Drosophila differentiation genes instrumental in tumor suppression

ELISABETH GATEFF[1]*, URSULA KURZIK-DUMKE[1], JASMINE WISMAR[1], THOMAS LÖFFLER[1], NEGUSSE HABTEMICHAEL[1], LUTZ KONRAD[2], STEPHAN DRESCHERS[1], SIBYLLE KAISER[3] and URSULA PROTIN[1]

[1]*Institut für Genetik, Johannes Gutenberg-Universität, Mainz, [2]Anatomisches Institut der Universität Marburg, Marburg and [3]I. Medizinische Klinik und Polyklinik, Johannes Gutenberg-Universität, Mainz, Germany*

ABSTRACT   Tumor suppressor genes of *Drosophila* are developmental genes which, in the homozygously mutated state, induce in one step malignant or benign neoplastic transformation of specific cell types. They act early in development and by this set the stage for cell specific differentiation of imaginal discs, adult optic neuroblasts, blood and gonial cells. The structure, expression and possible function of the following four tumor suppressor genes are discussed: *tumorous imaginal disc, lethal(3)malignant brain tumor, lethal(3)malignant blood neoplasm-1* and *benign(2)gonial cell neoplasm.*

KEY WORDS: *Drosophila, tumor suppressor genes, differentiation*

Introduction

During normal development, growth control, differentiation and the maintenance of the differentiated state are intricately coordinated. Despite the far-reaching discoveries of genes instrumental in cell cycle control (Nurse, 1990; Saint and Wigley, 1992; Orr-Weaver, 1994) and tumorigenesis (Hunter, 1991) very little is known about their developmental interactions. The aberrant coordination between cell growth and differentiation is most dramatically seen in cancer, where mitotically active and developmentally determined cells fail to differentiate and, consequently, engage in autonomous malignant or benign neoplastic growth.

In *Drosophila melanogaster* about 28 recessive-lethal genes have been identified which, in the mutated state, cause malignant or benign neoplastic transformation of specific cell-types in the embryo, the larva and the adult (Gateff, 1978a,b, 1982a, 1994; Gateff and Mechler, 1989; Bryant, 1993). Apparently, the genetic information, provided by the wild-type alleles of these genes, suppresses tumorous growth by promoting cell differentiation. The cloning of 12 of these genes supports this view. Most of the gene products are found in the cytoplasm or associated with the cell membrane, where most of them seem to be instrumental in cell-cell communication or signal transduction (Bryant et al., 1993).

We have cloned the following four tumor suppressor genes: (i) *lethal(2)tumorous imaginal discs*, (ii) *lethal(3)malignant brain tumor*, (iii) *lethal(3)malignant blood neoplasm-1* and (iv) *benign(2)gonial cell neoplasm*. In the following text we present pertinent data for each of the genes and discuss their possible functions in tumor suppression.

lethal(2)tumorous imaginal discs [*l(2)tid*] encodes the *Drosophila* homolog of the chaperone *DnaJ*

Mutations in the tumor suppressor gene *l(2)tid*⁺ cause in homozygotes malignant transformation of the imaginal discs and the death of the mutant larvae at the time of puparium formation (Kurzik-Dumke et al., 1992). The neoplastic imaginal discs, unable to differentiate, engage in an autonomous, lethal growth *in situ* as well as after transplantation *in vivo* into the abdomens of wild-type flies. The *l(2)tid*⁺ gene lays in polytene chromosome bands 59F4,5. An extensive genomic walk, performed in the polytene chromosome region 59F1-60A7 (Gundacker et al., 1993), allowed the molecular localization of the *l(2)tid*⁺ gene to a 20 kb genomic region (Kurzik-Dumke et al., 1995). cDNA-, exon- and transcript-analysis of this genomic region revealed three transcription units representing three genes. In order to identify which of the three genes represents the *l(2)tid*⁺ gene, Southern- and Northern analysis of hetero- and homozygous mutant animals from tumor-bearing alleles 1 and 2 and the non-tumorous fourth allele, together with the corresponding progenitor strains, were performed (Kurzik-Dumke et al., 1995). Southern analysis restricted the *l(2)tid*⁺ gene region from 20 to 10 kb of genomic DNA harbouring two closely interspaced genes, *l(2)tid*⁺ corresponding to cDNA 10D and the *lethal(2)neighbour of tid* [*l(2)not*] represented by cDNA 10F (Fig. 1A). The demonstration that cDNA 10D belongs to the *l(2)tid*⁺ gene was performed via transcript analysis of wild-type (Fig. 1B) and homozygous mutant larvae (Fig. 1C). In Northern blots from homozygotes of the two tumor-bearing *l(2)tid* alleles 1 and 2 the constitutively expressed 2.0 kb transcript, recognized by cDNA 10D, is absent (Fig. 1C, arrows). In contrast, the 0.82 kb larval-specific transcript, also

detected by cDNA 10D, and the constitutively expressed 2.2 kb transcript, identified by cDNA 10F (Fig. 1B,C), are present. Changes of the RNA expression pattern could also be seen *in situ* in the tumorous imaginal discs (U. Kurzik-Dumke, unpublished results).

In order to determine the organization of the *l(2)tid+* and *l(2)not+* genes, cDNAs 10D and 10F and the corresponding 7.0 kb genomic HindIII-fragment (Fig. 1A) were sequenced. The *l(2)tid+* gene spans a total of 2,539 nucleotides and resides on the opposite DNA strand in the 2.6 kb intron of the *l(2)not+* gene. The temporal and tissue-specific overlap of expression (Kurzik-Dumke, unpublished) and the nested arrangement of these two genes suggest, but do not prove, a functional relationship between the *l(2)tid+* and *l(2)not+* genes. Supporting this assumption are germ line transformation experiments, which showed rescue of the *l(2)tid* tumor phenotype only with a construct including both genes (Kurzik-Dumke *et al.*, 1995). The *l(2)tid+* gene, consisting of two exons (1,696 bps) which are subdivided by an intron of 142 bps (Fig. 1A), encodes a predicted Tid56 protein of 518 amino acids and a molecular weight of 56 kDa. It shows significant homology to all presently known DnaJ-homologous proteins from bacteria, yeast, and man (Kurzik-Dumke *et al.*, 1995; Fig. 1D). Ongoing investigations are concerned with the question whether the Tid56 protein belongs, like its *E. coli* and yeast homologs, to the hsp 70 chaperone machinery, and whether it possesses similar functions.

lethal(3)malignant brain tumor [*l(3)mbt*] belongs to the Polycomb-Group gene family and codes for a novel proline-rich zinc finger protein.

The homozygous third instar larvae of the temperature-sensitive *l(3)mbt*[ts1] allele exhibit, at the restrictive temperature (29°C), malignant brain tumors consisting of autonomously and invasively growing, differentiation-incompetent adult optic neuroblasts and ganglion-mother cells (Gateff, 1982a; Gateff *et al.*, 1993).

Shift-up experiments revealed an early embryonic temperature-sensitive period for tumor-suppression, encompassing the first 6 h of embryonic life. Shifts to the restrictive temperature during this period yielded 100% brain tumors (Gateff *et al.*, 1993). Between the 6th and 8th developmental hour an abrupt reduction of brain tumors down to 30% was observed. The brain tumor incidence thereafter declined gradually and reached zero around the mid second larval instar. Shifts performed during the third larval instar and the pupal period did not produce brain tumors anymore, but led to lethal adult brain defects (Gateff, personal observation) and female sterility (Gateff and Miyamoto, 1990).

The *l(3)mbt+* gene is located in polytene chromosome bands 97F3-11 (Gateff *et al.*, 1993). A successful P-element germ line transformation and the sequences of three overlapping cDNAs defined a genomic region of 6.8 kb containing the gene (Wismar *et al.*, 1995; Fig. 2B,C).

Developmental wild-type Northerns showed seven transcripts of 1.8 kb, 4.4 kb, 5.0 kb, 5.25 kb, 5.35 kb, 5.65 kb and 5.8 kb with developmental-specific expression (not shown). In adult females and in 0-3 h old embryos the maternal 5.25 kb and 5.0 kb transcripts are expressed strongly. In contrast, the 5.8 kb and 4.4 kb

transcripts show weak expression throughout development. During the next 3 h of embryonic life (3-6 h) two additional weakly expressed transcripts are detectable (5.35 kb, 5.65 kb). In mutant embryos of comparable age the 5.25 kb, 5.35 kb and 5.65 kb transcripts are missing. Thus we consider the absence of these three transcripts, which correspond well with the temperature-sensitive period for tumor suppression, responsible for the malignant neoplastic transformation of the adult optic neuroblast precursors.

l(3)mbt+ shows ubiquitous expression in stage 1 embryos (Fig. 2D), which in subsequent developmental stages becomes regionalized to the subcellular blastoderm cytoplasm (Fig. 2E), the germ band (Fig. 2F), and the central nervous system (Fig. 2G). In addition, other tissues express the *l(3)mbt+* gene, but at a lower level. While during the two first larval instars *l(3)mbt+* is expressed throughout the central nervous system, in the third instar larva only the presumptive adult optic neuroblasts in the brain show expression (Fig. 2H). In contrast, the mutant brain hemispheres exhibit ubiquitous expression (Fig. 2I) due to the neoplastic neuroblasts and ganglion-mother cells which invade all healthy portions of the brain.

The genomic organization of the *l(3)mbt+* gene, corresponding to the 5.25 kb transcript, is seen in Fig. 2C. Three exons define an open reading frame of 4431 bp and a putative protein of 1477 amino acids with a molecular weight of 163 kDa. The MBT163 protein is characterized by a high proline content of 9.3%. Proline is especially enriched (12%) within the N-terminal 632 amino acids (Wismar *et al.*, 1995). A search for conserved motifs revealed a novel single zinc finger of the CC-CC-type (Klug and Rhodes, 1987). Furthermore, three repeats of 99-105 amino acids, designated as *mbt*-repeats, are present in the C-terminal half of the putative MBT163 protein (Wismar *et al.*, 1995). These repeats show homology to repeats present in the *Pc-G* gene *sex comb on mid leg* (*Scm*; Bornemann *et al.,* 1996). In the C-terminal region a *SAM* (*Sterile α Motif*) box is present, which shows high homology to similar *SAM* boxes found in the *Drosophila Polyhomeotic* and the mouse *Rae28* genes (Ponting, 1995). By the aid of specific anti-MBT163 antibodies the protein is found in the nucleus. The above findings indicate strongly that the *l(3)mbt* tumor suppressor gene belongs to the *Pc-G* gene family.

lethal(3)malignant blood neoplasm-1 [*l(3)mbn-1*] encodes a cytoplasmic protein with Gly/Ser-rich repeats

Two blood-cell types originate in the wild-type larval hematopoietic organs: (i) the phagocytic plasmatocytes and (ii) the crystal cells involved in hemolymph coagulation and melanization (Rizki, 1978; Shresta and Gateff, 1982). The wild-type hematopoietic organs release a relatively small number of blood cells into the hemolymph, where most of them remain attached to the basement membranes.

Contrary to the well regulated wild-type hematopoiesis, recessive-lethal mutations in the *l(3)mbn-1+* gene cause an enormous overproliferation of plasmatocytes in the hematopoietic organs and after their release into the hemolymph (Shresta and Gateff, 1986). In contrast to their wild-type counterparts, the mutant plasmatocytes recognize "self" as foreign and phagocytose the tissues of the mutant larvae.
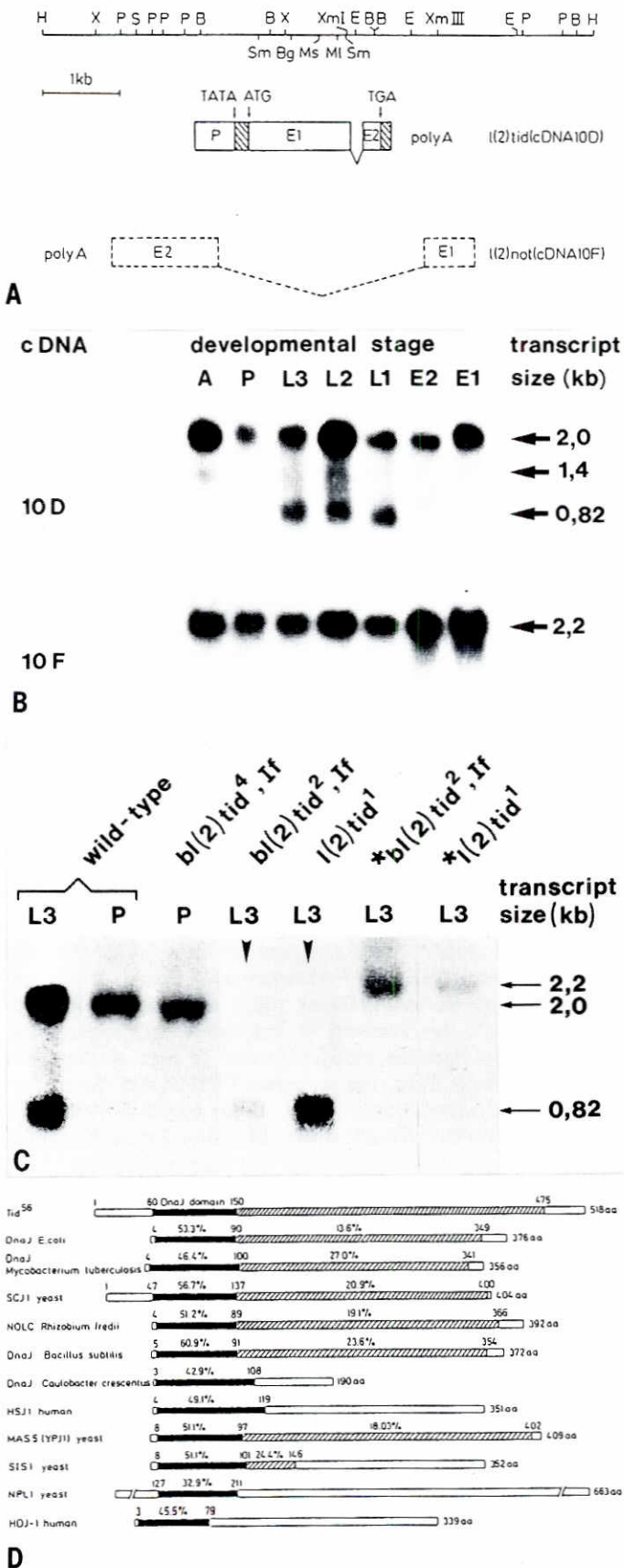
The *l(3)mbn*[+] gene is located in polytene chromosome bands 65A1,2. Its molecular position within this region was established via a 70 kb genomic walk (Fig. 3A), Southern and Northern analysis, a P-element insertional mutation (Fig. 3B) and the successful germ line rescue of the mutant phenotype (Konrad *et al.*, 1994) confirm the identity of the gene.

A 2.6 kb transcript is detected in the embryo and all three larval stages, while in the pupa and the adult two transcripts of 1.35 kb and 1.8 kb are found. We consider the protein encoded by the 2.6 kb transcript endowed with tumor suppressor function. The cDNA sequence, corresponding to this transcript, reconstructed from the sequences of three overlapping cDNAs (Fig. 3C), was compared with the genomic sequence. This analysis yielded a gene with 9 exons spanning approximately 5.5 kb of genomic DNA (Fig. 3D,E; Konrad *et al.*, 1994). The coding sequence is characterized by three classes of repeats, located in exons 6, 7 and 9 (not shown). The predicted MBN[83] amino acid sequence consists of 796 amino acids. Two putative transmembrane domains are present in the N-terminal region. The proline-rich central domain contains two repeat classes which show no homology to known repeats. In the C-terminal domain 7 Gly-Ser-rich repeats are prominent, exhibiting approximately 40% amino acid identity to the Gly-Ser-rich repeats, found in the tail domain of human cytokeratins K1 and K10 and mouse loricrin. However, the putative MBN[83] protein cannot be a cytokeratin, since it lacks the head and rode domain characteristic of cytokeratins.

The *l(3)mbn-1*[+] is expressed in the hematopoietic organs (Fig. 3E) and blood cells, but also in larval organs, such as the midgut, salivary glands and fat body (not shown). By the aid of a polyclonal antibody the MBN[83] protein can be detected in the cytoplasm of blood cells (Fig. 3F,G). Antibody staining of wild-type

Fig. 1. Molecular identification of the *l(2)tid*[+] gene. (A) *Structure of the nested l(2)tid*[+] *gene in relation to the l(2)not*[+] *gene and the 7.0 kb genomic HindIII-fragment which rescues the l(2)tid mutant phenotype. B, BamHI; Bg, BglII; E, EcoRI; H, HindIII; Ml, MluI; Ms, MstI; P, PstI; S, SalI; Sm, SmaI; X, XhoI; Xml, XmaI; XmIII, XmaIII.* **(B)** *Wild-type, developmental Northern probed with cDNAs 10D and 10F.* **(C)** *Northern blot of larval (L3) and pupal (P) poly(A+)mRNA from wild-type and mutant alleles 1,2 and 4, probed with cDNA10D (lanes 1 to 5) and cDNA10F (lanes 6,7, designated with asterisks). Note the absence of the constitutively expressed 2.0 kb transcript in alleles 1 and 2 (arrows) and its presence in wild-type larvae and pupae and in non-tumorous pharate adults derived from allele l(2)tid4 (lanes 1 to 3). The 2.2 kb transcript, detected by cDNA10F, is present in the tumor-bearing larvae of l(2)tid alleles 1 and 2 (lanes 6,7). A, adult; E1, embryo 0-10 h old; E2, embryo 10-20 h old; L1,2,3, larva 1,2,3.* **(D)** *Comparison of the homologous (black and striped bars) and non-homologous (open bars) regions of five prokaryotic (Bardwell et al., 1986; Ohki et al., 1986; Lathigra et al., 1988; Rothblatt et al., 1989; Wetzstein et al., 1990; Gomez et al., 1990; Krishnan and Pueppke) and six eukaryotic DnaJ proteins (Blumberg and Silver, 1991; Caplan and Douglas, 1991; Luke et al., 1991; Raabe and Manley, 1991; Sadler et al., 1989; Atencio and Yaffe, 1992; Cheetham et al., 1992) in relation to the Tid56 protein. The black bars represent the DnaJ-domain exhibiting the highest amino acid identity scores. Striped bars illustrate regions of lower homology, not present in all proteins, while white bars indicate non-homologous regions. The amino acid identities within these regions are indicated in percentages. The numbers above the bars represent the amino acids within the sequence delimiting the individual domains.*

embryos detects the protein in stage 1 and 2 embryos at the anterior and posterior poles and along a narrow ventral stripe (Fig. 3H). In subsequent preblastoderm and blastoderm stages the protein is found evenly distributed over the entire embryo including the pole cells (Fig. 3I). During gastrulation and germ band elongation the protein continues to be distributed ubiquitously (not shown). At stages 13 and 14 blood cells, expressing MBN[83] proteins, can clearly be seen in different regions of the embryo (Fig. 3J).
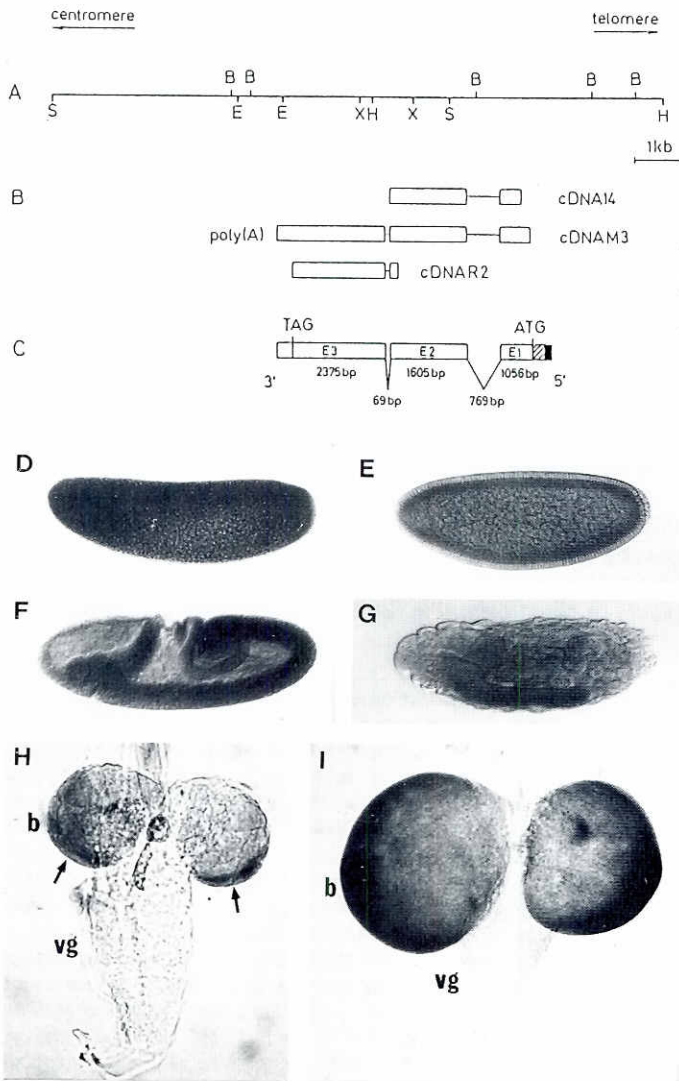
Fig. 2. *l(3)mbt*+: gene structure and *in situ* expression. **(A,B)** *Extent of cDNAs in relation to the 13 kb genomic SalI-HindIII fragment.* **(C)** *Exon-intron structure corresponding to the 5.25 kb transcript. The numbers indicate the length in bp of the single exons and introns. The shaded box represents 264 bp of the 5' untranslated region verified by PCR and the black box 161 bp up to the putative transcription start as identified via primer extension.* **(D-G)** *Whole-mounts of stages 1, 5, 9 and 17 wild-type embryos hybridized with digoxigenin-labeled cDNA14 antisense RNA.* **(H,I)** *Whole-mounts of wild-type and mutant brain-ventral ganglion (b, vg) complexes. Note the hybridization signal in the presumptive adult optic centers (arrows) of the wild-type brain (b) and the ubiquitous staining of the tumor-bearing mutant brain.*

benign(2)gonial cell neoplasm [b(2)gcn] causes germ line tumors in both sexes

The *b(2)gcn*+ gene, located in polytene chromosome band 60A3, was cloned via a 70 kb genomic walk and two overlapping deletions *Df(2R)OV1* and *Df(2R)OV2* (Fig. 4A). The deletion analysis demonstrated that *b(2)gcn*+ gene sequences are located within the 10 kb of DNA determined by the telomeric breakpoints of the above deficiencies (Fig. 4A). The cloning of the *b(2)gcn*+ gene was confirmed by germ-line rescue experiments, a P-element insertional mutation (Fig. 4A) and a revertant.
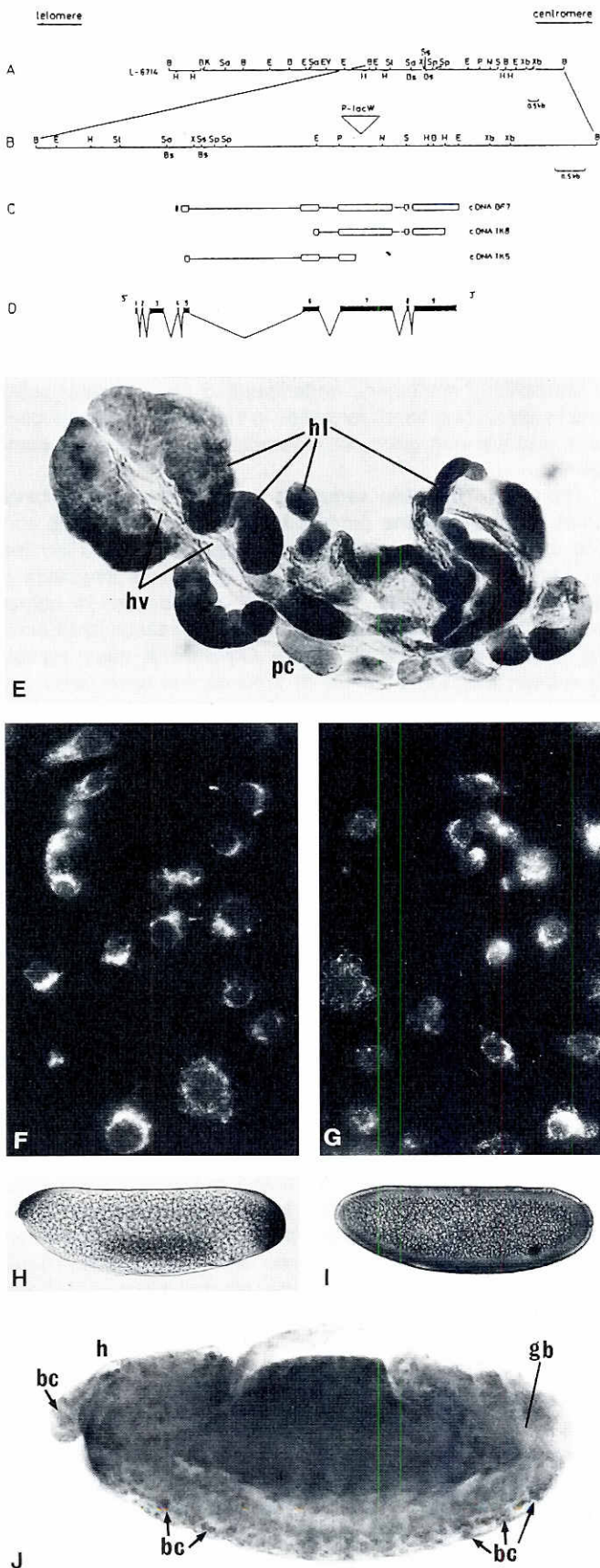
We have sequenced the *b(2)gcn*+ gene which spans approximately 11 kb of genomic DNA. The 3'end of the gene could be determined with the aid of 3 cDNAs which share sequences with 4.5 kb of the gene. For the 5'upstream 6.5 kb of the gene no cDNA could be isolated. Nevertheless, comparison of the genomic sequence with that of the three cDNAs revealed that they represent differential splice products.

b(2)gcn+ is expressed throughout development and codes for at least six transcripts (not shown), none of which is restricted to the germ line. In the wild-type ovary earliest *b(2)gcn*+ expression was detected in germarial region R2 (Fig. 4B). *b(2)gcn*+ expression is clearly reduced during oogenesis stages S1 and S2 and becomes stronger in stages S3 to S5. Stages 6 to 8 lack *b(2)gcn*+ expression. From stage 9 onward, maternal *b(2)gcn*+ mRNA is abundantly present in nurse cells, which at stage 12 is transported into the egg cytoplasm (not shown). In mutant ovaries no *b(2)gcn* mRNA was detected (Fig. 4C). The *b(2)gcn*+ gene is expressed ubiquitously throughout embryonic life (Fig. 4 D-F) and in the larval fat body (not shown).

Comparison of the putative *b(2)gcn*+ amino acid sequence, deduced from the sequences of the 3 cDNAs with known proteins revealed a high identity score of an Arg-Ser-rich domain to similar domains in U1snRNP 70K protein (Mount *et al.*, 1983), U2snRNP (Lamond, 1993), splice factors SC35 (Fu and Maniatis, 1992), ASF, U2AF (Zamore and Green, 1992) and *transformer* (*tra;* Burtis and Baker, 1989). All these proteins have been shown to be involved in pre-mRNA splice reactions. Present in the putative *b(2)gcn* protein is also another well known sequence motif, the so-called PRD-repeat, which has been found in *paired* (Frigerio *et al.*, 1986), *bicoid* (Berleth *et al.*, 1988), *daughterless* (Caudy *et al.*, 1988) and *forked* (Hoover *et al.*, 1993) gene products. The wide distribution of *b(2)gcn*+ transcripts and the above similarities imply a broad spectrum of *b(2)gcn* functions in the germ line and other cells.

Discussion

All efforts to prove the causal involvement of dominant cellular oncogenes, such as *abl* (Henkemeyer *et al.*, 1987), *src* (Simon *et al.*, 1985) or *ras* (Bishop and Corces, 1988) in the initiation of *Drosophila* malignant growth, have failed. Mutations in these genes exert only minor, non-lethal effects in the mutant animals. Cell cycle control genes, likewise, do not cause cancer in *Drosophila* (Orr-Weaver, 1994). Cancer as a multistep process, postulated for vertebrates (Hunter, 1991), also does not apply to *Drosophila* tumorigenesis. The *Drosophila* tumor suppressor genes are distinct in that they induce, in the homozy-

gously mutated state, malignant neoplastic transformation of a particular cell type in one step.

Twelve of the 28 *Drosophila* tumor suppressor genes are cloned, and the functional analysis of some of the gene products is well advanced. These studies revealed key functions of these genes in protein synthesis, cell-cell communication and signaling (Bryant, 1993). Vertebrate homologs to some of the genes also could be identified (Watson and Bryant, 1993). Recently Strand *et al.* (1995) cloned the human *l(2)gl* ortholog *hugl* gene and showed that, similarly to the *Drosophila* counterpart, the HUGL protein is part of a cytoskeletal network associating with non-muscle myosin II heavy chain and a serine kinase, thus supporting the idea that both proteins function in an identical manner.

Each of the four tumor suppressor genes, described above, transforms one of the following four cell-types and tissues: (i) the imaginal discs, (ii) the adult optic neuroblasts in the larval brain, (iii) the plasmatocytes, and (iv) the gonial cells of both sexes. The four putative gene products suppress tumorigenesis by promoting cell-specific differentiation. Based on amino acid homologies hypothetical functions can be predicted for three of the four genes.

For instance, the putative Tid56 protein contains a typical DnaJ domain (Kurzik-Dumke *et al.*, 1995), characterizing it as a member of the DnaJ chaperonine family of proteins (Fig. 1D), which have been shown to be involved in protein folding and transport (Schröder *et al.*, 1993; Stuart *et al.*, 1994). The prospect that tumor suppression may causally relate to cell-specific protein folding and transport represents an exciting new aspect of tumor formation. We assume that the Tid53 protein may be engaged in folding and/or transport of specific proteins important for imaginal disc differentiation. Consequently the loss of Tid56 function will prevent folding and/or transport of these proteins, thus rendering them non-functional. This in turn arrests the differentiation process at an early stage, when imaginal disc cells are dividing vigorously.

Recessive mutations in the *l(3)mbt*[+] gene also prevent differentiation, in this case, of adult optic ganglion mother-cells into optic neurons (Gateff, 1982a; Gateff *et al.*, 1993). Based on the presence of a single and novel zinc finger motif, three repeats homologous to repeats in the *Drosophila* PcG gene *sex combs on mid leg* (*scm*) and a SAM (*Sterile α Motif*) box present in the *scm* gene as well as in *Drosophila Polyhomeotic* and mouse *Rae28* proteins (Ponting, 1995), we assume that the MBT163 protein, belonging to the *PcG* gene family of negative regulators, is involved in silencing of specific groups of genes during early

Fig. 3. *l(3)mbn-1*[+]: Gene structure and *in situ* expression. (A,B,C) λ-*clone 6714 and the corresponding l(3)mbn1+ genomic region as defined by three cDNAs. The P-lacW insertion is shown in B.* **(D)** *Exon-intron structure of the gene.* **(E)** *Whole-mount of wild-type hematopoietic lobes (hl) located along the heart vessel (hv) exhibiting l(3)mbn-1+ expression in the hematopoietic lobes (hl). The pericardial cells (pc) do not express l(3)mbn-1+ mRNA.* **(F,G)** *Wild-type and mutant blood cells showing cytoplasmic fluorescence after hybridization with the polyclonal antibody PKA 16 directed against a 16 amino acid peptide from the central domain of the putative MBN83 protein.* **(H,I)** *Whole-mounts of embryo stages 1 and 5, showing characteristic staining with the above antibody.* **(J)** *Blood cells (bc) in the head (h) and between the epidermis and the germ band (bg; arrows) within a stage 14 embryo stained with the antibody PK 16.*

embryogenesis (0-6 h; Fig. 2D-F), when the body plan becomes established and the larval and presumptive adult nervous system singled out. This assumption is further supported by the nuclear localization of the protein (N. Habtemichael, unpublished results).

The *b(2)gcn*⁺ gene, expressed in both sexes (Gateff, 1982b), exhibits a complex transcript pattern which is not exclusively confined to the germ line (Kaiser, 1994; Protin, 1994). Due to the transcript complexity and the difficulties in finding cDNAs for all transcripts, the *b(2)gcn*⁺ gene structure is not yet completely established. The three cDNAs characterizing the 3' region, however, revealed an Arg-Ser-rich region which shows high identity scores to corresponding domains of splicing factors (Fu and Maniatis, 1992). The perspectives that the *b(2)gcn*⁺ gonial cell tumor gene may be involved in splicing, places it in the group of *Drosophila* sex determination genes such as *Sxl* (Schüpbach, 1985), which autoregulates female-specific splicing of its own pre-mRNA, and which in some alleles develops ovarian tumors. Since, in contrast to *Sxl*, *b(2)gcn*⁺ is not expressed exclusively in the germ line, we envision its involvement in splice processes characteristic for the different cell-types. If *b(2)gcn*⁺ is a part of the splicing machinery, understanding the particular splice event suppressing tumor formation in the male and female germ cells, and allowing germ cell differentiation, is indeed of prime interest.

The least informative sequence is that of the putative blood tumor suppressor gene product MBN[83], which shows no convincing overall homology to any known protein. The 7 C-terminal Gly-Ser-rich repeats, confining to the consensus sequence of the Gly-Ser-rich repeats, found in the tail domains of human cytokeratins K1 and K10, represent the only reasonable homology (Konrad *et al.*, 1994). Similar Gly-Ser-rich quasi repeats have been found in a variety of proteins and have been suggested to form loops which hypothetically may interact either with each other or with Gly-Ser-rich loops of other proteins (Steinert *et al.*, 1991). In the wild-type larvae the MBN[83] protein is found in the cytoplasm of blood cells, the fat body and the midgut. The fat body and the midgut express the protein also on their plasma membrane. Homozygous mutant larvae, in contrast, express MNB[83] protein only in the cytoplasm of blood cells, while fat body and midgut show neither cytoplasmic nor plasma membrane expression. These facts prompt the hypothesis that mutant plasmatocyte proliferation may be due to an autoimmune reaction. The MNB[83] protein on the plasma membrane of wild-type fat body and midgut may serve as a "self" recognition molecule. The lack of MNB[83] protein on the plasma membrane of the corresponding mutant tissues, on the other hand, may signal plasmatocytes to proliferate excessively and to phagocytose these tissues. Studies are presently underway to confirm or reject this hypothesis.

Furthermore wild-type and mutant embryonic blood cells also express MNB[83] (Fig. 3J). The mutant embryonic blood cells, however, are not tumorous. This fact strongly suggests that the embryonic and larval blood cell populations belong to different lineages and are thus endowed with different functions. Embryonic blood cells are involved in the deposition of the basal membranes (Tepass *et al.*, 1994), while larval blood cells play a role in defence and metamorphosis.

Fig. 4. Cytogenetic localization and *in situ* expression of the *b(2)gcn*⁺ gene. (A) *Cytogenetic localization of the b(2)gcn⁺ gene to polytene chromosome bands 60A1 and positioning of b(2)gcn⁺ sequences in the 10 kb region delimited by the telomeric breakpoints of Df(2R)OV1, which complements the b(2)gcn⁺ phenotype, and Df(2R)OV2, which does not complement the phenotype and extents Df(2R)OV1 with 10 kb in telomeric direction. In situ hybridization of digoxigenin-labelled antisense transcripts of cDNAE8 to:* **(B)** *whole-mount preparations of wild-type ovaries. Note the b(2)gcn⁺ expression in germarial region R2 and in oogenesis stages S3 to S5 and S9 (see also text).* **(C)** *Whole-mount preparations of mutant ovaries lacking b(2)gcn⁺ transcripts.* **(D,E)** *Whole-mounts of S5 and S11 wild-type embryos showing ubiquitous b(2)gcn⁺ expression.*

Conclusion

The four putative tumor suppressor gene products represent novel proteins, whose predicted functions involving protein folding and/or transport, gene regulation and cell-specific splicing have to be verified in the future. The genes are expressed not only in the target cells, but also in other cells and tissues which
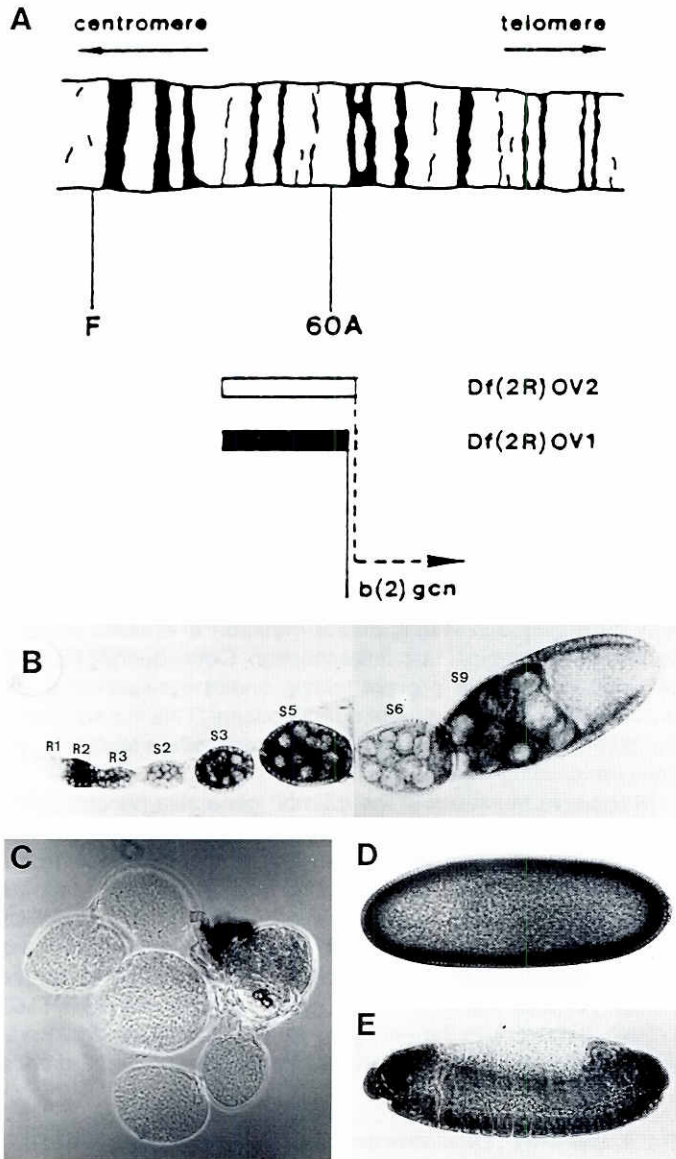
do not become malignant. This indicates strongly that each of the four genes may function in the tumorous and the unaffected cells in different pathways. The genes seem to be involved in the establishment and maintenance of the differentiated state. The interactions between these genes and the cell cycle genes have yet to be investigated. Contrary to mammalian tumors, 50% of which have a mutated *Rb* and *p53* gene (Hollstein *et al.*, 1991), each *Drosophila* tumor is causally related to only one recessive gene mutation when present in the homozygous state.

In view of the fact that the genetic principles establishing the body plan are universal, we would like to stress that the *Drosophila* tumor suppressor genes are an excellent tool to study the primary events leading to neoplastic growth, which may turn out to be also of importance for the understanding of vertebrate cancer initiation. We are convinced that, similarly to *Drosophila*, also in vertebrates a single mutation in a specific developmental gene is the primary cause for the malignant neoplastic transformation of a particular cell type.

References

ATENCIO, D.P. and YAFFE, M.P. (1992). MAS5, a yeast homolog of DnaJ involved in mitochondrial protein import. *Mol. Cell. Biol. 122:* 283-291.

BARDWELL, J.C.A., TILLY, K., CRAIG, E., KING, J., ZYLICZ, M., GEORGOPOULOS, C. (1986). The nucleotide sequence of the *Escherichia coli K12* dnaj gene: a gene that encodes a heat shock protein. *J. Biol. Chem. 261:* 1782-1785.

BERLETH, T., BURRI, M., THOMA, G., BOPP, D., RICHSTEIN, S., FRIGERIO, G., NOLL, M. and NÜSSLEIN-VOLHARD, C. (1988). The role of localization of bicoid RNA in organizing the anterior pattern of the *Drosophila* embryo. *EMBO J. 7:* 1749-1756.

BISHOP, J.G.III and CORCES, V.G. (1988). Expression of an activated ras gene causes developmental abnormalities in transgenic *Drosophila melanogaster*. *Genes Dev. 2:* 567-577.

BLUMBERG, H. and SILVER, P.A. (1991). A homologue of the bacterial heat shock gene *DnaJ* that alters protein sorting in yeast. *Nature 349:* 627-630.

BORNEMANN, D., MILLER, E. and SIMAR, J. (1996). The *Drosophila Polycomb* group gene *sex comb on midleg (scm)* encodes a zinc-finger protein with similarity to polyhomeotic protein. *Development.* (In press).

BRYANT, P.J. (1993). Toward the cellular functions of tumor suppressors. *Trends Cell Biol. 3:* 31-35.

BRYANT, P.J., WATSON, K.L., JUSTICE, R.W. and WOODS, D.F. (1993). Tumor suppressor genes encoding proteins required for cell interactions and signal transduction in *Drosophila*. *Development (Suppl.):* 239-249.

BURTIS, K.C. and BAKER, B.S. (1989). *Drosophila doublesex* gene controls somatic sexual differentiation by producing alternatively spliced mRNA5' encoding related sex specific polypeptides. *Cell 56:* 997-1010.

CAPLAN, A.J. and DOUGLAS, M.G. (1991). Characterization of YDJ1: A yeast homologue of the bacterial dnaJ protein. *J. Cell Biol. 114:* 609-621.

CAUDY, M., VÄSSIN, H., BRAND, M., TUMA, R., JAN, L.Y. and JAN, Y.N. (1988). *daughterless*, a *Drosophila* gene essential for both neurogenesis and sex determination, has sequence similarities to *myc* and the *achaete-scute* complex. *Cell 55:* 1061-1067.

CHEETHAM, M.E., BRION, J-P. and ANDERTON, B.H. (1992). Human homologues of the bacterial heat-shock protein DnaJ are preferentially expressed in neurons. *Biochem. J. 284:* 469-476.

FRIGERIO, G., BURRI, M., BOPP, D., BAUMGARTNER, S. and NOLL, M. (1986). Structure of the segmentation gene *paired* and the *Drosophila PRD* gene set as part of a gene network. *Cell 47:* 735-746.

FU, X-D. and MANIATIS, T. (1992). Isolation of a complementary DNA that encodes the mammalian splicing factor *SC35*. *Science 256:* 535-538.

GATEFF, E. (1978a). Malignant and benign neoplasms of *Drosophila melanogaster*. In *The Genetics and Biology of Drosophila, Vol. 2B* (Eds. M. Ashburner and T.R.F. Wright). Academic Press, New York, pp. 182-261.

GATEFF, E. (1978b). Malignant neoplasms of genetic origin in the fruit fly *Drosophila melanogaster*. *Science 200:* 1448-1459.

GATEFF, E. (1982a). Cancer, genes and development: the *Drosophila* case. In *Advances of Cancer Research, Vol. 37* (Eds. S. Weinhouse and G. Klein). Academic Press, London, pp. 33-69.

GATEFF, E. (1982b). Gonial cell neoplasm of genetic origin affecting both sexes of *Drosophila melanogaster*. In *Embryonic Development, Part B: Cellular Aspects* (Eds. M.M. Burger and R. Weber). A.R. Liss Inc., New York, pp. 621-632.

GATEFF, E. (1994). Tumor suppressor and overgrowth suppressor genes of *Drosophila melanogaster*. Developmental aspects. *Int. J. Dev. Biol. 38:* 565-590.

GATEFF, E. and MECHLER, B.C. (1989). Tumor suppressor genes of *Drosophila melanogaster*. In *Critical Reviews on Oncogenesis, Vol. 1* (Ed. E. Pimentel). CRC Press, Miami, pp. 221-245.

GATEFF, E. and MIYAMOTO, T. (1990). The tumor suppressor gene *lethal(3)malignant brain tumor l(3)mbt* of *Drosophila melanogaster*: Multiple functions during development. *Contrib. Oncol. 39:* 135-141.

GATEFF, E., LÖFFLER, T. and WISMAR, J. (1993). A temperature-sensitive brain tumor suppressor mutation of *Drosophila melanogaster*: Developmental studies and molecular localization of the gene. *Mech. Dev. 41:* 15-31.

GOMEZ, S.L., GOTER, J.W. and SHAPIRO, L. (1990). Expression of the *Caulobacter* heat shock gene *dnaK* is developmentally controlled during growth at normal temperatures. *J. Bacteriol. 172:* 3051-3059.

GUNDACKER, D., PHANNAVONG, B., VEF, O., GATEFF, E., KURZIK-DUMKE, U. (1993). Genetic and molecular characterization of breakpoints of five deficiencies in the genomic region 59F-60A. *Drosophila Inf. Serv. 72:* 129-131

HENKEMEYER, M.J., GERTLER, F.B., GOODMAN, W. and HOFFMANN, F.M. (1987). The *Drosophila* Abelson proto-oncogene homolog: identification of mutant alleles that have pleiotropic effects late in development. *Cell 51:* 821-828.

HOLLSTEIN, M., SIDRANSKY, D., VOGELSTEIN, B. and HARRIS, C.C. (1991). p53 mutations in human cancer. *Science 253:* 49-53.

HOOVER, K.K., CHIEN, A.J. and CORCES, V.G. (1993). Effect of transposable elements on the expression of *forked* gene of *Drosophila melanogaster*. *Genetics 135:* 507-526.

HUNTER, T. (1991). Cooperation between oncogenes. *Cell 64:* 249-270.

KAISER, S. (1994). *Klonierung, Expressionsanalyse und Sequenzierung des benign(2)gonial cell neoplasm Gens von Drosophila melanogaster*. Dissertation Thesis, Johannes Gutenberg-University, Mainz, Germany.

KLUG, A. and RHODES, D. (1987). Zinc fingers: a protein motif for nucleic acid recognition. *Trends Biochem. Sci. 12:* 464-468.

KONRAD, L., BECKER, G., SCHMIDT, A., KLÖCKNER, T., KAUFER-STILLGER, G., DRESCHERS, S., EDSTRÖM, J.E. and GATEFF, E. (1994). Cloning, structure, cellular localization and possible function of the tumor suppressor gene *lethal(3)malignant blood neoplasm-1* of *Drosophila melanogaster*. *Dev. Biol. 163:* 98-111.

KRISHNAN, H.B. and PUEPPKE, S.G. (1991). Nolc, a *Rhizobium fredii* gene involved in cultivar-specific nodulation of soybean, shares homology with a heat-shock gene *Mol. Microbiol. 5:* 737-746.

URZIK-DUMKE, U., PHANNAVONG, B., GUNDACKER, D., GATEFF, E. (1992). Genetic, cytogenetic and developmental analysis of the *Drosophila melanogaster* tumor suppressor gene *lethal(2)tumorous imaginal discs* [*l(2)tid*]. *Differentiation 51:* 91-104.

KURZIK-DUMKE, U., GUNDACKER, D., RENTROP, M. and GATEFF, E. (1995). Tumor suppression in *Drosophila* is causally related to the function of the *lethal(2)tumorous imaginal discs* gene, a *dnaJ* homolog. *Dev. Genet. 16:* 64-76.

LAMOND, A.I. (1993). The spliceosome. *BioEssays 15:* 595-603.

LATHIGRA, R.B., YOUNG, D.B., SWEETSER, D. and YOUNG, R.A. (1988). A gene from *Mycobacterium tuberculosis* which is homologous to the DnaJ heat shock protein of *E. coli. Nucleic Acids Res. 16:* 1636.

LUKE, M.M., SUTTON, A. and ARNDT, K.T. (1991). Characterization of SIS1, a *Saccharomyces cerevisiae* homologue of bacterial dnaJ proteins. *J. Cell Biol. 114:* 623-638.

MOUNT, S.M., PETTERSSON, I., HINTERBERGER, M., KARMAS, A. and STEITZ, J.A. (1983). The U1 small nuclear RNA-protein complex selectively binds a 5' splice site *in vitro. Cell 33:* 509-518.

NURSE, P. (1990). Universal control mechanisms regulating onset of M-phase. *Nature 344:* 503-508.

OHKI, M., TAMURA, R., NISHIMURA, S. and UCHIDA, H. (1986). Nucleotide sequence of the *Escherichia coli dnaJ* gene and purification of the gene product. *J. Biol. Chem. 261:* 1778-1781.

ORR-WEAVER, T.L.(1994). Developmental modification of the *Drosophila* cell cycle. *Trends Genet. 10:* 321-327.

PONTING, C.P. (1995). SAM: A novel motif in yeast sterile and *Drosophila* polyhomeotic proteins. *Protein Sci. 4:* 1928-1930.

PROTIN, U. (1994). *Genetische und molekulargenetishe Charakterisierung des benign(2)gonial cell neoplasm Tumorsuppressorgens von Drosophila melanogaster.* Dissertation Thesis, Johannes Gutenberg-University, Mainz, Germany.

RAABE, T. and MANLEY, J.L. (1991). A human homologue of the *Escherichia coli* DnaJ heat-shock protein. *Nucleic Acids Res. 19:* 6645.

RIZKI, T.M. (1978). The circulatory system and associated cells and tissues. In *The Genetics and Biology of Drosophila, Vol. 2b* (Eds. M. Ashburner and T.M.F. Wright). Academic Press, London, pp. 397-448.

ROTHBLATT, J.A., DESHAIES, R.J., SANDERS, S.L., DAUM, G. and SCHEKMAN, R. (1989). Multiple genes are required for proper insertion of secretory proteins. *J. Cell Biol. 109:* 2641-2652.

SADLER, I., CHIANG, A., KURIHARA, T., ROTHBLATT, J., WAY, J. and SILVER, P. (1989). A yeast gene important for protein assembly into the endoplasmic reticulum and the nucleus has homology to DnaJ, an *Escherichia coli* heat shock protein. *J. Cell Biol. 109:* 2665-2675.

SAINT, R. and WIGLEY, P.L. (1992). Developmental regulation of the cell cycle. *Curr. Opin. Genet. Dev. 2:* 614-620.

SCHRÖDER, H., LANGER, T., HARTL, F-U. and BUKAU, B. (1993). DNAK, DNAJ and GrpE form a cellular chaperone machinery capable of repairing heat-induced protein damage. *EMBO J. 12:* 4137-4144.

SCHÜPBACH, T. (1985). Normal female germ cell differentiation requires the female chromosome to autosome ratio and expression of *Sex-lethal* in *Drosophila melanogaster. Genetics 109:* 529-548.

SHRESTHA, R. and GATEFF, E. (1982). Ultrastructure and cytochemistry of the cell-types in the larval hematopoietic organs and hemolymph of *Drosophila melanogaster. Dev. Growth Differ. 24:* 65-82.

SHRESTA, R. and GATEFF, E. (1986). Ultrastructure and cytochemistry of the tumorous blood cells in the mutant *lethal(3)malignant blood neoplasm* of *Drosophila melanogaster. J. Invertebr. Pathol. 48:* 1-12.

SIMON, M.A., DRESS, B., KORNBERG, T. and BISHOP, J.M. (1985). The nucleotide sequence and the tissue-specific expression of *Drosophila c-src. Cell 42:* 831-840.

STEINERT, P.M., MACK, J.W., KORGE, B.P., GAN, S.-Q., HAYNES, S.R. and STEVEN, A.C. (1991). Glycine loops in proteins: Their occurrence in certain intermediate filament chains, loricrins and single-stranded RNA binding proteins. *Int. J. Biol. Macromol. 13:* 130-140.

STRAND, D., UNGER, S., CORVI, R., HARTENSTEIN, K., SCHENKEL, H., KALMES, A., MERDES, G., NEUMANN, B., KRIEG-SCHNEIDER, F., COY, J.F., POUSTKA, A., SCHWAB, M. and MECHLER, B.M. (1995). A human homologue of the *Drosophila* tumor suppressor gene *l(2)gl* maps to 17p11.2-12 and codes for a cytoskeletal protein that associates with nonmuscle myosin II heavy chain. *Oncogene 11,* 291-301.

STUART, R.A., CYR, D.M., CRAIG, E.A. and NEUPERT,W. (1994). Mitochondrial molecular chaperones: their role in protein translocation. *Trends Biochem. Sci. 19:* 87-92.

TEPASS, U., FESSLER, L.I., AZIZ, A. and HARTENSTEIN, V. (1994). Embryonic origin of hemocytes and their relationship to cell death in *Drosophila. Development 120:* 1829-1837.

WATSON, K.L. and BRYANT, P.J. (1993). *Drosophila* tumor suppressor genes and their human homologs. *Trends Cell Biol. 3:* 39-42.

WETZSTEIN, M. DEDIO, J. and SCHUMANN, W. (1990). Complete nucleotide sequence of the *Bacillus subtilis dnaK* gene. *Nucleic Acids Res. 18:* 2172.

WISMAR, J., LÖFFLER, T., HABTEMICHAEL, N., VEF, O., GEISSEN, M., ZIRWES, R., ALTEMEYER, W., SASS, H. and GATEFF, E. (1995). The *Drosophila* tumor suppressor gene *lethal(3)malignant brain tumor* encodes a proline-rich protein with a novel zinc finger. *Mech. Dev. 53:* 141-152.

ZAMORE, P.D. and GREEN, M.R. (1992). Biochemical localization of *U2 snRNP* auxiliary factor: an essential pre-mRNA splicing factor with a novel intranuclear distribution. *EMBO J. 10:* 207-214.