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The *black-pearl* gene of *Drosophila* defines a novel conserved protein family and is required for larval growth and survival

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Abstract

Using a transposon insertion line of the *Drosophila* Genome Project we have cloned the *black-pearl* gene (*blp*), analyzed cDNA clones, generated various mutants, and characterized their phenotypes. The *blp* gene codes for a protein of 15.7 kDa calculated molecular weight that has been conserved from yeast to plants and mammals with high homology. A domain of these new proteins shows distant similarity to DnaJ domains indicating a functionally relevant interaction with other proteins. The P element insertion in line P1539 lies within the 5′ untranslated leader of the *black-pearl* gene. Flies homozygous for this insertion are semi-lethal, escapers produce very few offspring and show melanotic inclusions in the hemocoel ('black pearls') similar to various melanotic 'tumor' mutants. Two small deletions confined to the *blp* gene and two EMS-induced mutations are homozygous lethal. These null mutants appear normal up to a prolonged first instar larval stage but fail to grow and die. Thus in *Drosophila* the *blp* gene is specifically required for larval growth. The evolutionary conservation in both unicellular and multicellular organisms suggests for the new protein family described here a fundamental role in cell growth.

Keywords: Transposon insertion; Null mutants; Growth arrest; Yeast; *Arabidopsis*; Human

1. Introduction

In the *Drosophila* genome project a large number of P transposon insertions that disrupt vital gene functions have been produced and mapped in order to generate multiple links between the genetic and physical maps of the *Drosophila* genome and to provide molecular access and knock-out utility for functionally relevant genes. During studies of the *Sap47* gene that maps cytologically to 89A8-B3 (Reichmuth et al., 1995) we have found that the P insertion of line l(3)01618 (P1539) from the *Drosophila* Genome Project lies just 1.2 kb upstream of the putative *Sap47* transcription start. The P1539 insertion has previously been shown to cause a recessive semi-lethal phenotype (Spradling et al., 1995). Also, comparison of embryos from heterozygous parents with those from homozygous female germ-line clones fertilized by heterozygous males has revealed that the P1539 insertion produces a paternally rescuable maternal effect and variable segmentation defects during embryonic development with a lethal phase during larval stages 1 and 2 (Perrimon et al., 1996). Here we show that the P1539 insertion affects a gene immediately adjacent to *Sap47*. The phenotype of homozygous P1539 escapers is characterized, and by generating null mutants we demonstrate that the insertion phenotype represents a hypo- or neo-morph of the *blp* gene while the wild-type allele is required for larval cell growth.

2. Materials and methods

2.1. Flies

The following *Drosophila melanogaster* strains were obtained from the stock centers at Bloomington (USA) or Umea (Sweden). PZ-insertion line, stock # P1539: P{ry^+,P\T:*lacZ*:*Hsp70* Km^r *ori* = PZ} l(3)01618, ry^{506}/TM3, $ry^{[RK]}$, *Sb, e*. Deficiency Po^4, stock # 3526: Df(3R)Po^4, *cv-c*/TM2;spa^{pol}; *y*. Stable source of transposase:

Abbreviations: aa, amino acid; BDGP, Berkeley *Drosophila* genome project; βGal, β-galactosidase; bp, base pair; kb, kilo base pairs; *blp*, black pearl gene; cDNA, DNA complementary to RNA; Da, dalton; EMS, ethyl methane sufonate; *Sap47*, synapse associated protein of 47 kD gene

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P{ry^+, Δ*2-3*}(99B), *Sb*. GFP-marked balancer TM3: stock # 4534: w*; Sb1/TM3, P{w + mC = ActGFP}JMR2, Ser^1. A detailed description of these and other strains used are given by http://fly.ebi.ac.uk:7081/ or Lindsley and Zimm, 1992.

2.2. Molecular genetic and histochemical procedures

Standard protocols were used according to Sambrook et al., 1989. Histochemical and immuno-histochemical techniques were employed as described by O'Kane and Gehring (1987); Buchner et al. (1986), respectively. Manufacturers' protocols were followed whenever commercial reaction kits were used.

2.3. Mutagenesis

For jump-out mutagenesis and generation of PZ revertants virgin P1539, ry^{506}/TM3, $ry^{[RK]}$, *Sb, e* were mated with P{ry^+,Δ*2-3*}(99B), *Sb*/TM2, *Ubx,e* males. F1 offspring were selected for *Sb* and e^+ to obtain P1539, ry^{506}/P{ry^+,Δ*2-3*}(99B), *Sb* flies. These were crossed to TM3, $ry^{[RK]}$, *Sb, e*/TM6B partners. F2 offspring with *ry* eyes can only result when the P1539 element is lost by remobilization or suffers a defect in its ry^+ gene. These P1539-jump-out, ry^{506}/TM3, $ry^{[RK]}$*Sb, e* flies were molecularly characterized by Southern analysis and/or sequencing and crossed to Po^4/TM6B for phenotypic characterization.

EMS mutagenesis followed the procedure of Ashburner (1989a,b). 100 wild-type males were starved for 12 h, then kept for 24 h on tissue saturated with 25 mM EMS in 5% sucrose and mass mated to virginal TM3, $ry^{[RK]}$, *Sb, e* balancer females. About 7000 F1 offspring selected for *Sb* were crossed individually to flies carrying a TM3, $ry^{[RK]}$, *Sb, e* -balanced deficiency that deletes parts of the *blp* and the *Sap47* genes (see below). F2 offspring from these 7.000 lines were screened for lack of Sb^+ flies indicating that the corresponding EMS-treated wild-type chromosome had suffered a lethal mutation that was not complemented by the *blp-Sap47* deficiency chromosome.

3. Results

3.1. Molecular analysis of the blp gene

With help of the bacterial plasmid sequences integrated into the modified PZ transposon (Mlodzik and Hiromi, 1992) it has been possible to clone genomic DNA adjacent to the insertion site of line P1539. Southern blots, restriction analysis, and sequencing identified the insertion site 1.2 kb upstream of the first known exon of the *Sap47* gene (Reichmuth et al., 1995; Becker, 1998). Northern blots of embryonic, larval and adult poly-A^+-RNA using the *Hind*III/*Xba*I fragment of the rescue plasmid (probe 1 in Fig. 1) reproduced the signals of the *Sap47* transcripts at 2.8, 4.5 and 4.8 kb (data not shown) obtained earlier with a *Sap47* cDNA probe (Reichmuth et al., 1995), but in addition identified

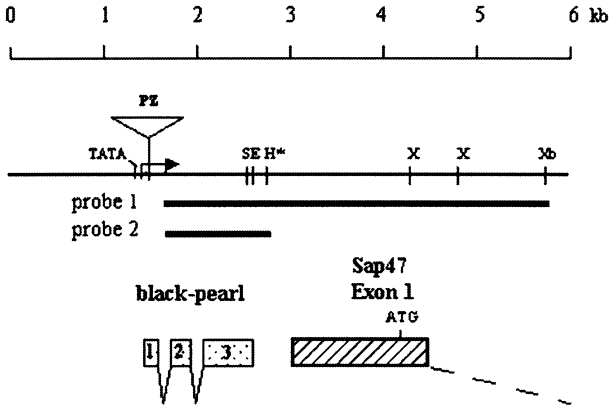

Fig. 1. Restriction map of the genomic region containing the *blp* gene and the first exon of the *Sap47* gene. The position of the PZ insertion in Line P1539 (blp^P) is indicated (triangle). The putative TATA box and start of transcription were obtained by computer analysis of genomic sequence. The fragments used as probes 1 and 2 for Northern analysis are indicated.

two new transcripts of 0.8 and 1.1 kb which by reprobing the blot (Fig. 2) using the 1.1 kb HindIII/HindIII fragment (probe 2 in Fig. 1) could be shown to derive from a gene lying upstream of the *Sap47* gene. As these transcripts appeared most abundant in embryos, the 1.1 kb fragment was used to screen an embryonic lambda-ZAP-II cDNA library (kindly provided by M. Noll, Zürich). A clone with a 686 bp cDNA insert was identified and sequenced. This 5′ incomplete cDNA was used to screen a second embryonic cDNA library (Resource Center/Primary Database (RZPD) # 603 of the German Human Genome Project) which led to the identification of two cDNAs of 763 and 942 bp, respectively, containing the complete open reading frame which translates into an inferred protein (BLP) of 141 amino acids and 15.7 kDa calculated molecular weight. The longest cDNA has been submitted to the EMBL/GenBank/DDBJ database (Accession No. AJ278732). Genomic sequencing and analysis (Burge and Karlin, 1997) revealed the putative transcription start, the PZ-transposon insertion site, two introns of 67 and 60 bp length, and the polyadenylation site a mere 25 bp upstream of the predicted TATA box of the *Sap47* gene (Figs. 1 and 3). Comparison with the

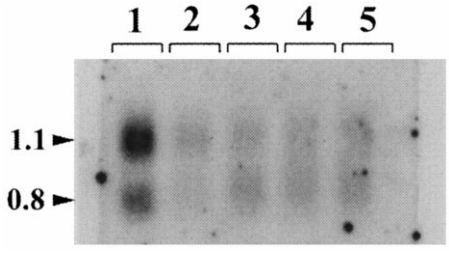

Fig. 2. Northern Blot analysis of wild type at different developmental stages using probe 2 of Fig. 1. The genomic 1.1 kb *Hind*III/*Hind*III-fragment detects two transcripts at 0.8 and 1.1 kb, respectively. Lane 1: 0–6 h embryo, 2: 18–24 h embryo, 3: larval stage 3, 4: male heads, 5: female heads.

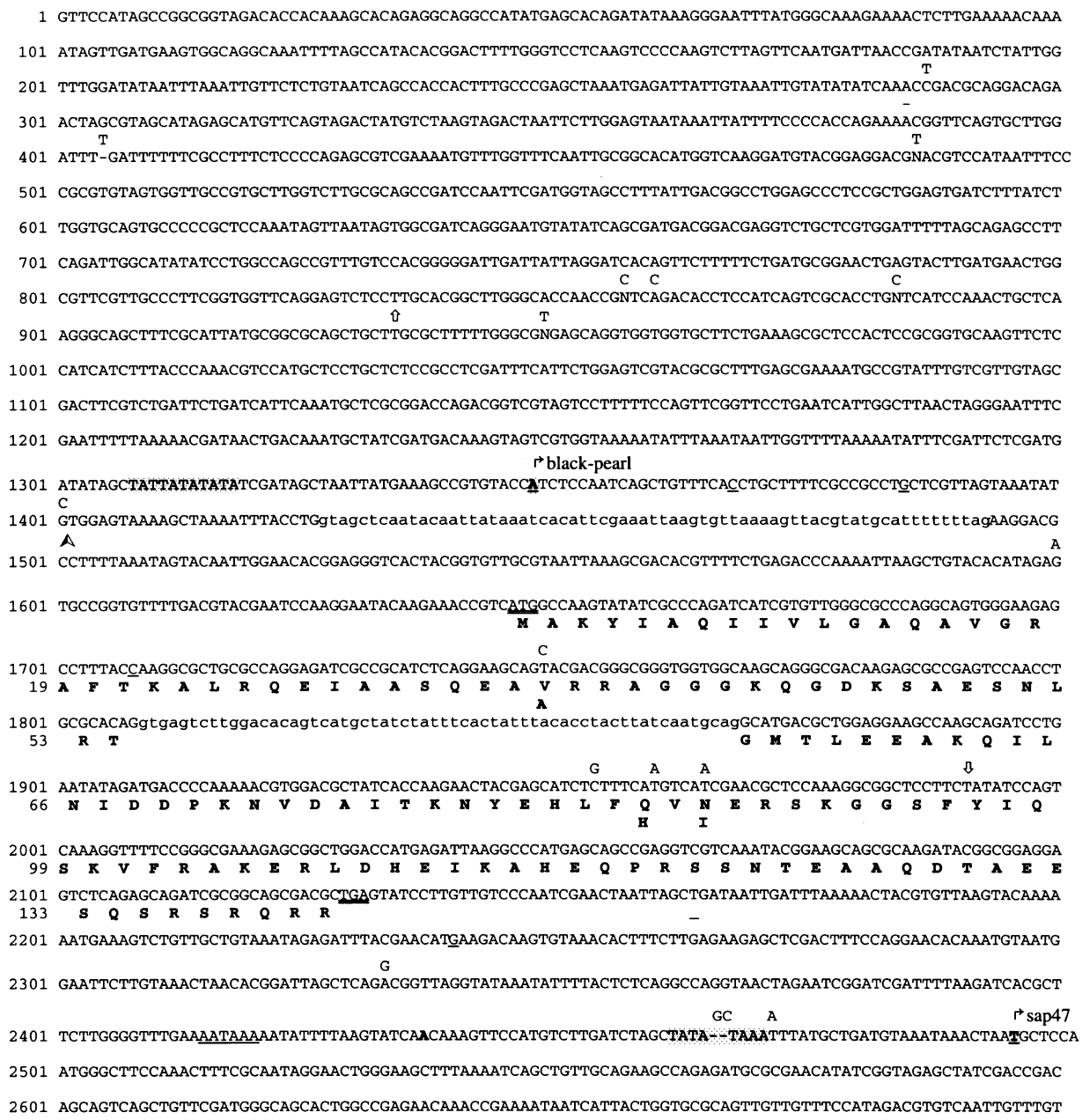

Fig. 3. Genomic sequence of the *blp* gene region. Computer analysis and comparison with cDNA clones identify putative TATA boxes (shaded) and transcription start sites (small arrows) of the *blp* and *Sap47* genes, the introns (lower case letters), the first ATG (putative translation start, double underlining), the encoded amino acid sequence (bold, one-letter code), the TGA translation stop (double underlining), as well as the polyadenylation site of cDNAs 1 and 3 (bold A at nt 2437) and the polyadenylation consensus (underlined) of the *blp* gene. The sequence between bp 835 and 1991 (marked by open arrows) has been replaced in the $blp^{\Delta 1}$ allele by 17 AT-rich base pairs of unknown origin. The P1539 insertion site in the blp^{P} mutant is indicated by the arrowhead before nt 1402. Bases different in the sequence now available from the BDGP are indicated above the sequence, interpretations of the differences with regard to the putative protein are given in section 3.1. The $5'$ end of the cDNAs 1, 2 and 3 are indicated by the underlined bases at positions 1708, 1368, and 1385, respectively, cDNA 2 ends at base 2240 (underlined) possibly due to internal priming during reverse transcription.

sequence now available from the Berkeley *Drosophila* Genome Project (BDGP, Adams et al., 2000) disclosed several base differences in non-coding parts and four in the coding region of the *blp* gene resulting three amino acid (aa) exchanges (valine to alanine at aa 35, glutamine to histidine at aa 85, asparagine to isoleucine at aa 87). At aa 35 this difference may be due to a polymorphism or a cloning/sequencing artefact since all three cDNAs and our genomic sequence encode a valine at this position. The other three base exchanges most likely represent a polymorphism as two cDNAs match our genomic sequence and one cDNA conforms at all three positions with the BDGP sequence. A TBLASTN search of public genetic data bases revealed that the *Drosophila* BLP shows 33% (35%) identity with a puta-

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D.M.BLP    -----MAKYI-AQIIVLGAQAVGRAFTKALRQEIAASQEAVRRAGGGKQGDKSAE------SNLRT
H.S.BLP    -----MAKYL-AQIIVMGVQVVGRAFARALRQEFAASRAAADARG--RAGHRSAAA-----SNLS-
D.M.BLPp   -----MARYL-AQIIILGAQLVGRALVKTMRQELQAFEDAARLQETLKANDPNSGR-----SAVAK
C.E.BLP    -----MPWRTALKVALAAGEAVAKALTRAVRDEIKQTQQAAARHA-ASTGQSASETRENANSNAKL
S.C.BLP    -----MAHRAFIQVIITGTQVFGKAFAEAYRQ--AASQSVKQGATNASRRGTGKGEYG--------
A.T.BLP    MGFMNWAGRLLANLIVMGSGIIGRAVFQAYRQ-ALANASKSGVAQEAMQNGVRQAGKA--------
A.T.JLP    MATPMIAGAAVAAAAVAGRY--GILAWQAFK-ARPRVPRMRRFYEGGFQSS----------------
A.T.UKP    MVAAIIAGAAVAAAAYAGKY--GIEAWQAFK-LRPVRPRMRKFYEGGFQAT----------------
D.M.UKP    ......................NEALKNLPKYDAESMAASKYYKGGFDPK----------------
D.M.CSP    ............................................................

D.M.BLP    GMTLEEAKQILNIDDPK---NVDAITKNYEHLFQVNERSKGGSFYIQSKVFRAKERLDHEIKAHEQ
H.S.BLP    GLSLQEAQQILNVSKL----SPEEVQKNYEHLFKVNDKSVGGSFYLQSKVVRAKERLDEELKIQAQ
D.M.BLPp   TMTLAEAQQILDVSDLT---NRQAIDTHYQHLFRVNDKSTGGSFYIQSKVFRAKERIDQELERTEL
C.E.BLP    GISLEESLQILNVKTPL---NREEVEKHYEHLFNINDKSKGGTLYLQSKVFRAKERIDEEFGRIEL
S.C.BLP    GITLDESCKILNIEESKGDLNMDKINNRFNYLFEVNDKEKGGSFYLQSKVYRAAERLKWELAQREK
A.T.BLP    -ITEQEARQILGVTEKT---SWEEILQKYDKLFENN--AKAGSFYLQSKVHRAKECL--EVVYRSQ
A.T.JLP    -MTRREAALILGVRESV---VADKVKEAHRRVMVANHPDAGGSHYLASKINEAKDMMLGKSNNSGS
A.T.UKP    -MNRREAALILGVRESV---AAEKVKEAHRRVMVANHPDAGGSHYLASKINEAKDMMLGKTKNSGS
D.M.UKP    -MNKREASLILGVSPSA---SKIKIKDAHKKIMLLNHPDRGGSPYLAAKINEAKD-FLDKAK
D.M.CSP    ......LYEILGLPKTA---TGDDIKKTYRKLALKYHPDKNPDNVDAADKFKEVNRAHSILSNQTK
                                                 ***

D.M.BLP    PRSSNTEAAQDTAEESQSRSRQRR
H.S.BLP    EDREKWQMPHT
D.M.BLPp   LVKTDDSHSILPTPPESSQFQCENKEPGQKSR
C.E.BLP    KEEKKKEENAKTE
S.C.BLP    NAKAKAGDASTAKPPPNSTNSSGADNSASSNQ
A.T.BLP    GNGTPS
A.T.JLP    AF
A.T.UKP    AF
D.M.UKP
D.M.CSP    RNIYDNYG...
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Fig. 4. Primary structure of putative BLP proteins as derived from genomic (*Drosophila* BLP paralogue D.M.BLPp, *C. elegans* C.E.BLP, *A. thaliana* A.T.BLP, *S. cerevisiae* S.C.BLP) or cDNA sequence (*Drosophila* D.M.BLP, human H.S.BLP). Significant homologies are also detected with DnaJ-like proteins (*A. thaliana* A.T.JLP) and an unknown protein (*A. thaliana* A.T.UKP) inferred from genomic DNA. The 'J' domain of the *Drosophila* cysteine string protein (D.M.CSP) is shown for comparison. Amino acids that are identical to the *Drosophila* BLP are shown on grey background to highlight conserved regions of the proteins, dots indicate sequence without similarity, dashes represent alignment gaps. Note that the functionally important amino acids HPD (asterisks) of the 'J' domain are not present in the BLP protein family.

tive yeast (*C. elegans*) protein inferred from genomic sequences and 56% identity with murine and human proteins inferred from cDNA clones (expressed sequence tags) (Fig. 4). The human EST sequence has now been verified by genomic sequence from the Human Genome Project at chromosomal position 16p13.3. Highly conserved protein domains (shaded in Fig. 4) are found at the N-terminus and near the C-terminus. Mouse and rat mRNA sequences can be inferred from multiple expressed sequence tags cloned from a wide variety of tissues (embryo, fetal liver/spleen, placenta, mammary gland, lung, heart) but have not been verified genomically.

At the amino acid level a distant similarity to DnaJ domains has been observed (Fig. 4) which can be readily detected using default thresholds in PSIBLAST (Altschul et al., 1997). Starting with *S. pombe* sequence as a query, members of the DnaJ family are found in the second iteration with statistical significance (expected ratio of false positives $E = 10^{-4}$). The closest members of the large DnaJ family (Bork et al., 1992) are all single domain proteins with a length of 110–160 residues, i.e. similar to the family described here. However, residues important for J-domain function, e.g. the HPD motif, are not conserved so that we conclude that this is only a structural relationship with no reliable functional information (cf. Section 4).

3.2. LacZ gene expression in the blp^P mutant

The modified PZ transposon inserted in line P1539 (now renamed blp^P) has been designed to allow visualization of enhancer activity operating on the weak transposase promotor of a transposase-lacZ gene construct that encodes an active bacterial β-galactosidase (βGal) (Mlodzik and Hiromi, 1992). Due to a nuclear localization signal of the transposase-βGal fusion protein, Xgal or immunohistochemical anti-βGal staining reveals the perikarya of cells in which an active enhancer interacts with the transposase promotor. Fig. 5A illustrates the distribution of βGal in whole mount preparations of late embryos (stage 15–17, Hartenstein, 1993). Small cell clusters in the antennal maxillary complex, in the brain, and along the ventral midline are clearly stained. Nine groups of at least two cells can be discerned along the ventral midline. In whole mounts of second instar larval brains a few perikarya can be

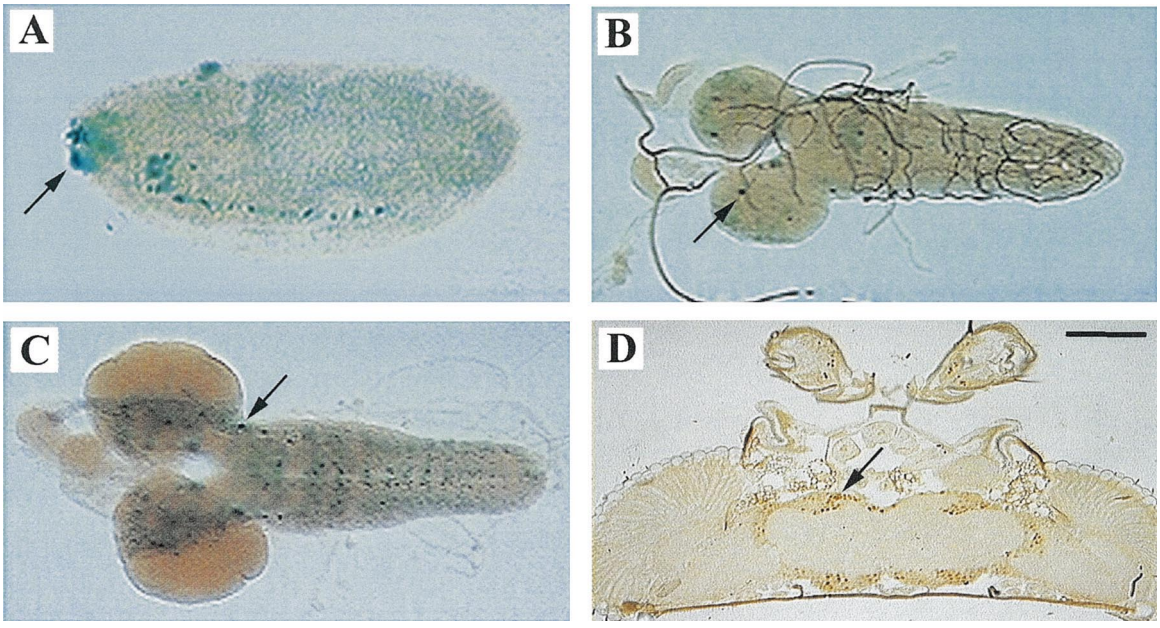

Fig. 5. X-Gal (A–C) and anti-β-galactosidase antibody (D) staining of late stage 13 embryo (A), second (B) and third (C) larval central nervous system, and horizontal adult brain section (D) demonstrate selective βGal expression in blp^P homozygotes. (The embryo in A may be either hetero- or homozygous). Scale bar, A: 70 μm, B: 80 μm, C,D: 100 μm.

recognized in the two hemispheres and in the ventral ganglion (Fig. 5B). A large number of cells stain in the brain and ventral ganglion of third instar larvae, exempting, however, the optic lobes (Fig. 5C). Four nuclei show particularly strong labeling (arrows). In addition to the clear nuclear staining, diffuse activity of βGal is observed in the central brain and in segmental stripes of the ventral ganglion. No staining was observed in eye or leg imaginal discs. In frozen sections of adult heads (Fig. 5D) a large number of perikarya in the cellular rind of the brain contain βGal.

Tissue in-situ hybridization using antisense RNA transcribed in vitro from *blp* cDNA failed to show any detectable staining above background in a series of experiments that produced clear positive results with two antisense RNAs from different genes. Also, antisera raised in rabbits and mice injected with bacterially expressed BLP-histidine-tag fusion protein detected the fusion protein in Western blots of induced bacterial lysates but failed to produce significant stainings in Western blots or tissue sections of *Drosophila*.

3.3. The semi-lethal phenotype of the blp^P line

Among 9885 adult offspring from TM3, ry balanced blp^P parents kept at 25°C, 464 flies (4.8%) were homozygous for the PZ insertion (‘escapers’). On average, these required 5 to 6 days longer to develop. At 18°C about the same fraction of homozygotes reach adulthood (5.2% of 15,577 flies) but eclosion is delayed by 14 days compared to balanced animals. Flies heterozygous for the blp^P mutation show no obvious phenotype.

At the transition from second to third larval stage all homozygotes develop pigment encapsulated cell clusters which gradually lose their cellular appearance and transform into dark brown to black inclusion bodies of variable sizes (‘*black pearl*s’, Fig. 6). These melanotic ‘tumors’ are found at arbitrary sites apparently without contact to the surrounding tissue. In surviving adults the ‘tumors’ are located predominantly in the abdomen, less frequently in the thorax, but stop growing and appear to be benign without obvious effects on longevity. All aspects of the phenotype are reverted to wild type when the PZ element is lost in a transposon remobilization experiment.

3.4. Knock-out mutagenesis of the blp gene

By remobilizing the PZ element of the strain blp^P (cf. Section 2) we have found among 22,500 F1 offspring 300 balanced ry^- flies. The jump-out chromosomes were analyzed for phenotypic traits in combination with the Po^4 chromosome (Nelson and Szauter, 1992) in which the region 88F7, 8-89A1; 89A11-13 has been deleted. Chromosomal in situ hybridization using genomic DNA covering both the *blp* and the *Sap47* genes as a probe had verified that both genes are deleted in the Po^4 chromosome (data not shown). Most of the lines established from the 300 flies could not be discriminated from wild-type controls. For these, Southern blots of restriction fragmented genomic DNA indicated a precise jump-out of the P element. Eight lines were homozygous lethals and did not complement each other. By Southern blotting it could be shown that two jump-out chromosomes, $blp^{\Delta 1}$ and $blp^{\Delta 2}$, had lost the PZ element and suffered a small deletion of about 1.2 kb around the insertion site. Sequencing of PCR amplified DNA from heterozygous adults mapped the deficiency of

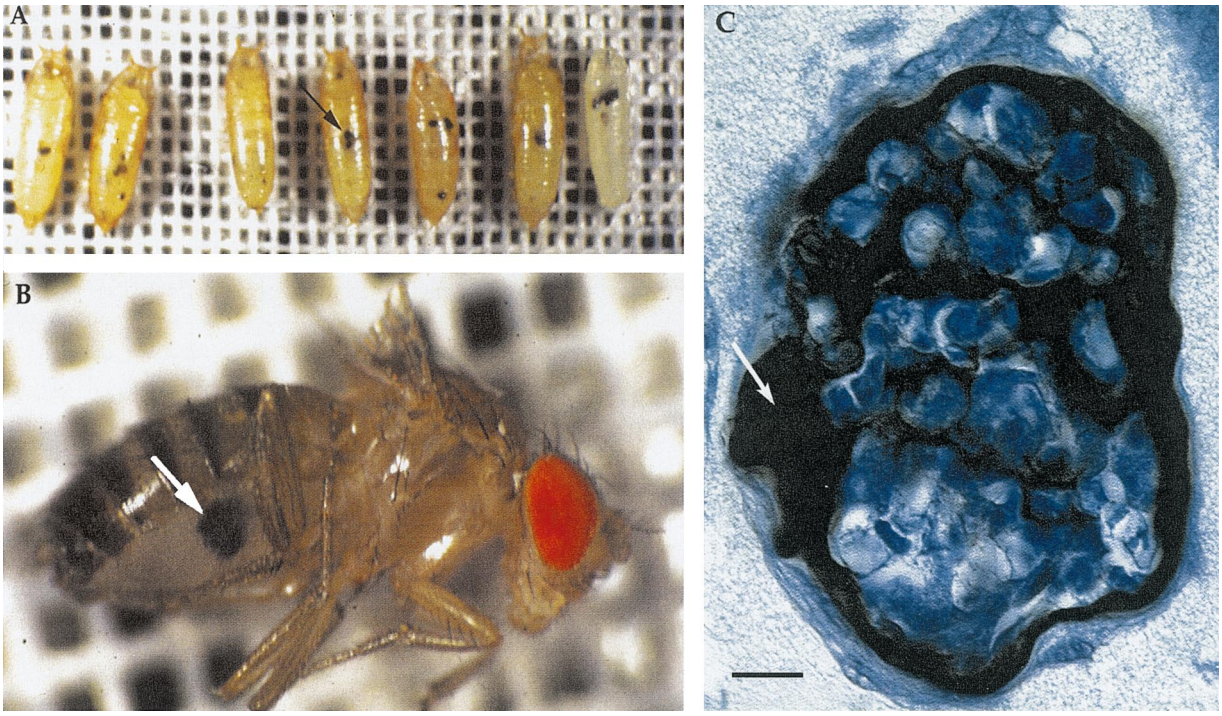

Fig. 6. Pupae (A) and adults (B) homozygous for the PZ insertion of line P1539 (blp^{P}) with melanotic 'tumor' inclusions (arrows). (C) Hematoxilin staining of a paraffin section through an inclusion containing large amounts of pigment (arrow). Scale bar, A: 1 mm, B: 280 μm, C: 10 μm.

$blp^{\Delta 1}$and $blp^{\Delta 2}$. A sequence of 1157 bp (between open arrows in Fig. 3) has been replaced by a stretch of 17 bp of unknown origin in line $blp^{\Delta 1}$ and 1275 bp have been deleted in line $blp^{\Delta 2}$. These deletions eliminate most or all of the presumed *blp* promoter, the untranslated leader and more than half of the coding region. The $blp^{\Delta 1}$ and $blp^{\Delta 2}$ chromosomes thus represent molecular null alleles of the *blp* gene. A third deletion extended into the *Sap47* gene and thus constitutes a *blp-Sap47* double mutant chromosome.

In an EMS mutagenesis, 7000 lines were screened for lethality over this *blp-Sap47* double mutant chomosome. Two chromosomes with lethal mutations were isolated and identified as alleles for the *blp* gene (blp^{E1} and blp^{E2}) by complementation analysis. No differences to wild type were seen in Southern blots indicating that these alleles presumably represent point mutations.

3.5. The blp null phenotype

Both deletions $blp^{\Delta 1}$ and $blp^{\Delta 2}$ as well as the two EMS alleles blp^{E1} and blp^{E2} are homozygous first instar lethals but heterozygotes show no obvious dominant phenotype. With help of the green fluorescent protein (GFP) marked balancer chromosome TM3-GFP homozygous *blp* first instar larvae could be identified. As quantified for $blp^{\Delta 1}$, about 98% of these embryos hatch but fail to grow such that second instar larvae are found only in extremely rare cases. Third instar larvae have never been observed. Ninety percent of homozygous *blp* larvae die within 7 days after hatching (Fig. 7), while their balanced siblings (wild-type control) pupariate under the same conditions after 5 days. Raising larvae on medium stained with food color indicates, however, that *blp* mutant first instar larvae take up normal amounts of food. Serial 2 μm sections of EPON-embedded first instar larvae show no obvious morphological defect (data not shown). No obvious motor differences are observed.

4. Discussion

4.1. Mutant phenotypes

While targeted mutagenesis (Walter, 1992; Eberle et al., 1998; Lenz-Böhme et al., 1997) had failed to uncover

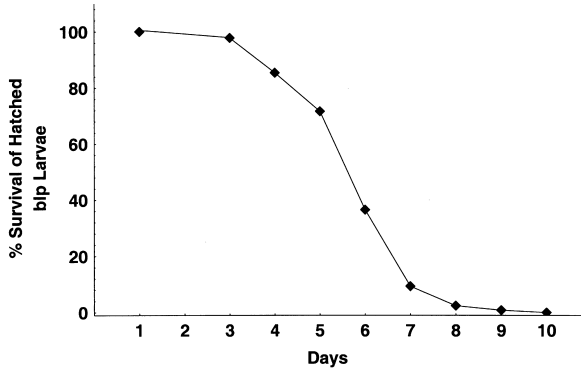

Fig. 7. Survival curve for homozygous *blp*-null mutant first instar larvae that successfully hatched but fail to grow ($n = 89$).

among 34,000 lines a transposon insertion in the *Sap47* genomic region, we detected, among 11 strains with a P element that had been mapped to region 89A-B by in situ hybridization as part of the *Drosophila* genome project, the line l(3)01618 (P1539) with a PZ insertion just 1.2 kb upstream of the presumed *Sap47* transcription start site. Western blots and immunohistochemistry using an anti-SAP47 monoclonal antibody (Reichmuth et al., 1995) showed no recognizable influence of the P insertion on SAP47 expression (data not shown). Molecular analysis revealed that the insertion occurred in the 5′ non translated leader of an independent gene, *blp*, which is situated immediately upstream of the *Sap47* gene. By generating two deletion mutants and two EMS alleles we show that the observed phenotype of the PZ insertion line reflects a hypomorphic or neomorphic mutation with residual *blp* gene function. Insertion of a 14 kb transposon containing various bacterial and eukaryotic gene fragments into the untranslated leader of a single copy gene is likely to reduce mature mRNA levels due to prolonged transcription. In fact, since the PZ insertion lies upstream of the presumed translation start site, transcription from some promotor-like sequences within the PZ element may result in an mRNA from which the correct protein could be translated, albeit with a different spatial and temporal expression pattern. Thus the complex phenotype of homozygous P1539 flies, semi-lethality, delayed development, melanotic inclusions, and reduced fertility of homozygotes, cannot yet be interpreted at the molecular or cellular level. To quantify semilethality we have counted among offspring of balanced parents only about 5% P1539 homozygotes (instead of 33% expected if the insertion had no effect on viability). It is also at present not possible to decide whether semi-lethality, delayed development, and melanotic inclusions are all direct consequences of *blp* misexpression or if, e.g. delayed development may more indirectly result in some of these phenomena. It is intriguing to note that mutations in more than 25 different gene loci of *Drosophila* have been identified that lead to melanotic inclusions similar to the blp^P phenotype (for review cf. Sparrow, 1978). Some of these genes have been cloned and molecularly analyzed. Since their gene products show no functional similarity or common properties, no inference is possible as to the function of the BLP protein. Rather, it seems likely that various ways of interference with normal development may lead to this malfunction of the fly's immune system. For this reason characterization of the null mutants generated here in principle should provide more information about the function of the BLP protein than the blp^P phenotype. The *blp*-null phenotype shows conspicuous similarity to that of *Drosophila peter pan* mutants which cannot synthesize the conserved protein PPAN. In *Drosophila* PPAN appears to play an essential role in cell growth (Migeon et al., 1999), while yeast homologues of PPAN seem to be transported to the nucleus (Kim and Hirsch, 1998) and are required for cell division (Yu and Hirsch, 1995). No information on yeast BLP homologues is presently available. However, as larval growth in *Drosophila* is mainly due to cell growth by endoreplication rather than by mitotic cell division (Britton and Edgar, 1998), it is tempting to speculate on the basis of the described phenotypic similarity between *ppan* and *blp* null mutants in *Drosophila* that the BLP protein family may also be involved in cellular growth.

The conspicuous distribution of lacZ staining due to the PZ insertion (Fig. 6) is suggestive but due to the possibility of complex interactions of the weak transposase promotor with unknown transcription factors regulating *blp* expression or with enhancers of other genes of the region, the β-Gal staining may not faithfully reflect the distribution of BLP in *Drosophila*. The stainings show no similarities to the rather homogeneous distribution of the SAP47 protein which is found throughout development in most or all neurons (Reichmuth et al., 1995, and unpublished data). A selective expression of the *blp* gene in a specific subset of neurons would, however, not contradict a general function of BLP in cellular growth as independent functions of the same protein are frequently observed in different developmental stages. Tissue in situ hybridization and the generation of antibodies to resolve this issue were unsuccessful. Our failure to detect selective mRNA localization in embryos could be explained by assuming low homogeneous expression of the *blp* mRNA in all tissues, which would be difficult to discern from unspecific background staining, but still could add up to give the strong Northern signal seen in Fig. 2. However, technical problems specific for the *blp* anti-sense probe cannot be ruled out.

4.2. A novel protein family

The fact that the *Drosophila* BLP protein shows high homologies with inferred proteins of yeast, *A. thaliana*, *C. elegans*, mouse and human implies that this novel gene family has been highly conserved in evolution. Since the amino acid sequence shows weak but significant similarity to the 'J' domain of DnaJ-like proteins (Fig. 4) one may speculate that BLP interacts with other proteins, possibly as a co-chaperone, a role that has been demonstrated for several members of the DnaJ protein family (Silver and Way, 1993). The DnaK (HSP70) family is most likely not the target of BLP family proteins since the functionally important HPD motif of the 'J' domain is not found in BLP proteins. Yet, the conservation of several amino acids in this domain extends even to more complex proteins with a 'J' domain such as the cysteine string protein of *Drosophila* (Zinsmayer et al., 1990; Eberle et al., 1998) as shown in Fig. 4.

From systematic genome sequencing of various organisms it has become clear that as much as half the genes of higher eukaryotes code for proteins of entirely new families. To obtain information on functional aspects of novel conserved protein families genetic model systems can be employed for the targeted elimination and experimental

manipulation of the corresponding genes. The present work represents a first major step in this approach to study the function of the BLP protein family. Assuming that maternal RNA cannot provide sufficient protein through embryonic and first instar larval development our data suggest that in *Drosophila* BLP may not be required for cellular survival in general. The issue is confounded, however, by the fact that a BLP paralogue (Fig. 4) is encoded in the *Drosophila* genome at position 7C4–7C5 on the X chromosome (Adams et al., 2000). Possibly, one member of the new protein family described here may be sufficient to maintain general cellular function. Targeted mutagenesis of the *blp* paralogue and mosaic techniques can now be employed to clarify these questions.

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