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Tumor suppressor genes encoding proteins required for cell interactions and signal transduction in *Drosophila*

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SUMMARY

Tumor suppressor genes, whose products are required for the control of cell proliferation, have been identified by their mutant phenotype of tissue overgrowth. Here we describe recent work on the molecular identification of tumor suppressor genes that function in two different cell types of the *Drosophila* larva: the blood cells, and the undifferentiated epithelial cells of developing imaginal discs. Mutations in the *aberrant immune response8* (*air8*) gene lead to overproduction and precocious differentiation of blood cells. This gene encodes the *Drosophila* homolog of human ribosomal protein S6. The mutant phenotype is consistent with a role for S6 in the control of cell proliferation, and is compatible with findings from mammalian cells where alterations in S6 expression and phosphorylation are associated with changes in cell proliferation. Mutations in the *discs large* (*dlg*) gene cause neoplastic overgrowth of imaginal discs in the larva. The mutant discs show loss of septate junctions and of apical-basal cell polarity, and they also lose the ability to differentiate cuticular structures. The *dlg* protein product (DlgA) is localized at septate junctions between epithelial cells, and cDNA sequencing indicates that the gene product includes a domain with homology to guanylate kinase (GUK). Two mammalian homologs of this gene have been identified, and one of them (PSD-95/SAP90) encodes a component of synaptic densities in the brain; this protein therefore resembles the DlgA protein in being located in a specialized cell junction that functions in information transfer between cells. Mutations in the *fat* gene cause hyperplastic imaginal disc overgrowth, in which the overgrowing disc tissue retains its epithelial structure and its ability to differentiate. Some of the excess disc tissue is shed as vesicles suggesting a loss of cell adhesion. In support of this hypothesis, the predicted gene product shows homology to cadherins in its extracellular domain. However, the *fat* protein is much larger than known cadherins. As in human cancer, somatic loss of the normal alleles of tumor suppressor genes can lead to tumor formation in *Drosophila*; an example of this is provided by the *warts* (*wts*) locus. The *wts* gene was identified by the dramatic overgrowth of mitotic recombination clones that are homozygous for a *wts* deletion. In these clones the cuticle intrudes between epithelial cells, suggesting an alteration in cell adhesion. The study of these and other tumor suppressor genes in *Drosophila* is providing new evidence supporting the critical role of cell interactions and specialized apical junctions in controlling epithelial cell proliferation.

Key words: *Drosophila*, imaginal discs, *discs large*, *aberrant immune response 8*, cell adhesion

INTRODUCTION

Cell proliferation is controlled by cell-autonomous mechanisms involving cyclins and cell division control genes, but it is also affected by factors originating outside the cell including both diffusible signals and contact-mediated signals from adjacent cells. Disruption of proliferation control in cancer cells is often associated with loss of cell polarity, adhesion and junctions, suggesting that the maintenance of these features is important for the operation of the signalling mechanisms controlling proliferation and differentiation.

An important source of information regarding these aspects of cell biology is provided by the identification and analysis of oncogenes and tumor suppressor genes, and their protein products. At least nine human tumor suppressor genes have now been identified by analyzing the changes in somatic cell DNA that occur during tumor development (see Bryant, 1993 for review). Structural analysis of these genes indicates that their protein products are involved in cell adhesion, signal transduction, and cell cycle control, although none of their products had been previously recognized in studies of normal cells. By revealing mechanisms and molecules that have not been identified in studies of normal cells, the study of tumor suppressor genes is contributing not only to our understanding of oncogenesis, but also to basic cell biology.

Recently it has become clear that *Drosophila* can make an important contribution in this area, because simple traditional genetic procedures can be used to identify mutations that cause overgrowth of specific tissues and thus to identify tumor suppressor genes (Gateff, 1978; Gateff, 1982; Bryant,

1993). Furthermore, molecular genetic techniques available for *Drosophila* facilitate the identification and characterization of the gene products. Sequence comparison can then be used to identify human homologs as candidate tumor suppressor genes.

Mutations in most of the known *Drosophila* tumor suppressor genes are recessive lethals that show either hyperplastic or neoplastic overgrowth of specific tissues, followed by death of the animal during the late larval or early pupal stage. The tumor suppressor genes functioning only in the gonads are exceptional in that the tumors form in the adult and cause sterility but not lethality. Over 50 tumor suppressor genes have been identified; they function in the embryo, in the developing gonads, in the developing brain, in developing imaginal discs (embryonic tissues that develop inside the larva and differentiate into parts of the adult fly during metamorphosis), and in the developing hematopoietic system. Eight of these genes have been cloned and characterized, and three of them show clear homology to human genes, none of which had been recognized previously as a tumor suppressor gene (Bryant, 1993). In this paper we describe recent findings on these three genes.

THE RpS6 GENE, IDENTIFIED BY THE *aberrant immune response8* (*air8*) MUTATION

The *aberrant immune response8* (*air8*) mutation was recovered in a P-element mutagenesis screen that led to the identification of over 20 genes in which mutations lead to melanotic tumor formation (Watson et al., 1991). The mutations are called *aberrant immune response* (or *air*) since genetic alterations rather than foreign challenges lead to the activation of cellular defense mechanisms and the formation of melanotic tumors (Fig. 1). The *air* mutations fall into two classes; the phenotype of the first class (including *air8*) includes overgrowth of the hematopoietic organs (lymph glands) indicating that the corresponding genes function in cell proliferation control in that tissue. The second class of *air* mutations has no observable effect on the lymph glands, but the mutant animals develop melanotic tumors associated with specific tissues. Mutations in this class are thought to result in "autoimmune responses" against the affected tissues (Watson et al., 1991).

The *air8* mutation inhibits growth of most larval organs but causes overgrowth of the lymph glands (Fig. 2; Watson et al., 1992). It also causes overproduction of blood cells from the lymph gland (K.L.W., unpublished data) and the precocious transformation of plasmatocytes to lamellocytes during larval life, an event that normally occurs during the pupal stage (Fig. 3; Shrestha and Gateff, 1982; Rizki and Rizki, 1984). The lamellocytes aggregate to form large melanotic tumors, and death occurs during the late larval stage (Figs 1, 3; Watson et al., 1991; Watson et al., 1992). Transplantation of *air8* hemocytes into the abdomens of wild-type females leads to bloating, melanotic tumor formation and significantly more host lethality than wild-type hemocyte injections (K.L.W., unpublished data). Also, explanted *air8* hemocytes show indefinite proliferation in vitro (K.L.W. and D. Peel, unpublished data). In contrast to its effect on the hematopoietic system, the *air8* mutation appears to block cell growth in other tissues. Mitotic recombination clones of *air8* mutant cells fail to survive both in the germline and developing imaginal disc cells, indicating a requirement of the normal gene product for cell survival and/or proliferation in these tissues (Watson et al., 1992).

The gene identified by the *air8* mutation was the first tumor suppressor functioning in the *Drosophila* hematopoietic system to be characterized at the molecular level. Sequencing of *air8* cDNAs from an adult female library revealed an open reading frame encoding 248 amino acids with 75% identity and 95% similarity to both human and rat ribosomal protein S6 (Fig. 4A; Watson et al., 1992). Two different P-element insertions from different melanotic tumor strains (*air8* and *WG1288*) map to the 5′ transcribed but untranslated region of the *Drosophila* RpS6 gene. On northern blots, the 1 kb RpS6 transcript is barely detectable in *air8* mutant larvae yet this transcript is abundant in wild-type animals throughout development and in *air8* revertant animals that were produced by excision of the P-element (Watson et al., 1992).

Although mammalian S6 has not been recognized as a tumor suppressor, there are several indications that it may play a role in cell proliferation control and tumorigenesis. The human rpS6 gene has been mapped to 9p21, a region in which genetic alterations are associated with various cancers including acute lymphoblastic leukemia and acute myelogenous leukemia (Antoine and Fried, 1992). In addition, S6 is overexpressed in a variety of tumors such as human colon carcinoma and adenomatous polyps (Pogue-Geile et al., 1991; Barnard et al., 1992). Most intriguingly, the S6 protein contains a cluster of serine residues in the carboxy terminus that shows developmentally regulated and mitogen-induced phosphorylation (Traugh and Pendergast, 1986; Sturgill and Wu, 1991); it is not yet known whether the *Drosophila* protein is similarly modified. In quiescent mammalian cells, most ribosomes exist as inactive monosomes and S6 is not phosphorylated (Traugh and Pendergast, 1986). However, shortly after cells are stimulated

Fig. 1. Hemizygous *air8* third-instar larvae showing the melanotic tumor phenotype. From Watson et al. (1992).

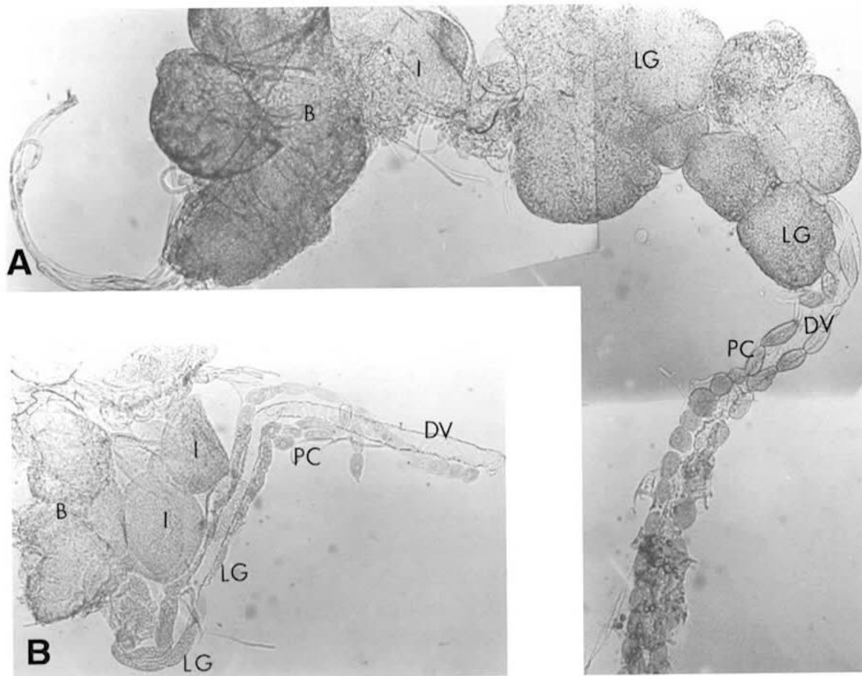

Fig. 2. Brain (B), imaginal discs (I), dorsal vessel (DV) and lymph gland (LG) of third-instar larvae. (A) *air8*. Four pairs of hypertrophied lymph-gland lobes line the dorsal vessel in the anterior region and pericardial cells (PC) are found posterior to the lymph glands. (B) Corresponding parts of a wild-type larva at the same magnification. From Watson et al. (1991).

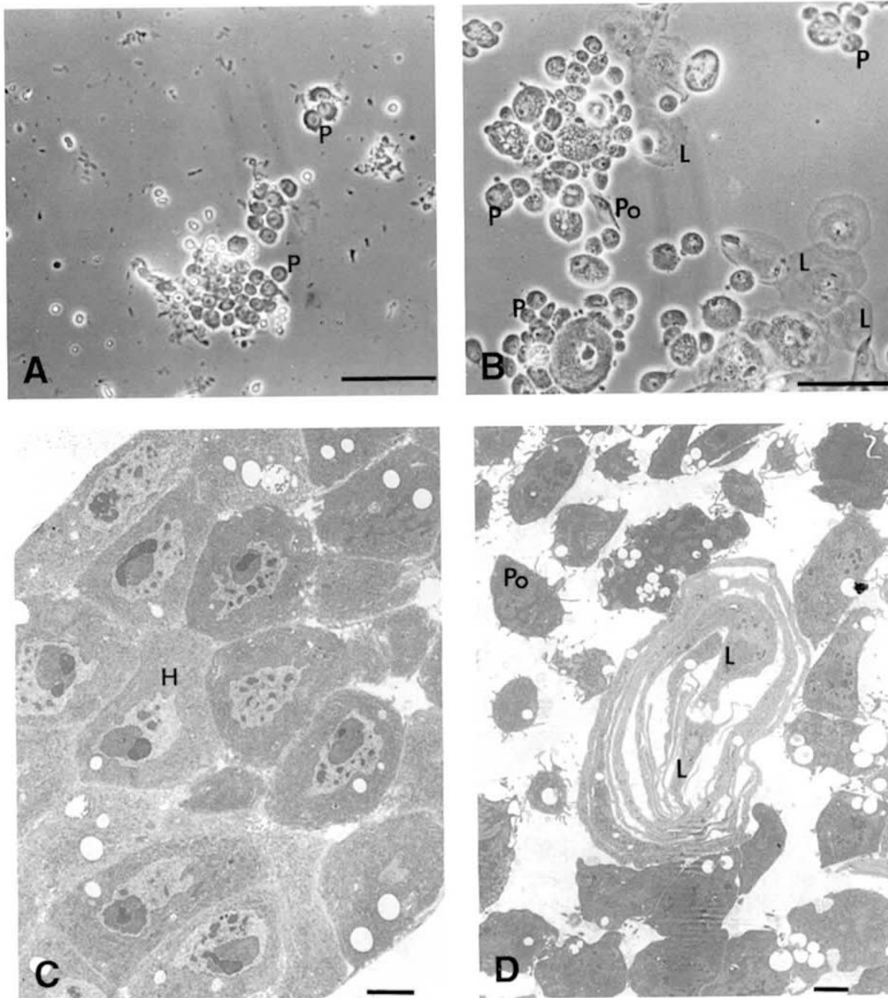

Fig. 3. (A) Phase contrast view of circulating hemocytes in the hemolymph of a wild-type third-instar larva. P, plasmatocytes. Bar, 50 μm. (B) Phase contrast view of circulating hemocytes of an *air8* larva including a plasmatocyte (P), lamellocyte (L), and a podocyte (Po), the latter representing an intermediate in the transformation of a plasmatocyte to a lamellocyte. Bar, 50 μm. (C) Electron micrograph showing hemocytes (H) in a lymph gland lobe of a wild-type larva. Bar, 1.5 μm. (D) Electron micrograph showing an *air8* lymph gland lobe. At least three lamellocytes, L, are encapsulating each other and many podocytes (Po) in transition to lamellocytes can be seen. Bar, 1.5 μm. Modified from Watson et al. (1992).

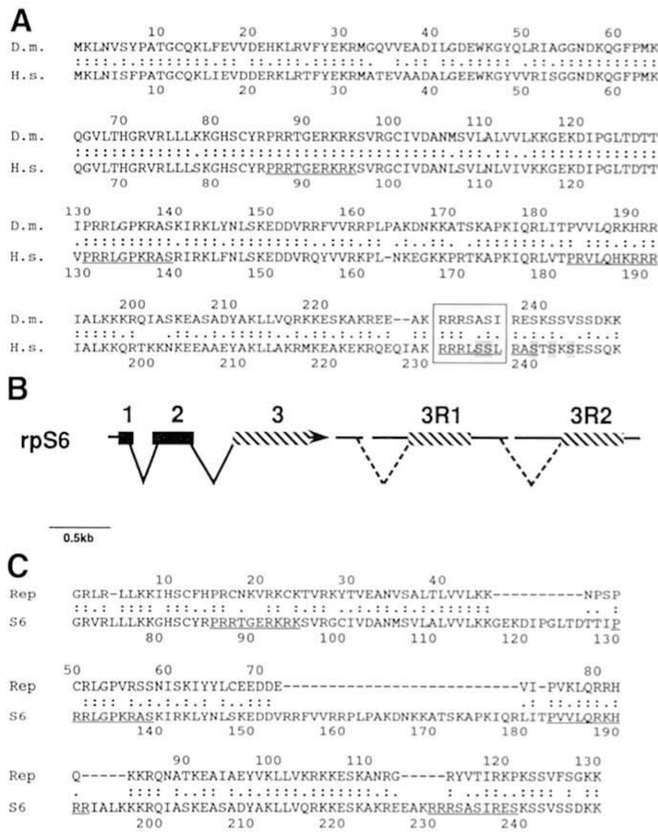

Fig. 4. (A) Sequence alignments of predicted *Drosophila* and human S6 ribosomal protein sequences. Alignments were generated using the FASTA program (Pearson and Lipman, 1990). The four copies of the ten amino-acid motif that may serve as a nuclear localization signal (Chan and Wool, 1988) are underlined. Residues that are subject to phosphorylation in the human protein are shaded (Wettenhall et al., 1988). The consensus recognition sequence for the mitogen-activated 70K S6 kinase (Ferrari et al., 1991; Flotow and Thomas, 1992) is boxed. :, identical residues; ., conservative substitutions. From Watson et al. (1992). (B) Schematic diagram of the genomic arrangement of the *Drosophila* RpS6 gene and its two downstream repeats 3R1 and 3R2 (K. L. W. and P. J. B., unpublished data). (C) Sequence alignment of the two identical predicted repeats (Rep) with the predicted S6 amino acid sequence over the region of overlap. Conventions as in A. Note that the C-terminal tail in the repeats contains only four serine residues, compared with seven in *Drosophila* and human S6.

to proliferate by treatment with serum growth factors, insulin, tumor promoting agents, or transforming viruses, protein synthesis increases as a result of higher rates of translation initiation (Traugh and Pendergast, 1986; Ballou et al., 1988). S6 becomes multiply phosphorylated in an orderly manner after mitogen stimulation and during development, and ribosomal subunits containing phosphorylated S6 become preferentially incorporated into polysomes (Traugh and Pendergast, 1986; Heinze et al., 1988). It has been suggested that phosphorylation of specific residues in S6 may control the state of cells by regulating the selective translation of mRNAs (Mutoh et al., 1992). The S6 protein resides in the mRNA binding domain of the 40S subunit where it might be expected that any subtle conformational and/or charge change induced by S6 phosphorylation could have a significant effect on ribosome function (Ballou et al., 1988; Traugh and Pendergast, 1986).

Two distinct families of S6 kinases, $pp90^{rsk}$ and $pp70^{S6k}$, have been shown in vivo and in vitro to phosphorylate S6 directly via two distinct intracellular signalling pathways (Erikson, 1991). In one of these pathways, $pp90^{rsk}$ and S6 are downstream targets in a serine/threonine phosphorylation cascade that has been identified in a variety of systems (Lange-Carter et al., 1993). Recently, $pp70^{S6k}$ function was shown to be necessary for cell cycle progression through G1 and its principal target, S6, is phosphorylated during this transition (Lane et al., 1993). These results provide additional support for the hypothesis that S6 phosphorylation may regulate cell proliferation.

The growth inhibition seen in most organs of *air8* animals, and the failure of mutant clones to survive in the germ line and imaginal discs, is consistent with a defect in protein synthesis. However, it is surprising to find that tumor formation in the hematopoietic system appears to occur in the absence of the RpS6 transcript and presumably S6 protein. One possible explanation is that a different protein is able to substitute for S6 in the ribosomes of hematopoietic cells, and this hypothesis is supported by the finding that the genomic DNA immediately flanking the 3′ end of the RpS6 coding region contains two tandem repeats (3R1 and 3R2) of a sequence closely related to RpS6 intron 2, exon 3 and 3′ flanking DNA (Fig. 4B). The existence of these repeats can be explained by two unequal crossover events, leading first to a duplication of part of RpS6, followed by duplication of the copy (K.L.W., unpubl.). The intron 2/exon 3 boundaries, the open reading frame and the putative polyadenylation signals are retained in 3R1 and 3R2, suggesting that the repeats are transcribed. Conceptual translation of the repeats predicts peptides showing 62.3% amino acid identity plus an additional 25.4% similarity with S6 (Fig. 4C). However, there are interesting differences between the repeats and S6 including deletions and residue changes that predict S6 isoforms with altered functions. In particular, there are residue alterations in the putative nuclear localization signals and loss of three of the C-terminal serine residues that may be phosphorylated in S6 (Fig. 4C). Preliminary results suggest that the RpS6 repeats are transcribed (K.L.W., unpublished data) and if these transcripts are translated, the alternative isoforms might be capable of assembling into ribosomes. Tissue-specific isoforms of ribosomal proteins have not been reported, but if they exist they could explain the tissue-specific phenotype caused by mutations in the RpS6 gene.

THE *lethal(1)discs-large* (*dlg*) LOCUS

Recessive lethal mutations in the *dlg* gene cause neoplastic overgrowth of the imaginal discs in the larva, followed by death of the animal in the early pupal stage (Woods and Bryant, 1989, 1991). The overgrowing imaginal discs, which are normally single-layered epithelia, become disorganized masses and lose the ability to develop into adult parts even after transplantation into normal hosts. Strong

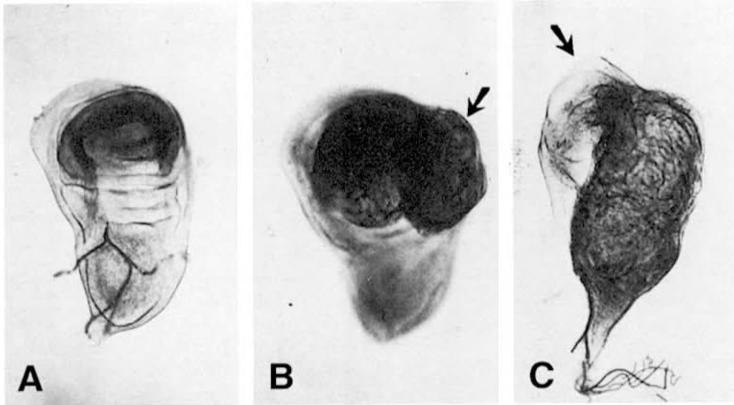

Fig. 5. Late third-instar wing imaginal discs. (A) Wild-type. Note the flat shape. (B) dlg^{XI-2}, showing loss of epithelial structure over part of the disc (arrow) but normal structure elsewhere. (C) dlg^{XI-2} wing disc with only a small area of normal epithelial structure (arrow). From Bryant (1987).

alleles disrupt epithelial structure over the entire disc whereas with weaker alleles only part of the tissue loses epithelial structure and overgrows (Figs 5, 6A). When the entire disc is affected, it can grow to at least three times the normal cell number during the extended larval period (Woods and Bryant, 1989). Normal imaginal disc cells are highly polarized epithelial cells connected by junctional complexes including adherens and septate junctions, but in the imaginal discs of larvae carrying a *dlg* loss-of-function mutation, apical-basal polarity is almost completely lost and the septate junctions are missing (D. F. W. and P. J. B., unpublished data). The brain and lymph gland also overgrow in the mutant and the larvae become bloated, but the remainder of the larval organs appear normal.

The *dlg* gene encodes a 960-amino acid protein (DlgA) that is expressed in epithelial cells, as well as other tissues including the hematopoietic organs and the nervous system, throughout development (Woods and Bryant, 1989; Woods and Bryant, 1991). Immunocytochemical analysis shows that in epithelial cells the DlgA protein is localized in a subapical belt of the lateral cell membrane (Fig. 6B), at the position of the septate junction (Woods and Bryant, 1991). Thus the DlgA protein appears to be localized in septate junctions and is required for their formation. The role of septate junctions is unknown, although they have often been considered the invertebrate equivalent of vertebrate tight junctions (Noirot-Timothee and Noirot, 1980). Tight junctions are known to provide a transepithelial barrier to diffusion of small molecules and to prevent diffusion of membrane components between the apical and basolateral membrane domains (Schneeberger and Lynch, 1992). Septate junctions also provide a barrier to transepithelial diffusion of lanthanum (Green and Bergquist, 1982) and appear to separate membrane domains as indicated by the behavior of lipophilic markers (Wood, 1990).

Sequence comparisons based on the *dlg* cDNA sequence indicate that the encoded protein includes a series of modules each of which is also found in other proteins (Fig. 7). These domains and their possible functions are as follows:

DHR

There are three tandem copies of the DHR motif in the N-terminal half of the predicted DlgA protein (previously reported as a "filamentous" domain based on its sequence similarity to some filamentous proteins; Woods and Bryant, 1991). The motif was named GLGF based on an amino acid sequence present at the N-terminal end of the motif in DlgA and one of its mammalian homologs (Cho et al., 1992). However, since the GLGF sequence itself is absent from most other copies of this motif, we suggest the alternative name **DHR** (Discs-large Homologous Region). A single copy of this domain is present in nitric oxide synthase (NOS; Cho et al., 1992), in a human cytosolic tyrosine phosphatase (Gu et al., 1991), and in the cytoplasmic domain of a human brain transmembrane protein encoded by a gene potentially involved in Friedreich ataxia (Duclos et al., 1993). Most of these proteins are localized at the cell surface, probably in association with the cortical cytoskeleton. Since DlgA and at least two of its mammalian homologs (see below) are also membrane associated, we propose that the DHR repeat is involved in directing this localization.

OPA

The OPA trinucleotide repeat is found in many genes that are developmentally regulated or show tissue-specific expression, in a variety of organisms from yeast to mammals (Wharton et al., 1985). It is translated in several reading frames to give tracts that are rich in specific amino acids. In *dlg* it is predicted to give rise to tracts of serines and alanines.

SH3

There is a single copy of the SH3 (*src*-homology region 3; Musacchio et al., 1992) domain in the predicted DlgA protein. Mutations in the SH3 domain of v-*src* can restore transforming activity in otherwise defective mutants (Dezélée et al., 1992), indicating that this domain is important for regulation of protein activity. SH3 binds a ten-amino acid proline-rich peptide motif that has been found in several signal transduction proteins (Ren et al., 1993) including one that is similar to the GTPase activating protein (GAP) associated with the *ras*-related protein *rho* (Cicchetti et al., 1992). Other binding targets for SH3 include the GTP-hydrolyzing motor protein dynamin (Booker et al., 1993), and guanine nucleotide exchange factors (Olivier et al., 1993; Rozakis-Adcock et al., 1993).

PEST

The PEST domain was originally described in proteins that had a high rate of degradation in the cell and was therefore interpreted as a signal for rapid turnover (Rogers et al., 1986). However, this interpretation is open to question since the *Notch* protein, which contains both PEST and OPA

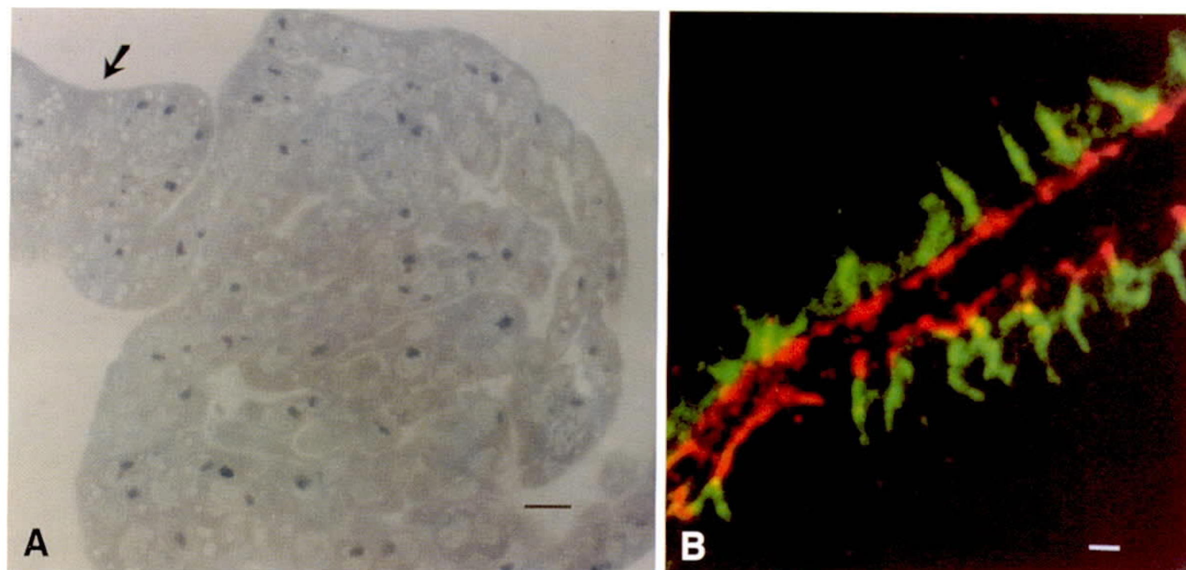

Fig. 6. (A) A section of a dlg^{XI-2} wing disc showing partial loss of epithelial structure (see Fig. 5b). The arrow points to a region of the disc where the cells remain columnar, while the overgrowing region is highly folded and the cells are cuboidal. Bar, 5 µm. (B) Confocal section of a wild-type late third-instar wing disc stained with antibodies against *dlg* (green staining) and against phosphotyrosine (PY; red staining). This optical section shows the apical surfaces of two cell layers both facing a luminal space. The *dlg* protein is restricted to the septate junctions and PY is highly enriched in the adherens junctions. Bar, 1 µm.

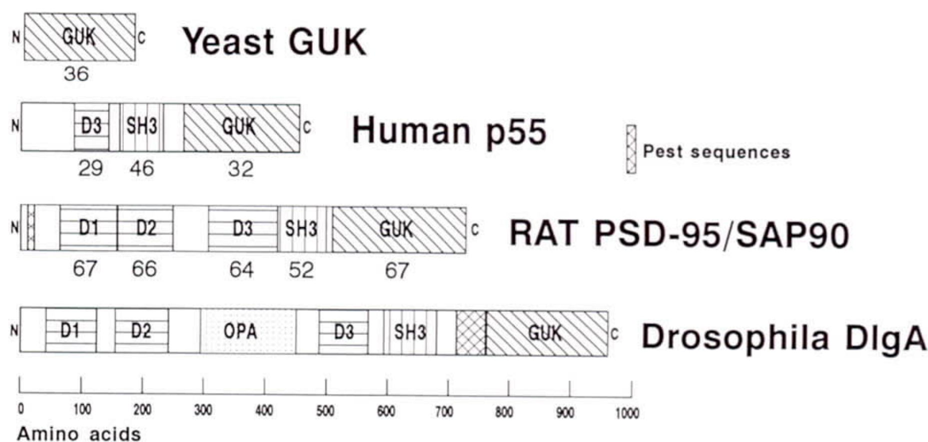

Fig. 7. Comparison between the predicted organization of the DlgA protein and that of its yeast and mammalian homologs. The alignments were identified by the standard BLAST algorithm (Altschul et al., 1990). Percentages shown refer to amino-acid identity with the corresponding domain of DlgA. Yeast GUK from Berger et al. (1989); PSD-95 from Cho et al. (1992); SAP90 from Kistner et al. (1993); DlgA from Woods and Bryant (1991); p55 from Ruff et al. (1991). D1-D3, DHR repeats; SH3, src-homology 3 domains.

sequences, is apparently stable for several cell cycles (Kidd et al., 1989).

GUK

The DlgA protein contains an approximately 179 amino-acid region at the carboxyl terminus with significant sequence similarity to the soluble enzyme guanylate kinase (GUK) of yeast (Berger et al., 1989; 36% identity) and pig (Zschocke et al., 1993; 35% identity) (Fig. 8). This enzyme transfers a phosphate group from ATP to GMP, converting it to GDP. If the DlgA protein has GUK activity, it could regulate the production of guanine nucleotides that act as messenger molecules within the cell (Woods and Bryant, 1991). The DlgA GUK domain shows conservation or conservative substitution of all of the amino acids that have been shown to interact with the GMP substrate as well as the B motif that is thought to bind the Mg^{++} cofactor in the yeast enzyme (Fig. 8; Stehle and Schulz, 1990, 1992). However, in DlgA the A motif GxxGxGK, which in the yeast enzyme binds the ATP phosphate donor, is interrupted by a three amino-acid deletion (Koonin et al., 1992). This deficiency in the ATP-binding site (a domain that is otherwise highly conserved in a wide variety of kinases) suggests that the DlgA protein may have lost the ability to bind ATP and may have acquired a novel function requiring GMP binding. Whatever the exact catalytic function of the DlgA protein, its potential involvement in guanine nucleotide metabolism or regulation suggests a relationship to signal transduction mechanisms known to control cell proliferation, such as those mediated by $pp21^{ras}$ (Woods and Bryant, 1991).

The *dlg* gene identifies a family of related proteins - the MAGUKs

Recent findings indicate that the *dlg* gene has homologs in mammals and that there is at least one other related gene in

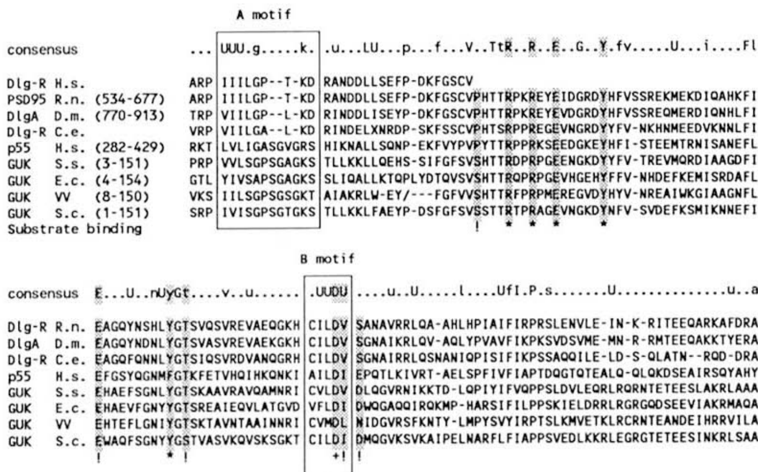

Fig. 8. Alignment of the amino acid sequences of guanylate kinases with the GUK domains of p55, Dlg-A and Dlg-R proteins. The alignment was generated using the multiple alignment program OPTAL (Gorbalenya and Koonin, 1989). The consensus includes invariant amino acid residues (upper case), and residues found in all but one sequence (lower case); U - a bulky hydrophobic residue (I, L, V, M, F, Y, W); X - any residue. A and B motifs (see text) are boxed. Amino acid residues implicated in specific molecular interactions in yeast GUK (Stehle and Schulz, 1990, 1992) are indicated as follows: *, binding to phosphate; !, binding to guanine ring; +, binding to magnesium; these residues and their counterparts in the other sequences are shaded. D.m. - *Drosophila melanogaster*, C.e. - *Caenorhabditis elegans*, E.c. - *Escherichia coli*, S.c. - *Saccharomyces cerevisiae*, H.s. - *Homo sapiens*, R.n. - *Rattus norvegicus*, S.s. - *Sus scrofa*, VV - *Vaccinia* virus (In *Vaccinia* virus the GUK-related sequences are found in two distinct reading frames, shown by a slash, and the enzyme is probably not functional). Arg-295 of p55 was originally reported as Pro, but this was due to a sequencing error (A. Husain-Chishti, personal communication). From Koonin et al. (1992) with the addition of S.s. (Pig) GUK from Zschocke et al. (1993).

Table 1. Proteins that co-purify with p55 and PSD-9 5/ SAP90, and proteins immunolocalized at *Drosophila* septate junctions

Protein	Co-purifies with p55	Co-purifies with PSD-95/SAP90	Enriched at septate junctions
Spectrin	+	+	+
Actin	+	+	+
Tubulin	NT	+	+
Protein 4.1	+	NT	+
Dematin	+	NT	NT
Fasciclin III	NA	NA	+
S/T kinase	+	+	NT

The p55 data are from erythrocytes (Ruff et al., 1991); the PSD-95/ SAP90 from neurons (Cho et al., 1992), and the septate junctions from *Drosophila* imaginal disc epithelium (D. F. W and P. J. B., unpublished data). The information on the localization of protein 4.1 in *Drosophila* was kindly provided by R. Fehon. NA, not applicable - protein has not been reported in this tissue; NT, not tested.

Drosophila. These genes all encode membrane-associated guanylate kinase homologs, which we abbreviate **MAGUK**s. Each MAGUK consists of three distinct domains (Fig. 7). (1) One or three copies of DHR, (2) an SH3 domain and (3) the guanylate kinase-like domain (GUK). The OPA and PEST domains present in the DlgA protein are not present in the other MAGUKs identified so far.

Mammalian PSD-95/SAP90

The first mammalian MAGUK was identified as a partial cDNA sequence (1779) from a collection of cDNAs made from human brain RNA (Adams et al., 1992). The partial sequence shows that 1779 is the human homolog of a gene recently identified in the rat that encodes a component of synaptic junctions (PSD-95/SAP90) in the adult brain (Cho et al., 1992; Kistner et al., 1993). The predicted PSD-95/SAP90 protein shows strong homology (about 67% identity) to DlgA throughout its length and we therefore refer to it as a Dlg-R (discs large-related) protein. Unlike DlgA, which is expressed in a variety of tissues including the nervous system, PSD-95/SAP90 appears to be expressed only in the nervous system in postnatal rats. However, it is intriguing to note that, like the product of *dlg*, PSD-95/SAP90 is localized in a specialized junction, the synaptic junction, that is involved in information transfer between cells. Also like the product of *dlg*, the PSD-95/SAP90 sequence shows strong conservation of the GMP-binding site but has the same deficiency in the putative ATP-binding site (Fig. 8), suggesting that its catalytic activity, if any, is similar to that of the *Drosophila* protein (Koonin et al., 1992). We have no information about PSD-95/SAP90 expression in embryonic stages of development, so it is still possible that it is expressed in epithelial cells and is involved in cell interactions controlling proliferation in these early stages.

Human p55

A second human MAGUK is the heavily palmitoylated protein p55, which was purified from red blood cell membrane ghosts and shows striking similarity to the DlgA protein in the GUK region, the SH3 region, and one DHR repeat (Ruff et al., 1991; Bryant and Woods, 1992). p55 is expressed in most cell types in addition to blood cells and thus could be involved in cell growth control. We have recently cloned a *Drosophila* p55 homolog (D. F. W and P. J. B., unpublished data) and the partial predicted amino acid sequence shows approximately 60% identity to that of human p55. Thus, there are two types of MAGUK in both mammals and *Drosophila*, suggesting that these proteins may have distinct and conserved functions. The ATP-binding site in p55 does not contain a deficiency like that seen in DlgA, but it does show a K to R substitution (Fig. 8) that might affect enzyme activity. The p55 gene is located near the tip of the long arm of the X chromosome, between the genes encoding the blood-clotting protein factor VIII and the enzyme glucose-6-phosphate dehydrogenase (Metzenberg and Gitschier, 1992). No tumor suppressor genes have been discovered in this chromosome region, but at least 19 genetic diseases are associated with the interval and one of them, dyskeratosis congenita, is associated with precancerous lesions (leukoplakia) and a high risk of cancer (McKay et al., 1991; Marsh et al., 1992).

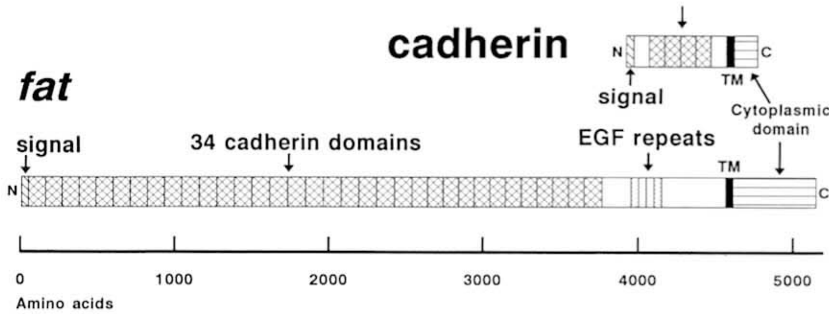

Fig. 9. Comparison between the predicted organization of the *Drosophila fat* protein and a typical vertebrate cadherin (Mahoney et al., 1991). The predicted *fat* product contains 34 extracellular domains with sequence similarity to the four extracellular domains of typical cadherins. It also contains four epidermal growth factor (**EGF**) repeats that are absent from typical cadherins. In the predicted *fat* protein the first cadherin domain occurs immediately after the signal sequence, whereas in typical cadherins the signal sequence is followed by approximately 100 amino acids that are cleaved off to produce the mature protein. There is no significant homology between the predicted *fat* protein and typical cadherins in the cytoplasmic domain. C, carboxy terminus; N, amino terminus; TM, transmembrane domain. After Mahoney et al. (1991).

Proteins interacting with MAGUK proteins

The p55 and PSD-95/SAP90 proteins were purified by subcellular fractionation, and in both cases several co-purifying proteins were identified (Table 1). This association may result from specific interactions between the MAGUK protein and the co-purifying molecules, or it may simply be a result of similar physico-chemical properties. The former explanation seems more likely for at least some of these proteins since they remain tightly associated with the MAGUK even after treatment with sarcosyl, a relatively harsh detergent (Cho et al., 1992). Furthermore, some of these proteins have *Drosophila* homologs that are found, along with DlgA, at the septate junctions (Table 1). These results suggest that the MAGUKs are integral components of large protein networks that are restricted to specific junctional complexes or other membrane-associated structures in the cell.

Most of the MAGUK-interacting proteins thus far identified appear to be part of the cytoskeletal network, perhaps reflecting the importance of these proteins in maintaining cytoarchitecture. Other associated proteins include cell adhesion molecules (FasIII; Patel et al., 1987) and signal transduction proteins (serine/threonine kinases; Ruff et al., 1991; Cho et al., 1992). In the case of the synaptic density, where PSD-95/SAP90 is localized, the kinase activity (type II Ca^{2+}/calmodulin-dependent protein kinase) has been shown to be necessary for induction of long-term potentiation of synaptic transmission (Malinow et al., 1989). There are probably additional proteins associated with the MAGUKs, including some expected to interact with the SH3 domain, whose identities and functions remain to be elucidated. Furthermore, if MAGUK proteins are involved in transduction of extracellular signals, it seems likely that transmembrane proteins will be involved upstream of them in the signalling pathway. The identity of these elements has yet to be discovered.

THE *fat* LOCUS

In lethal mutations of the *fat* locus all of the imaginal discs show hyperplastic overgrowth, in that the overgrowing imaginal discs retain their single-layered epithelial structure and their ability to differentiate (Bryant et al., 1988; Mahoney et al., 1991). The gene potentially encodes a 5147-amino acid protein, and it is transcribed in the embryonic ectoderm and larval imaginal discs (Mahoney et al., 1991). The predicted *fat* gene product is a large transmembrane protein with strong sequence homology to calcium-dependent cell adhesion molecules (cadherins; Fig. 9). However, the product is much larger than typical cadherins, with 34 extracellular domains corresponding to the four domains of typical cadherins, as well as four EGF-like repeats and other cysteine-rich regions in the extracellular domain. The cytoplasmic domain shows no clear homology to that of typical cadherins. The structure of the predicted protein suggests that cell adhesion, dependent on a cadherin-like molecule, is required for the cell communication that controls tissue growth. Parts of the imaginal disc epithelium are shed as vesicles in *fat* mutants (Bryant et al., 1988), supporting the idea that these mutations partially disrupt cell adhesion.

Cadherins have not typically been considered as tumor suppressor gene products, but recent work is suggesting strongly that the E-cadherin (=uvomorulin, homologous to L-CAM) gene located at 16q22.1 (Natt et al., 1989) may correspond to a tumor suppressor gene identified by loss of heterozygosity in this genetic region in hepatocellular, breast, and prostate carcinomas (Sato et al., 1990; Zhang et al., 1990; Carter et al., 1990; Tsuda et al., 1990; Field, 1992). E-cadherin expression is lost in head and neck and hepatocellular carcinomas (Schipper et al., 1991; Shimoyama and Hirohashi, 1991), and E-cadherin loss is correlated with invasiveness of carcinoma and epithelial cell lines (Behrens et al., 1989; Frixen et al., 1991). Non-invasive carcinoma and epithelial cell lines become invasive when treated with anti-E-cadherin antibodies (Behrens et al., 1989; Frixen et al., 1991); and invasiveness of carcinoma cell lines can be prevented by transfection with E-cadherin cDNA (Frixen et al., 1991). These results are highly suggestive of a role for this cell adhesion molecule in tumor or metastasis suppression. The finding that mutations in the *fat* gene cause overgrowth supports the idea that the cadherin losses reported in cancer cells do, indeed, contribute to the malignant phenotype.

SOMATIC LOSS OF TUMOR SUPPRESSOR FUNCTION

In some cases, loss of heterozygosity for a tumor suppressor gene in individual cells of a heterozygote, caused by

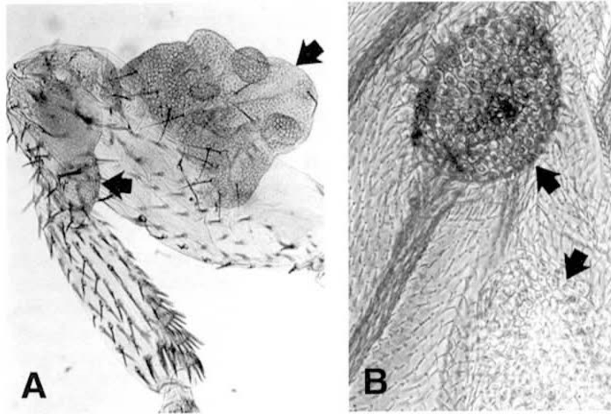

Fig. 10. The *wts* phenotype is shown in mitotic recombination clones (arrows) homozygous for *Df(3R)A177der20*, induced by gamma irradiation. Clones are marked by homozygous recessive Ki^+, which produces normal (long, straight) bristles in a background of Ki^s heterozygous cells that produce short, curved bristles. Polygonal ridges of cuticle surround cells within clones on the leg (A) and on the wing (B), where the unusual rounded shape of the clones is also apparent (R. W. J. and P. J. B., unpublished data).

experimentally induced mitotic recombination, leads to overgrowing clones of cells in specific tissues. This has been demonstrated for both *dlg* (Woods and Bryant, 1991) and *fat* (Mahoney et al., 1991), and the phenomenon raises the possibility of identifying new tumor suppressor genes by directly examining the effects of mutations in mitotic recombination clones. Recent advances in *Drosophila* genetics make screening mitotic recombination clones very efficient. The screen takes advantage of transgenic flies developed by Golic and Lindquist (1989) in which a yeast target-specific FLP recombinase gene has been placed under the control of a *Drosophila* heat-shock promoter and integrated into the *Drosophila* genome. Yeast FLP recombinase target sequences (FRTs) also have been integrated into several proximal sites on *Drosophila* chromosomes. Heat shocking flies carrying the FLP recombinase construct and homozygous for a FRT site leads to a high frequency of mitotic recombination at the FRT site (Golic, 1991). To use this system for screening, chromosomes are mutagenized in the parental generation and then made homozygous in mitotic recombination clones of the F_1 generation. The phenotype of the mutant clones is then examined, and chromosomes that give overgrowing clones are recovered by breeding from the affected F_1 flies.

We have recovered a deficiency for the *warts* gene (*wts*) using this screen. Mitotic recombination clones homozygous for such deficiencies produce spectacular outgrowths from the body surface (Fig. 10). The clones are larger than normal and tend to split into several rounded fragments, whereas control clones are intact, but irregular and elongated on both legs and wings. The large size of *wts* clones indicates that the constituent cells undergo more divisions than normal, and the rounded shape of mutant clones suggests that the division of mutant cells is not preferentially oriented as it appears to be in wild-type imaginal discs (Bryant and Schneiderman, 1969). The texture of the cuticle in *wts* clones is very unusual - each polygonal cell outline is marked by a thick ridge of cuticle (Fig. 10). The apparent intrusion of cuticle between cells suggests that there may be a failure of adhesion or other abnormality in the apical region of the epithelial cells. The *wts* gene has been localized to 100A3-7 on chromosome 3 based on the cytology of a series of overlapping deficiencies that give the *wts* phenotype in mitotic recombination clones (R. W. J and P. J. B., unpublished data).

The mitotic recombination screen is efficient because it allows immediate detection of candidate chromosomes in F_1 flies without the necessity of breeding each F_1 individual as is required in other screens for lethal mutations. The screen is ideally suited to the identification of tumor suppressor genes that function in imaginal discs.

CONCLUSIONS

The preceding examples show that the *Drosophila* model system can be used effectively to identify and characterize tumor suppressor genes that have human homologs. Perhaps not surprisingly, these genes encode proteins at various positions in the cell which might act at different points in the pathways of signalling and signal transduction that control cell proliferation. In the case of epithelial cells, a requirement for cell adhesion in proliferation control is suggested by the overgrowth caused by mutations affecting the *fat* transmembrane protein and possibly the *dlg* membrane-associated protein. This requirement is consistent with experimental work indicating that contact-dependent cell interactions control proliferation in epithelial tissue (Bryant, 1987). The involvement of guanine-nucleotide mediated signal transduction is suggested by the nature of the predicted protein produced by the *dlg* locus. In the hematopoietic system, little experimental work has been done on the mechanisms controlling cell proliferation and differentiation, but the evidence from the RpS6 gene suggests that phosphorylation cascades and translational control mechanisms will turn out to be important in these cells. The cloning and characterization of more tumor suppressor genes in *Drosophila* seems likely to provide a wealth of new information on how cell proliferation is controlled by cell signalling in a variety of tissues and organs.

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