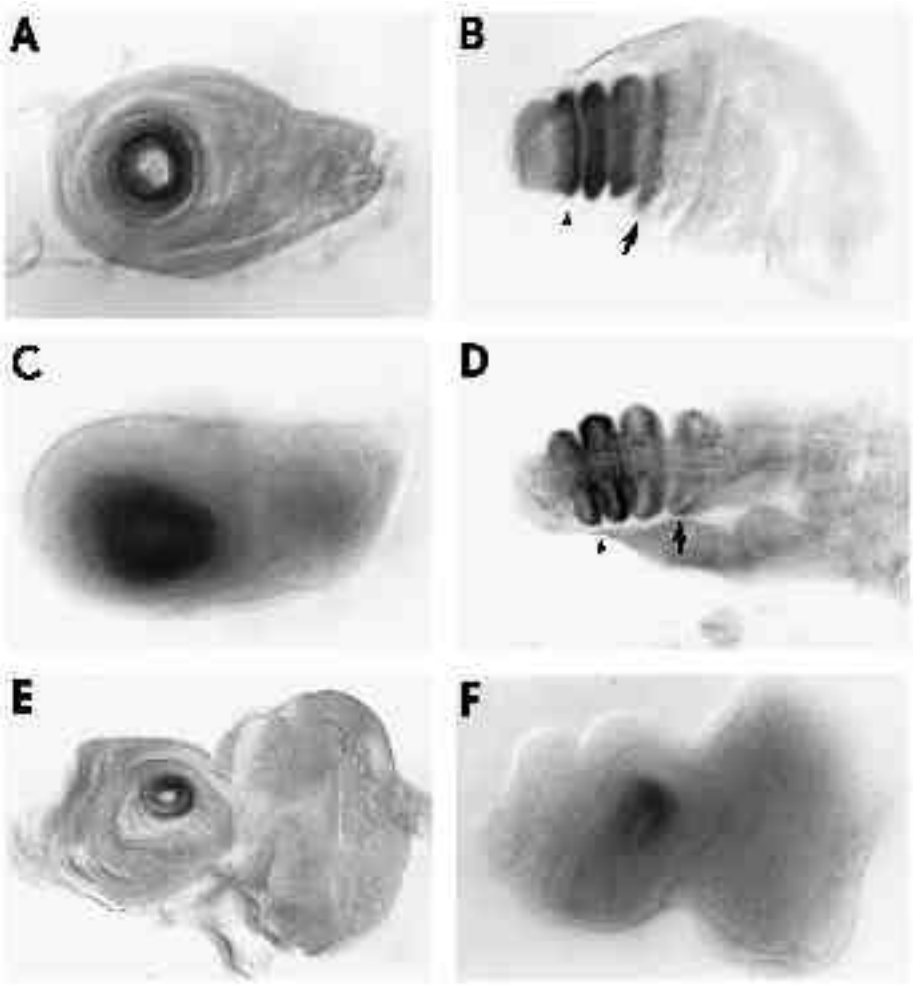

Fig. 3. *bab* expression in leg and antenna imaginal discs. (A) Anti-β-gal antibody staining of a prothoracic leg disc of a bab^{A128}/+ late third instar larva. β-gal is distributed in a concentric domain around the center of the disc. Several rings of cells stain with varying intensity in the expression domain. (B) Anti-β-gal antibody staining of a leg disc of a bab^{A128}/+ early prepupa (about 2 hours PP; distal is to the left). The expression domain is from the distal margin of tarsal fold 1 (arrow) trough tarsal fold 4 (arrowhead). A gradient of staining intensity is seen from distal to proximal. Tarsal folds 3 and 4 have the strongest staining, tarsal fold 2 is lower and tarsal fold 1 lower still. Furrows are stained more weakly than the ridges of the segmental folds. (C) In situ hybridization of a digoxigenin-labeled *bab* DNA probe (genomic fragment E199, Fig. 6A) to the prothoracic leg disc of a wild-type late third instar larva. The *bab* transcript is concentrically distributed around the center of the disc. (D) Staining of a wild-type prepupal leg disc (4-5 hours PP; distal is to the left) with the polyclonal rat anti-BAB r2 antibody. The BAB protein is distributed in a graded manner in the tarsal folds 1 (arrow) through 4 (arrowhead). The antigen is located in the nuclei of the epithelial cells. The anti-BAB antibody staining pattern is similar to the anti-β-gal staining pattern seen in panel B. (E) Anti-β-gal antibody staining of an eye-antenna disc of a bab^{A128}/+ third instar larva shows two strongly stained rings of cells around the center of the antennal portion of the disc. (F) In situ hybridization of a digoxigenin-labeled *bab* DNA probe (genomic fragment E199, Fig. 6A) to a wild-type eye-antenna disc of a third instar larva detects a concentric domain of expression around the center of the antennal primordium. Whole-mount preparations of imaginal discs were viewed with Nomarski optics.

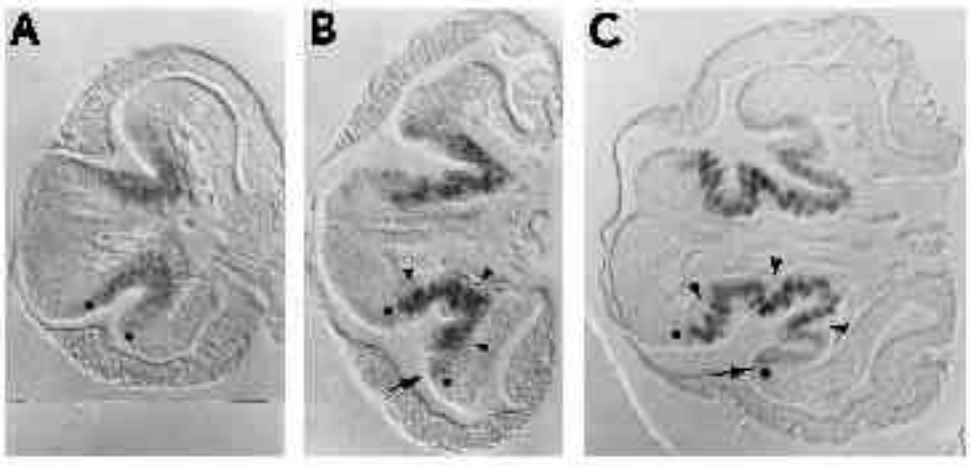

Fig. 4. *bab* expression at different stages of leg disc development. (A) Weak antibody staining is detected in the furrow between the two central folds of the leg disc of a mid third instar larva. (B) In mid-late third larval instar, three folds, indicated by arrowheads, develop from the region of the tarsal primordium where β-gal is detected. Staining intensity has become generally stronger at this stage. The arrow points to the distal margin of tarsal fold 1 which shows weaker staining than the other folds. There are differences between each of the folds in the staining level of the nuclei. (same magnification as in A). (C) Leg disc of a late third instar larva. Antibody staining is restricted to the central region of the tarsal primordium, from the distal margin of tarsal fold 1 through tarsal fold 4. The staining level is higher in the cells at the ridges than in the furrows of all tarsal folds. The ridges of the different tarsal folds vary in staining intensity. The highest level is in tarsal fold 3, less in 4, even less in 2 and lowest in 1. Cross-sections (anterior-posterior) of anti-β-gal antibody-stained bab^{A128}/+ imaginal discs were photographed using Nomarski optics. β-gal is localized to the nuclei of the cells. An arrow points to tarsal fold 1, arrowheads denote tarsal folds 2-4; dots indicate the boundaries of the expression domain. Distal is always to the left.

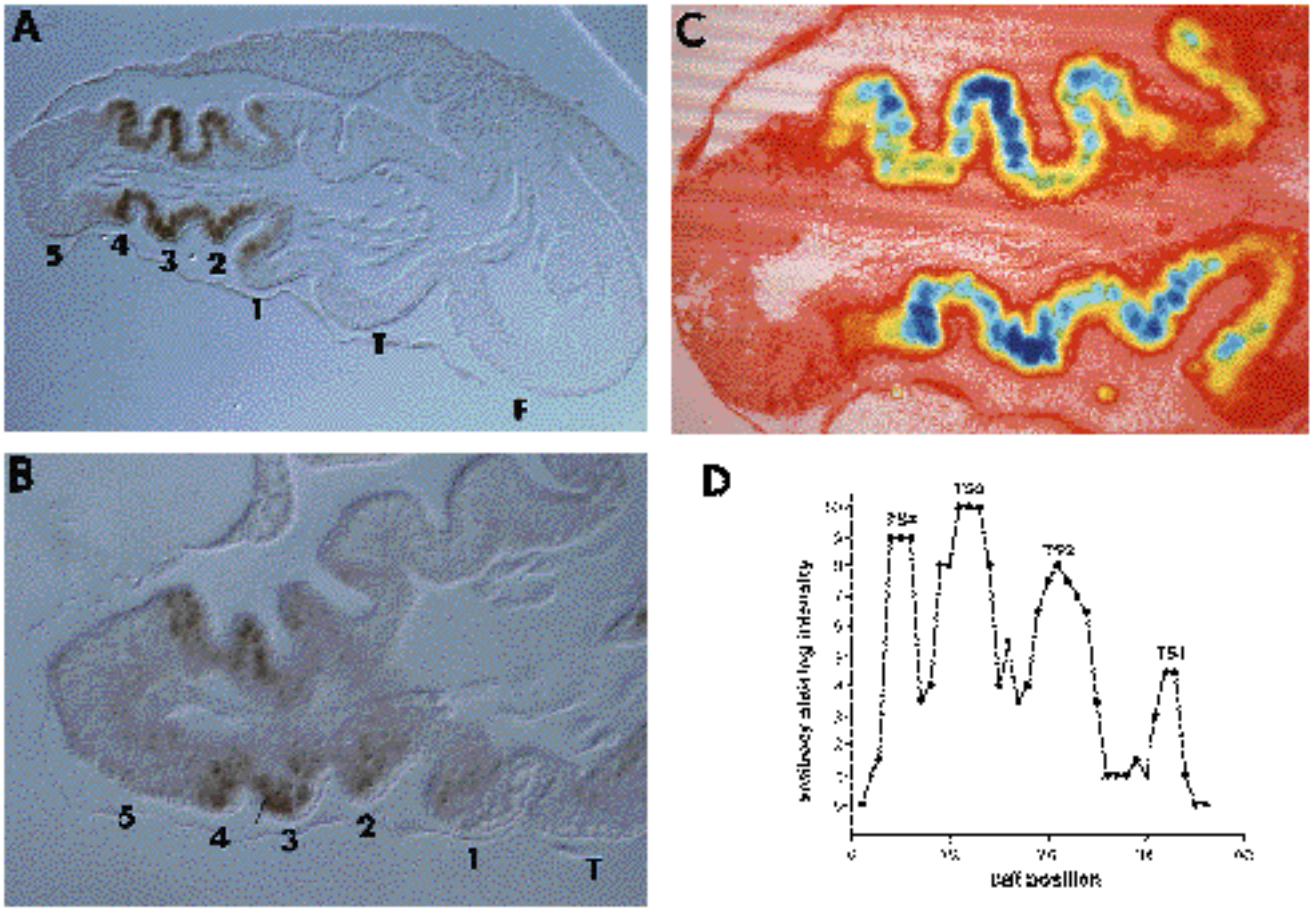

Fig. 5. Graded and wave-like distribution of the *bab* gene product in the tarsal primordium. (A) Distribution of β-gal in the tarsal region of a bab^{A128}/+ prepupal leg disc (about 3 hours PP) and (C) its processed pseudo-colored image. The tarsal primordium shows alternatively high and low staining levels; the maxima correspond to the ridges (arrow), the minima to the furrows (arrowhead) of the tarsal folds. Each of the four folds has a bell-shaped graded distribution of β-gal. In addition, the ridges of the different tarsal folds vary in staining intensity, showing the highest level in tarsal fold 3, less in 4, even less in 2 and lowest in 1. (B) Distribution of the anti-BAB r2 antibody in a wild-type prepupal leg disc (about 2 hours PP). A similar graded and wave-like staining pattern is seen as in A. Note the heavily stained dots in the nuclei of the antigen expressing cells (arrow). (D) Schematic representation of β-gal distribution in the leg imaginal disc shown in panel A. The relative level of anti-β-gal antibody staining in each cell nucleus of the *bab* expression domain was defined as described in Materials and methods. The graph illustrates the graded and wave-like expression profile. Longitudinal sections (dorsal-ventral) of antibody-stained imaginal discs. T, tibia; F, femur; numbers denote tarsal folds 1-4. Distal is always to the left.

cells in the furrows (Figs 4C and 5A,B). Secondly, there is a graded distribution of the *bab* product throughout the expression domain. The highest levels of the *bab* product are found at the ridge of TS3. TS2 shows lower expression levels than TS3, and the ridge of TS1 has the lowest levels. The staining intensity in TS4 was never found to be higher than in TS3 but either equal or lower (Figs 3B,D, 4C and 5A,B).

In order to facilitate a cell-by-cell analysis of the complex *bab* expression pattern, sections of anti-β-gal-stained prepupal leg discs of bab^{A128} flies were examined with an image processor which translated the staining intensity of each cell into a color value (Fig. 5C). Fig. 5D shows the processed profile of anti-β-gal staining intensity which corresponds to the imaginal disc shown in Fig. 5A. It illustrates the segmentally repeated high and low levels and the graded distribution of staining intensity. This pattern was reproduced in all ten leg discs examined by this method.

The *bab* gene shares a homologous domain with the *Drosophila* genes *tramtrack* and *Broad-Complex*

The P-*lacZ* insertion of the bab^{P} allele was used as a tag for the cloning of chromosomal DNA adjacent to the insert. This DNA was then used as a probe to map the *bab* locus by chromosome in situ hybridization (Fig. 6C) and to screen both a *Drosophila* genomic library and a number of cDNA libraries (see Materials and methods). Conclusions from these data are shown in Fig. 6A. The sites of the P-*lacZ* insertions of bab^{P} and bab^{A128} were mapped within the 4.2 kb *Eco*RI genomic fragment E199 (data not shown). Analysis of the 8 cDNAs isolated indicates that they are incomplete (data not shown). Compilation of sequence data from the cDNAs and the genomic clones allowed us to identify a 1.7 kb open reading frame that is adjacent to the P-*lacZ* insertion site (Fig. 6A; data not shown). This open reading frame may not represent the entire BAB protein-

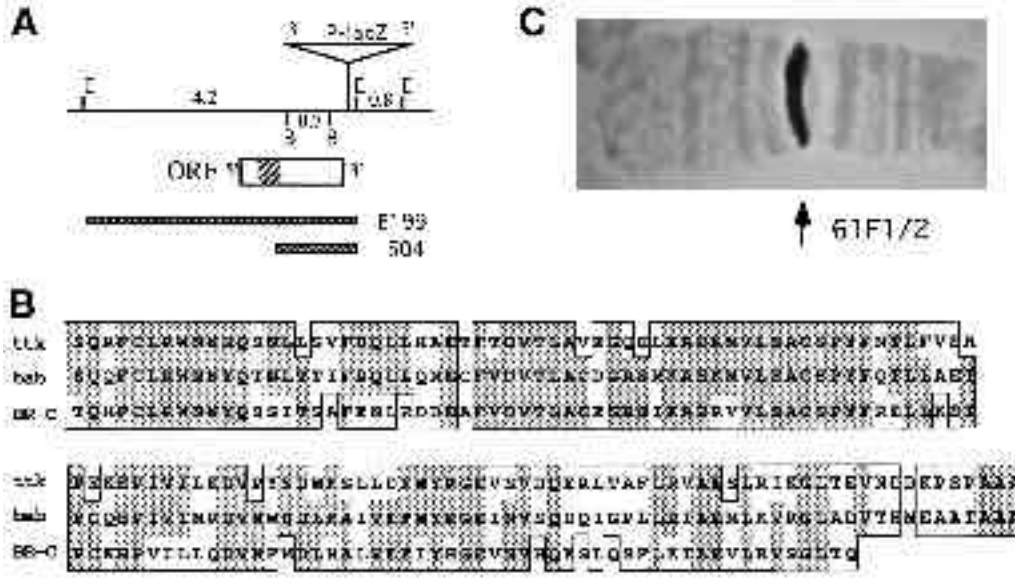

Fig. 6. Molecular analysis of the *bab* gene. (A) Physical map of the region surrounding the P-*lacZ* insertion site of the bab^{P} allele. *Eco*RI [E] and *Bam*HI [B] restriction enzyme sites and the distance in kilobases between them are shown. The location of the 1.7 kb open reading frame [ORF] is indicated. The crossed-hatched region within the open reading frame marks the location of the BTB domain. The 0.7 kb *Bam*HI restriction enzyme fragment within the open reading frame was used to make the BAB-fusion protein which was used for immunization. The fragments that were used as probes for tissue in situ hybridization, the genomic *Eco*RI fragment E199 and the subclone 504, are shown in hatched bars. (B) *bab* shares a strongly conserved domain with the *Drosophila* genes *ttk* and *BR-C*. The predicted amino acid sequence of the putative BABprotein is shown in comparison with the protein sequences of *ttk* and *BR-C*. Only the region of homology between the three genes, the BTB domain, is shown. The identical amino acids in the BTB domain are shaded, the conserved amino acids are boxed. The *ttk* protein sequence shown is from amino acids 3 to 129 (Harrison and Travers, 1990; Read and Manley, 1992). The *BR-C* protein sequence shown consists of amino acids 4 to 117 (DiBello et al, 1991). The N and C termini of the BAB protein have not been identified and the location of the conserved domain within the BAB protein is not known. (C) The *bab* locus was cytologically mapped via chromosome in situ hybridization. The panel shows the tip of the left arm of the third chromosome with the hybridization signal in 61F1-2.

coding region, and the analysis of additional cDNAs is required. However, we believe that the sequences identified are part of the *bab* gene for three reasons. First, the P-*lacZ* insert of the bab^{P} allele is located immediately 3′ of the identified open reading frame. Second, the distribution of the detected RNA and protein is equivalent to the β-gal expression pattern of the enhancer trap lines (as shown in Fig. 3), and this expression pattern is consistent with the defects observed in the ovaries (Godt, Sahut, Couderc, Laski, unpublished observations) and limbs of *bab* mutants. Third, the anti-BAB r2 antibody which is directed to a portion of the open reading frame (Fig. 6A) does not detect the BAB protein in the imaginal tissues of flies homozygous for the strong alleles bab^{PR24} or bab^{PRDS} but does detect it in wild-type revertants of the bab^{P} allele (data not shown).

Computer analysis of the 1.7 kb open reading frame identified a strong similarity to two other *Drosophila* genes, *tramtrack* (*ttk*) (Harrison and Travers, 1990; Read and Manley, 1992) and *Broad Complex* (*BR-C*) (DiBello et al., 1991). The amino acid sequences of the relevant region of BAB, TTK and BR-C are shown in Fig. 6B. There is a 57% identity over 127 amino acid residues between BAB and TTK, 59% over 112 amino acid residues between BAB and BR-C, and 55% over 113 amino acid residues between TTK and BR-C. Both *ttk* and *BR-C* are genes that encode a family of zinc-finger proteins that are thought to be transcription factors (*ttk*: Harrison and Travers, 1990; Read et al., 1990 and 1992; Brown et al., 1991; Jiang et al., 1991; Read and Manley, 1992; *BR-C*: Galceran et al., 1990; DiBello et al., 1991). The highly conserved region (which we have named the BTB domain after *BR-C*, *ttk* and *bab*) is not part of the zinc-finger domain in the TTK and BR-C proteins, and its function is unknown.

DISCUSSION

Our present study shows that *bab* is required and expressed in a distinct proximal-distal domain of the limbs; the central region of the tarsus of the leg and the basal cylinder of the antenna. The domain of *bab* activity in limbs is apparently identical to the domain defined by the phenotype and expression pattern of *rotund* (Cavener et al., 1986; Kerridge, 1981; Kerridge and Thomas-Cavallin 1988; Agnel et al., 1989, 1992). In addition, this domain is characterized by the gene *deadpan*, which is expressed in a distal circumferential stripe in each of the segments TS1 to TS4 (Bier et al., 1992). *bab* and *rotund* appear to act rather late in limb development, in contrast to genes that control the whole proximal-distal axis and appear to be required from embryogenesis onward, such as *Dll* (Sunkel and Whittle, 1987; Cohen and Jürgens, 1998a,b) and *wg* (Simcox et al., 1989; Baker, 1988a,b; Couso et al., 1993). The subdivision of the tarsal primordium is a late event in the pattern formation of the leg and is also an evolutionary recent step. Primitive insects only have one tarsal segment and the number of tarsal segments differs widely among more advanced insects (Borror and White, 1970).Taken together, this indicates that the *bab*/*rotund* domain is a distinct field for pattern formation during leg and antenna development.

bab is required for the specification of tarsal segments

Comparison of the bristle pattern in the tarsal segments of wild-type and *bab* mutant flies suggests that loss-of-function *bab* mutations cause a homeotic transformation of the three central tarsal segments (TS2-TS4) towards the basitarsus (TS1). This indicates a serial homology of segments in the tarsus. A similar conclusion has also been reached by Stern (1954) based on the finding of an extra sex comb on TS2 in *extra sex comb-aristapedia* double mutants. It may be assumed, therefore, that TS1-TS4 have the same basic pattern information that is modulated by *bab* activity in TS2-TS4 in order to give them a specification different from that of TS1. Our data suggest a role for *bab* as a homeotic gene that is required along the proximal-distal axis of the legs to direct the developmental fate of TS2, TS3, and TS4.

The haploinsufficient transformation effect of *bab* alleles indicates that the specification of the tarsal segments depends on *bab* dosage. Also, the phenotypic series of *bab* alleles can be interpreted as a consequence of sequentially reduced levels of *bab* expression. The comparison of different *bab* alleles indicates a graded requirement for *bab* activity along the proximal-distal axis that becomes apparent in the higher sensitivity of TS2 compared to TS3 and TS4 transformation towards TS1. Given the dosage dependence, a simple explanation for this observation would be a graded distribution of the *bab* gene product along the proximal-distal axis. This explanation is corroborated by the different levels of *bab* expression that are observed in TS1-TS4. TS1 contains the lowest level of *bab* product and is considered the ground state. TS2, which is most sensitive to homeotic transformation to TS1, has a lower level of *bab* expression than TS3 and TS4. We propose, therefore, that the sensitivity to homeotic transformation towards TS1 is correlated with the concentration of the BAB protein in the different tarsal segments. It is unclear if this correlation holds for TS4, which contains equal or slightly lower levels of *bab* expression than TS3. In TS4, whose small size seems to allow the production of only one SCB, the usage of this morphological marker might not be sensitive enough to always detect a transformation to TS1.

Is *bab* a morphogen?

There are two possible explanations for how *bab* may act in a concentration-dependent manner for the specification of tarsal segments. *bab* may promote a binary decision between a TS1 fate and a non-TS1 fate, or *bab* may act as a morphogen and provide different positional values for the specification of TS2, TS3 and TS4. The latter possibility is supported by the graded distribution of the *bab* product. In addition, the analysis of metathoracic tarsal segments of weak and intermediate *bab* mutants shows a change in the bristle pattern of TS3 that can be interpreted as a transformation of TS3 towards TS2 rather than TS1. We suggest, therefore, that *bab* may provide, depending on its concentration, different positional values for the specification of TS2 and TS3. It will be possible to assess the ability of the BAB protein to act as a morphogen by studies of *bab* overexpression and by examination of segment-specific molecular markers in *bab* mutants.

bab is the first gene for which a graded expression along the proximal-distal axis is described. The BAB protein distribution in the tarsal primordium reflects the distribution of the *bab* transcript, which indicates that the pattern of *bab* expression is regulated at the transcriptional level. The polar coordinate model proposes the formation of distal limb structures under the control of a circular coordinate which integrates anterior-posterior and dorsal-ventral positional values (French et al., 1976; Bryant et al., 1981). Mutations in *wg*, for example, which encodes a secreted and diffusible protein (for review see Nusse and Varmus, 1992) and is believed to define circumferential positional values, have a drastic effect on the proximal-distal axis (Couso et al., 1993). However, it is not clear how genes like *wg* could induce a gradient of a nuclear protein along the proximal-distal axis. Whether *Dll*, which likely acts upstream of *bab* and is proposed to provide positional information along the proximal-distal axis (Cohen et al., 1989), directly regulates *bab* expression is not known, but considering that the tarsus is a distinct *Dll* expression domain in the third larval instar (Cohen, 1993), *Dll* might be involved in defining the domain and/or the pattern of *bab* expression.

bab is expressed in a wave-like manner and is involved in segmentation of the leg and antenna

The analysis of *bab* mutations indicates that *bab* has, in addition to its requirement for segment specification, a second function in limb development. *bab* mutations cause segmentation defects in the central region of the tarsus and in the homologous part of the antenna, the basal cylinder. Because our strongly hypomorphic *bab* mutations cause more severe segmentation defects as hemizygotes than as homozygotes, we assume that amorphic *bab* mutations might lead to even stronger defects in segmentation.

At the onset of metamorphosis, the *bab* product is distributed in a wave-like pattern in the tarsal primordium. Each tarsal fold shows a bell-shaped expression pattern of *bab* with the maximum level of expression at the ridge and the minimum in the furrow. Considering the segmentation defects in the tarsus of *bab* mutants, we propose that the wave-like pattern may be involved in the segmentation process of the tarsus. A number of theoretical models have utilized chemical wave patterns to explain how segmentation could occur (for review see Wolpert, 1989). In these models, the waves compose a prepattern that reflect the structures that will develop, and the segment boundaries are proposed to be specified by the troughs or peaks of the wave pattern. Analysis of the molecular mechanisms of segmentation in the *Drosophila* embryo has shown that wave-like prepatterns are not part of the segmentation process (for review see Akam, 1987). Our finding that a wave-like pattern of *bab* exists in the developing tarsus suggests that segmentation in the tarsus occurs by a mechanism that differs from segmentation in the embryo.

In this context, we have to ask whether the wave-like *bab* expression pattern can be considered a prepattern for segmentation of the tarsus. The wave-like pattern of the *bab* product at the onset of metamorphosis develops from a rather uniform distribution in the mid third larval instar. The wave-like pattern appears to form in parallel to the development of the tarsal folds. This, together with the observa-

tion that the segments of the tarsus seem to be already defined by the mid third larval instar (Schubiger, 1974), suggests that the wave-like distribution of the *bab* product is not likely to be a classical prepattern. In contrast to the embryo, however, pattern formation in the tarsal primordium occurs in a proliferating epithelium where a stable molecular prepattern is not expected. It has been suggested that new positional values in the growing imaginal disc are generated by intercalation between values that already exist (Bohn, 1970; Cohen and Jürgens 1989a). The development of the wave-like *bab* expression pattern may reflect this process. Early folding defects in the tarsal region of *bab* mutant imaginal discs (D. Godt, unpublished observation) indicate that *bab* is involved in the morphogenetic folding process. We therefore propose that in the growing field of the tarsal primordium, the morphogenetic process of segmentation and the development of the *bab* gene expression pattern may occur in a mutually dependent manner.

The BAB protein shares a highly conserved domain with two transcription factors

The genetically defined *bab* locus corresponds to genomic DNA that we have cloned from the region 61F1-2. The β-gal expression pattern of three *bab* enhancer trap lines and the expression pattern of the transcript and protein from a gene in this region are similar. Further support for the identification of this gene as *bab* was gained by showing that strong *bab* mutations do not express the prospective protein at a detectable level.

Part of the open reading frame encodes a domain of about 115 amino acids, named the BTB domain, that shows 57% and 59% identity to the aminoterminal region of the transcription factors encoded by *BR-C* and *ttk*. *BR-C* is an ecdysone-inducible 'early' gene (Ashburner, 1972; Chao and Guild, 1986; Galceran et al., 1990) and is required for proper morphogenesis during imaginal development in a number of different tissues (Fristrom et al., 1981; Kiss et al., 1988; Restifo and White, 1991, 1992). *ttk* acts as a repressor of pair-rule genes in the embryo (Read et al., 1992; Xiong and Montell, 1993; Brown and Wu, 1993) and is required during eye development (Xiong and Montell, 1993). The BR-C and TTK proteins contain both a BTB domain and a zinc-finger domain (Harrison and Travers, 1990; DiBello et al., 1991; Read and Manley, 1992). Due to the incomplete nature of our cDNAs, we do not know whether *bab* also encodes a zinc-finger domain. However, the observations that *bab* encodes a nuclear protein and that *bab* mutations cause homeotic transformations in legs suggest that *bab* functions as a transcriptional regulator.

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Note added in proof

The DNA sequence that encodes the BTB domain of the bric à brac protein can be found in GenBank under accession no. U01333.