Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 11302–11306, December 1992
Cell Biology

Drosophila homolog of the human S6 ribosomal protein is required for tumor suppression in the hematopoietic system

(melanotic tumor/tumor suppressor gene/invertebrate immunity/P element)

KELLIE L. WATSON*†, KENNETH D. KONRAD*‡, DANIEL F. WOODS*, AND PETER J. BRYANT*

*Developmental Biology Center, University of California, Irvine, CA 92717; and ‡Advanced Development Unit, Beckman Instruments Inc., Fullerton, CA 92634

Communicated by Edward B. Lewis, August 26, 1992 (received for review July 30, 1992)

ABSTRACT **The tumor suppressor gene lethal(1)aberrant immune response 8 (*air8*) of *Drosophila melanogaster* encodes a homolog of the human S6 ribosomal protein. P element insertions that prevent expression of this gene cause overgrowth of the lymph glands (the hematopoietic organs), abnormal blood cell differentiation, and melanotic tumor formation. They also cause delayed development, inhibit growth of most of the larval organs, and lead to larval lethality. Mitotic recombination experiments indicate that the normal S6 gene is required for clone survival in the germ line and imaginal discs. The S6 gene produces a 1.1-kilobase transcript that is abundant throughout development in wild-type animals and in revertants derived from the insertional mutants but is barely detectable in the mutant larvae. cDNAs corresponding to this transcript show a 248-amino acid open reading frame with 75.4% identity and 94.8% similarity to both human and rat S6 ribosomal protein sequences. The results reveal a regulatory function of this ribosomal protein in the hematopoietic system of *Drosophila* that may be related to its developmentally regulated phosphorylation.**

Genetic procedures in *Drosophila* can be used to rapidly identify genes that have important roles in controlling development, and these genes are often found to have mammalian homologs that are already known to be clinically important or may be found to be clinically important in the future. This approach holds promise for identification of tumor suppressor genes required for the control of cell proliferation during development. So far, ≈25 tumor suppressor genes have been identified in *Drosophila* by recessive lethal mutations that cause tumor formation in various proliferating cell populations in the larva (1–5). Three of the tumor suppressor genes that function in imaginal discs have been cloned, and two of these show homology to known human genes. Thus, the *dlg* gene encodes a cell junction-associated protein (6) that has at least two different homologs in mammals (7–9), and the *fat* gene encodes an enormous transmembrane protein with homology to vertebrate cadherins (10). In these cases, a role for the human homolog in cell growth control is not known but now seems likely. The third example, the *lgl* gene, encodes a cell-surface protein with no clear homologies (11). The fact that all three of these *Drosophila* tumor suppressor genes encode cell membrane-associated proteins indicates that cell proliferation in imaginal discs and other tissues is controlled at least in part by cell–cell interactions requiring gene products at the membrane. A human tumor suppressor gene (*DCC*) important in colorectal carcinoma encodes a cell adhesion molecule (12), again suggesting the importance of cell–cell interactions in controlling tissue growth.

Hematopoietic tissues produce blood cells by a stem-cell mechanism and release them into circulation. Cell proliferation control mechanisms in these tissues are different from the mechanisms operating in solid tissues, such as imaginal discs, that normally cease growth at a specific final size. In *Drosophila*, genes necessary for proliferation control in hematopoietic tissues have been identified by overproduction of blood cells in mutant larvae, and in mutant larvae the blood cells aggregate to form melanotic tumors. One example is identified by the mutation lethal(1)aberrant immune response 8 (*air8*; polytene map location, 7C4-9), which was recovered in a genetic screen designed to identify X chromosome-linked tumor suppressor genes in which transposable-element insertions cause lethal phenotypes associated with melanotic tumor formation (1). In *air8* mutant larvae, the hematopoietic organs (lymph glands) show dramatic overgrowth involving both cell enlargement and overproliferation (1). The mutant blood cells aggregate and differentiate into multilayered cellular capsules, which are colored black by accumulated melanin (13). Other tissues in the mutant larvae fail to reach their normal final size, and the mutants die as late larvae. In this paper, we report that the *air8* gene encodes a *Drosophila* homolog of the mammalian S6 ribosomal protein, which is unusual among ribosomal proteins in showing developmentally regulated phosphorylation that is often associated with cell growth and tumorigenesis.§ The results provide a striking confirmation for the suggestion that this protein is involved, at least in some tissues, in the regulation of cell growth. Although we have used allele designations in this paper, based on the evidence presented here, the *air8* gene should be renamed *rpS6*.

MATERIALS AND METHODS

Mutations and Stocks. The mutations analyzed in this paper are associated with insertion of transposable elements—a P element (14) in stock *air8* (1), and a *P[lArB]* element in stock *Is(X)Pry007C-WG1288* (*WG1288*) (15). Descriptions of all other mutations and chromosomes can be found in ref. 16.

Germ-Line Clone Analysis. The germ-line function of the *air8* gene product was determined by the dominant female-sterile technique (17). In this technique, the flies were heterozygous for a dominant female-sterile mutation *Fs(1)K1237* (18) that blocks egg development. Mitotic recombination clones that are homozygous for the wild-type allele of *Fs(1)K1237* can produce eggs, but in this experiment they were also homozygous for *air8* and were thereby used to determine the effects of this mutation on germ-line clone development. y^1 w^1 *air8/Binsn* or y^1 w^1 females were crossed to v^{24} *Fs(1)K1237* males and the F_1 progeny were irradiated (1000 rads; 1 rad = 0.01 Gy; Isomedix, Parsippany, NJ, ^{137}Cs source) during the mid-to-late first instar of development to induce mitotic recombination. The resulting heterozygous v^{24}

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

†To whom reprint requests should be addressed.

§The sequence reported in this paper has been deposited in the GenBank data base (accession no. L01658).

Fs(1)K1237/y^1 w^1 air8 or *v^{24} Fs(1)K1237/y^1 w^1* females were mated, examined for egg production, and dissected to search for vitellogenic ovaries, which would indicate the presence of homozygous germ-line clones.

Hemocyte and Lymph Gland Preparations. Circulating hemocytes were obtained from third-instar larvae by removing a small volume of hemolymph and transferring it into a large drop of mineral oil on a glass slide. Lymph glands were dissected in Ringer's solution, fixed in 0.05 M cacodylate/4% sucrose/4% glutaraldehyde for 1 h at room temperature, postfixed with osmium tetroxide, counterstained with lead citrate and uranyl acetate, embedded in plastic, and sectioned for electron microscopy.

Genomic DNA/cDNA Clones. λEMBL3 phage and pJB8 cosmid clones containing DNA from the 7C polytene interval were kindly provided by A. Lambertsson, T. Johansson, and S. Andersson (University of Umeå, Umeå, Sweden). An adult female cDNA library was constructed in LambdaZAP II by directionally cloning into the *Eco*RI/*Not* I sites. Approximately 10^5 plaques containing recombinant clones were blotted onto Hybond-N membrane (Amersham) by standard methods, baked at 80°C for 2 h, and hybridized as described below. Using a *Drosophila* S6 probe, positive signals were obtained from the adult female cDNA library at a frequency of 0.07%.

Southern and Northern Blot Analyses. DNA was purified from animals, phage, plasmids, and cosmids and transferred to Biotrans (ICN) filters according to established techniques (19). Prehybridization in 5× Denhardt's solution/5× standard saline citrate (SSC), hybridization in 1× Denhardt's solution/5× SSC, and washes in 0.1× SSC/0.1% SDS were performed at 65°C. Total RNA was extracted from whole animals by the LiCl/urea method (20) as adapted for small amounts of *Drosophila* tissue by J. Rawls and G. Richards (personal communication), and poly(*A*)$^+$ RNA was isolated by using the PolyAtract mRNA isolation system (Promega). The mRNAs were blotted from 1% agarose/formaldehyde gels onto Nytran (Schleicher & Schuell) membranes, UV-crosslinked, hybridized in a buffer containing 1% bovine serum albumin, 0.5 M Na_2HPO_4 (pH 7.2), and 7% SDS and washed in 0.1% SDS/0.1× SSC at 65°C. Probes were labeled with [α-^{32}P]dCTP (NEN) using a Prime-It kit (Stratagene).

PCR. DNA was amplified under the following conditions: 100 ng of template DNA, 100 ng of each primer DNA, 1.25 mM dNTPs, 1× buffer (Promega), and 2.5 units of *Taq* DNA polymerase (Promega). The temperature cycle 94°C for 1 min, 45°C–70°C (depending on the primer sets) for 2 min, and 72°C for 3 min was used for 35 cycles followed by 65°C for 2 min and 72°C for 10 min for 1 cycle in an M. J. Research MiniCycler. The amplification products were isolated from agarose gels and used as templates for double-stranded cycle sequencing (Life Technology, Grand Island, NY).

DNA Sequencing. Genomic DNA derived from λEMBL3-57 and λEMBL3-64 was subjected to dideoxynucleotide chain-termination sequencing with Sequenase (United States Biochemical) using a series of 17- to 20-mer oligonucleotide primers. S6 sequence information was obtained from sequencing cDNA clones isolated from an adult female library using probes A and B (see Fig. 3). The cDNAs were PCR amplified with oligonucleotide primers and the amplified products were subjected to double-stranded cycle sequencing. The locations of the P elements in the mutant strains were determined by PCR amplification and double-stranded cycle sequencing of the mutant genomic DNAs surrounding the P insertion sites.

RESULTS

Mutant Phenotype. In *air8* hemizygotes, both embryonic and larval development are much slower than normal and the ring gland, salivary glands, gastric cecae, and most imaginal discs fail to reach normal size before the late-larval lethal phase (1). The ventral ganglion fails to condense and the brain hemispheres are undersized. Mutant animals die at a variety of larval stages, up to and including the late third larval instar when large melanotic tumors can be seen in the body cavity (Fig. 1). The melanotic tumor phenotype results from abnormalities in the production and differentiation of blood cells (hemocytes) in the mutant larvae. The hemocyte population in normal larvae consists almost entirely of small round cells called plasmatocytes, which differentiate into large flat lamellocytes shortly after puparium formation (21) (Fig. 2). The normal function of lamellocytes is to encapsulate foreign bodies (13) and phagocytose cell fragments derived from the tissue remodeling that occurs during metamorphosis (22). The hemocyte population of *air8* third-instar larvae contains large numbers of lamellocytes (Fig. 2), suggesting precocious differentiation of these cells from plasmatocytes as in many other melanotic tumor strains (23–25).

The source of hemocytes is the lymph gland, which consists of paired sacs associated with the dorsal blood vessel (13). In *air8* mutant larvae, the lymph gland shows tremendous hyperplasia (1), and lamellocytes are found within it, whereas they are never observed in wild-type lymph glands. The lamellocytes in the mutant lymph gland are spiral in cross section (Fig. 2), suggesting a form of self-encapsulation that may be related to the abnormal cellular events leading to tumor formation. The abnormal lymph glands are frequently melanized and this property appears to be tissue autonomous since dissociated *air8* lymph gland tissue injected into wild-type adult flies causes melanotic tumors to form in the hosts, whereas wild-type lymph gland tissue does not have this effect (K.L.W., unpublished data).

The effect of *air8* on development of cell clones was investigated by producing homozygous *air8* mitotic recombination clones in heterozygous larvae. Adult females derived from these larvae showed no clones of vitellogenic cells in the ovary, whereas the controls produced a high frequency of such clones (Table 1). Mutant heterozygotes also failed to

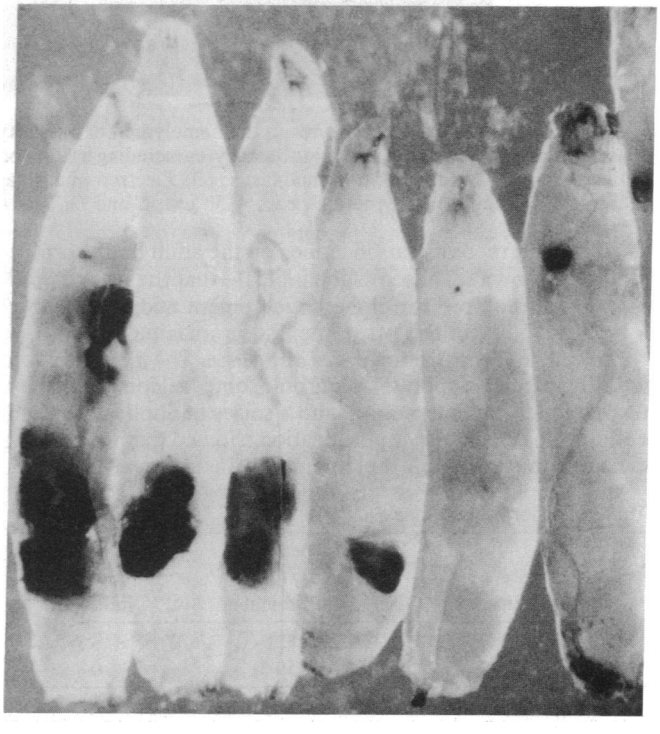

FIG. 1. Hemizygous *air8* third-instar larvae showing the melanotic tumor phenotype. (×18.)

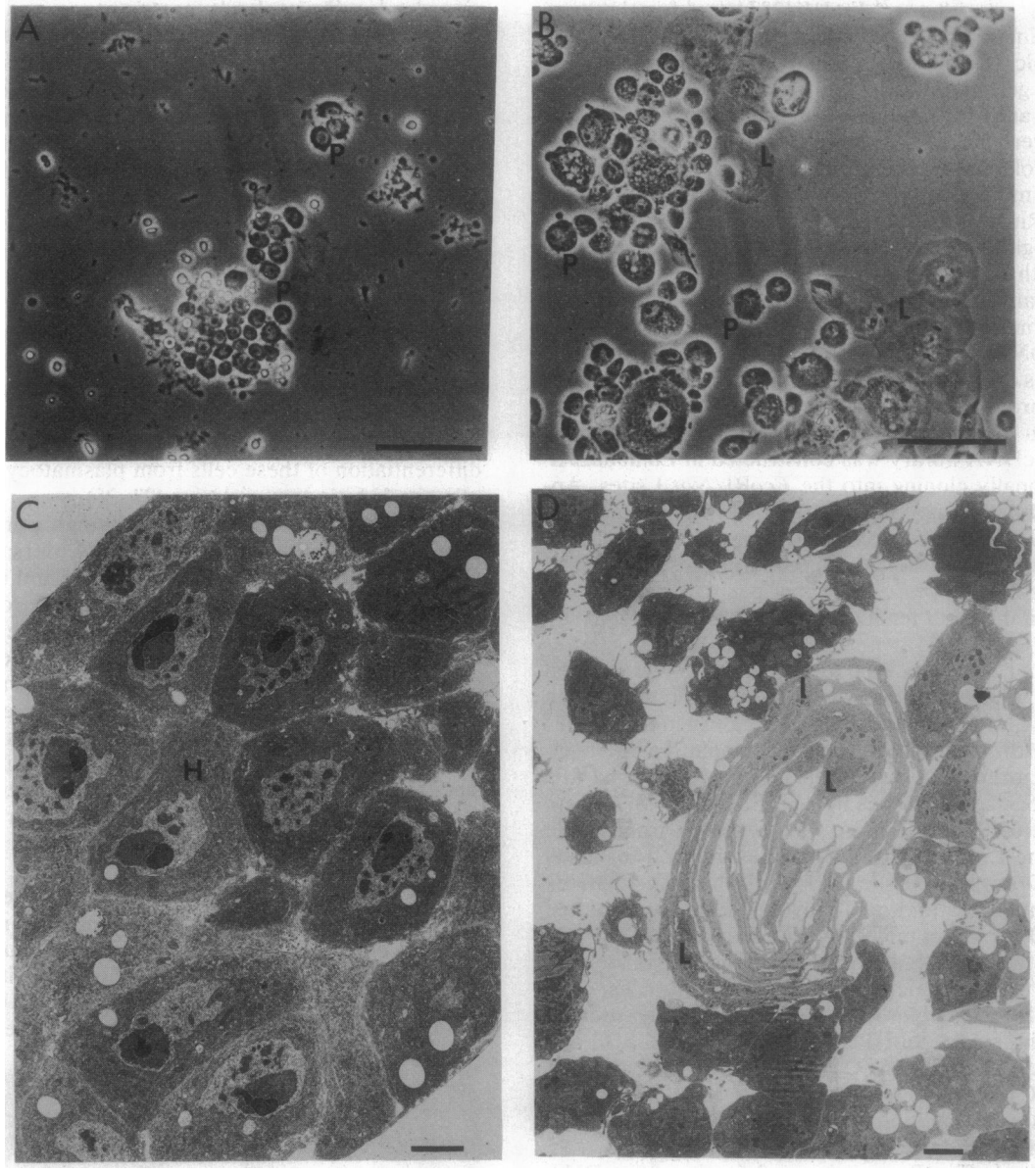

FIG. 2. (*A*) Circulating hemocytes in the hemolymph of a wild-type third-instar larva viewed under phase contrast. P, plasmatocytes. (*B*) Phase-contrast view of *air8* circulating hemocytes including a lamellocyte (L) and plasmatocyte (P). (*C*) Electron micrograph showing hemocytes (H) in a lymph gland lobe of a normal larva. (*D*) Electron micrograph showing an *air8* lymph gland lobe. At least three lamellocytes (L) are encapsulating each other. (*A* and *B*, bars = 50 μm; *C* and *D*, bars = 1.5 μm.)

show mitotic recombination clones on the adult body surface (data not shown). These results indicate that the normal *air8* function is required for clone development and presumably for cell viability in the germ line and in imaginal discs.

Molecular Identification of the *air8* Gene. The *air8* mutation was localized to polytene chromosome region 7C4-9 by testing for complementation with a series of duplications and deficiencies (1). *In situ* hybridization showed that the mutant chromosome carries a P element (14) insertion at this location, whereas the element is missing from a chromosome in which the mutation was reverted by exposure to P transposase (15) (data not shown). Genomic Southern blots show that phages λ57 and λ64 (Fig. 3) recognize restriction fragment length differences in the *air8* chromosome consistent with the presence of a P element insert, whereas the revertant chromosome appears normal (data not shown). Probing of a developmental Northern blot with genomic DNA fragment B (Fig. 3) revealed a 1.1-kilobase (kb) transcript expressed strongly throughout development, with an abundance comparable to that of ribosomal protein 49 and actin (Fig. 4). The *air8* mutant lane on the Northern blot showed barely detectable levels of this transcript, whereas the revertant showed expression at a level indistinguishable from that of wild type.

Five different cDNAs were isolated by probing an adult female cDNA library with genomic probes A and B (Fig. 3) and sequencing of these cDNAs showed that they all carry the same open reading frame encoding a protein of 248 amino acids. The presumed full-length cDNAs measure ≈1 kb (Fig. 3). PCR amplification and sequencing of *air8* DNA revealed

Table 1. Germ-line clone analysis

Mutant chromosome	Clone-bearing females/total irradiated females	% clone induction
y^1 w^1	22/252	8.7
y^1 w^1 *air8*	0/159	0

Homozygous mutant clones were produced by γ-ray-induced mitotic recombination in a dominant female-sterile [*Fs(1)K1237*] background and were identified by the presence of at least one vitellogenic ovariole.

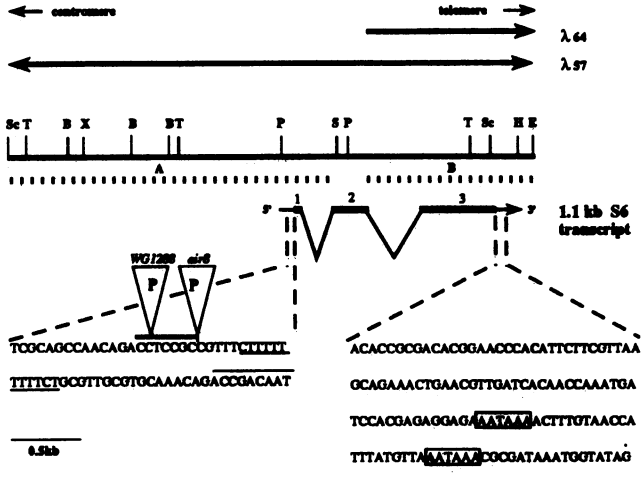

FIG. 3. Schematic representation of the S6 gene. Genomic DNA in λEMBL3-57 and λEMBL3-64 is indicated above the restriction map. B, *Bgl* II; E, *Eco*RI; H, *Hin*dIII; P, *Pvu* II; S, *Sal* I; Sc, *Sac* I; T, *Pst* I; X, *Xho* I. Genomic probe A was isolated from λ57 and detects two different transcripts, only one of which, S6, is altered in *air8* animals. Genomic probe B is represented in both λ57 and λ64. Sequences of untranslated regions are shown at the bottom. The 5′ upstream region of S6 lacks an obvious TATA box but contains a cap site with a pyrimidine motif (underlined) and a consensus sequence (overlined) flanking the initiator codon important for efficient translation that is found in many ribosomal protein genes (26, 27). Insertion sites of P elements in *air8* (triangle) and *WG1288* (triangle with open bar) are indicated in the untranslated DNA (P elements not to scale). Precise insertion site of the *WG1288 P[lArB]* element was unresolved due to a small duplication at the insertion site. In the nontranslated trailer, there are two putative polyadenylylation signals (boxed), the second of which occurs 17 nucleotides from the poly(A) addition site (dot) and is preceded by a TTT stretch and TGT repeats, which mediate efficient cleavage and polyadenylylation (28). The three exons (exon 1, encoding amino acids 1 and 2; exon 2, encoding amino acids 3–70; exon 3, encoding amino acids 71–248) are indicated with solid bars and direction of transcription is indicated with an arrow.

the presence of a P element in the 5′ untranslated region of this gene (Fig. 3). P element sequences were not detected in this region of the revertant chromosome. A second allele of this locus, *WG1288*, which produces a melanotic tumor phenotype similar to that of *air8*, has a *P[lArB]* element (15) inserted in the 5′ noncoding region within 10 nucleotides of the P insertion site in the *air8* strain (Fig. 3).

Homology with the S6 Ribosomal Protein Gene. The amino acid sequence predicted from the transcription unit affected by *air8* shows 75.4% identity, and 94.8% similarity if conservative substitutions are counted, with the S6 ribosomal protein sequence of human (30) and rat (31) (Fig. 5). The sequence includes four copies of a 10-amino acid motif for which the consensus sequence includes an initial proline residue and 4–6 basic amino acids and is postulated to be a nuclear localization signal (31). It also contains seven serine residues in the C-terminal 16 amino acids, similar to the serine-rich C terminus of the human S6 protein in which at least four serines have been shown to be subject to phosphorylation (34), and a consensus recognition sequence for the mitogen-activated 70-kDa S6 kinase (35). The sequence shows 61.1% identity with that of *Schizosaccharomyces pombe* ribosomal protein S6 (28), and 61.0% identity with that of *Saccharomyces carlsbergensis* ribosomal protein S10 (36).

The presence of a ribosomal protein gene in interval 7C4-9 is consistent with the possibility of ribosomal protein gene cluster, since ribosomal proteins S14A and -B have also been mapped in this interval (37) ≈40 kb away from the S6 gene. The S6 gene contains two introns near its 5′ end, reminiscent

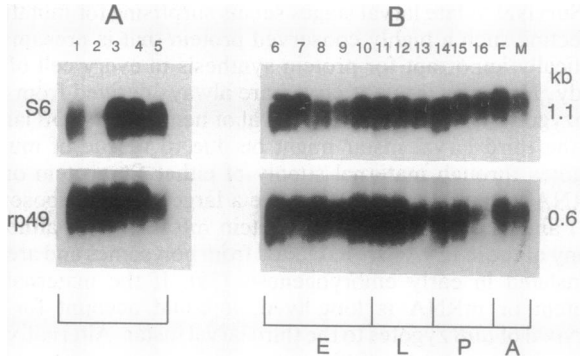

FIG. 4. Northern blot analysis of the S6 1.1-kb mRNA in various genotypes and throughout development in wild-type animals. Filters containing 2.5 μg of poly(A)$^+$ RNA per lane were hybridized with probe B (see Fig. 3). (*A*) A 1.1-kb transcript is seen in *air8*/+ heterozygotes (lane 1), *WG1288*/+ heterozygotes (lane 3), another P insertion mutation P/+ heterozygotes (lane 4), and *air8*^{REV} hemizygotes (lane 5) but is barely detectable in *air8* hemizygotes (lane 2). The weak signal may be due to remnant maternal transcripts or transcription from the P promoter. (*B*) Developmental profile of S6 expression in wild-type animals including embryonic (E) stages at 4–8, 8–12, 12–16, 16–20, and 20–24 h (lanes 6–10); larval (L) stages L1 (24–36 h), L2 (48–60 h), and L3 (wandering); pupal (P) stages P1 (24-h pupae), P2 (48-h pupae), P3 (72-h pupae); and adult (A) females (lane F) and males (lane M). Probes for both actin (data not shown) and ribosomal protein 49 (rp49) (0.6 kb) (29) were used as controls. When these filters were probed with an S6 cDNA (isolated from a LambdaZAP II adult female library) containing exons 1 and 2 with 5′ untranslated sequence, a similar result was obtained (data not shown). Early embryos (0–3 h) also contain abundant S6 mRNA detected with this probe (data not shown).

of the 5′ introns that are a common feature of ribosomal protein genes in yeast (26).

DISCUSSION

In mutant animals loss of expression of the S6 ribosomal protein gene causes growth inhibition in some tissues, and mitotic recombination experiments also show that the S6 gene product is required for egg development in the ovary and for cell survival in developing imaginal discs. Surprisingly, loss of the same gene product in the hematopoietic system does not cause cell lethality but instead leads to lymph gland hyperplasia, precocious differentiation of plasmatocytes into lamellocytes, and melanotic tumor formation. This pleiotropic syndrome eventually leads to larval death.

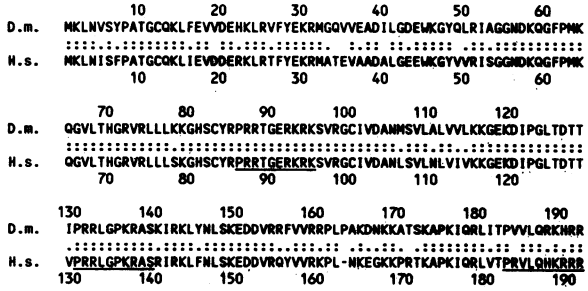

FIG. 5. Sequence alignments of *Drosophila* (D.m.) and human S6 (H.s.) ribosomal protein sequences. Alignments were generated by the FASTA program (32). The four copies of the 10-amino acid motif (31) mentioned in the text are underlined. Residues that are subject to phosphorylation in the human protein (33) are shaded. Consensus recognition sequence for mitogen-activated 70-kDa S6 kinase (34, 35) is boxed.

Survival to late larval stages seems surprising for mutations affecting such a highly conserved protein that is presumably critically important for protein synthesis in every cell of the body. However, mutant larvae are always derived from heterozygous mothers, so the survival of hemizygous *air8* larvae to the third larval instar might be due to rescue of mutant zygotes through maternal supply of either S6 protein or S6 mRNA in the egg. Embryos have a large store of ribosomes (38) and maternal ribosomal protein mRNAs (39), although many of these RNAs are excluded from polysomes and are not translated in early embryogenesis (39). If the maternal S6 protein or mRNA is long-lived, it could account for the survival of *air8* zygotes to the third larval instar. Alternatively, the minute amount of S6 transcription in mutant larvae, detected on Northern blots, may be directed from a weak promoter in the P element and might provide enough gene product to allow development to late larval stages.

If the lymph gland required less S6, it might be able to grow while other tissues are growth impaired. However, this interpretation does not easily account for the ability of *air8* lymph glands to produce tumors in wild-type hosts, which indicates an autonomous effect of the mutation on lymph gland differentiation.

Our results support previous indications that S6 is important in controlling cell growth and proliferation. For example, S6 and other ribosomal phosphoproteins are overexpressed in human colon carcinomas and liver metastases (40). Furthermore, S6 is unusual among ribosomal proteins in that it shows developmentally regulated phosphorylation of a cluster of serine residues at the C terminus. In quiescent mammalian cells, this phosphorylation is stimulated by treatments that increase protein synthesis and cell proliferation such as serum growth factors, insulin, tumor promoting agents, transforming viruses, mitogens, and chemical carcinogens (see ref. 41 for review). S6 ribosomal protein is rapidly dephosphorylated when growth is arrested by serum deprivation, contact inhibition, or heat shock (41, 42). It has therefore been suggested that S6 and its specific kinases (35) may have important regulatory roles in controlling cell growth through the selective translation of particular classes of mRNAs (41), although the latter have not been identified.

In insects, S6 phosphorylation is stimulated by 20-hydroxyecdysone in the fat body of fly larvae (43, 44) and by prothoracicotropic hormone in the prothoracic gland of moth larvae (45); in both cases, the stimulation is inhibited by juvenile hormone (44, 45). Juvenile hormone treatment can cause a dramatic increase in frequency and earlier onset of melanotic tumor formation in the *tu-bw* melanotic tumor strain (46) and can also induce such tumors in wild-type *Drosophila* (47). It will now be important to determine whether these effects are due to stimulation of blood cell production or to enhanced differentiation and melanization and whether they are associated with altered S6 expression or phosphorylation.

We thank A. Lambertsson, T. Johansson, and S. Andersson for the gift of phages and cosmids; P. Coassin, J. Rampal, and D. Johnson for generous assistance in producing oligonucleotide primers; and B. Hamkalo, M. O'Connor, and G. Serbedzija for many helpful suggestions. This investigation was supported by a research grant from the National Science Foundation, and by predoctoral fellowships to K.L.W. from the National Institutes of Health and the American Cancer Society, Orange County Chapter.

1. Watson, K. L., Johnson, T. K. & Denell, R. E. (1991) *Dev. Genet.* **12**, 173–187.
2. Mechler, B. (1990) *Cancer Surv.* **9**, 505–527.
3. Bryant, P. J. & Schmidt, O. (1990) *J. Cell Sci.* **97**, Suppl. 13, 169–189.
4. Mechler, B. M., Strand, D., Kalmes, A., Merz, R., Schmidt, M. & Török, I. (1991) *Environ. Health Perspect.* **93**, 63–71.
5. Gateff, E. A. & Mechler, B. M. (1989) *CRC Crit. Rev. Oncog.* **1**, 221–245.
6. Woods, D. F. & Bryant, P. J. (1991) *Cell* **66**, 451–464.
7. Koonin, E. V., Woods, D. F. & Bryant, P. J. (1992) *Nature Genet.*, in press.
8. Cho, K.-O., Hunt, C. A. & Kennedy, M. B. (1992) *Neuron*, in press.
9. Bryant, P. J. & Woods, D. F. (1992) *Cell* **68**, 621–622.
10. Mahoney, P. A., Weber, U., Onofrechuk, P., Biessmann, H., Bryant, P. J. & Goodman, C. S. (1991) *Cell* **67**, 853–868.
11. Merz, R., Schmidt, M., Török, I., Protin, U., Schuler, G., Walther, H. P., Krieg, F., Gross, M., Strand, D. & Mechler, B. M. (1990) *Environ. Health Perspect.* **88**, 163–167.
12. Fearon, E. R., Cho, K. R., Nigro, J. M., Kern, S. E., Simons, J. W., Ruppert, J. M., Hamilton, S. R., Preisinger, A. C., Thomas, G., Kinzler, K. W. & Vogelstein, B. (1990) *Science* **247**, 49–56.
13. Rizki, T. M. (1978) in *The Genetics and Biology of Drosophila*, eds. Ashburner, M. & Wright, T. R. F. (Academic, New York), Vol. 2b, pp. 397–452.
14. O'Hare, K. & Rubin, G. M. (1983) *Cell* **34**, 25–35.
15. Bellen, H. J., O'Kane, C. J., Wilson, C., Grossniklaus, U., Pearson, R. K. & Gehring, W. J. (1989) *Genes Dev.* **3**, 1288–1300.
16. Lindsley, D. L. & Zimm, G. G. (1992) *The Genome of Drosophila melanogaster* (Academic, New York).
17. Perrimon, N., Engstrom, L. & Mahowald, A. P. (1989) *Genetics* **121**, 333–352.
18. Komitopoulou, K., Gans, M., Margaritis, L. M., Kafatos, F. C. & Masson, M. (1983) *Genetics* **105**, 897–920.
19. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), 2nd Ed., p. 1-I.47.
20. LeMeur, M., Glanville, N., Mandel, J. L., Gerlinger, P., Palmiter, R. & Chambon, P. (1981) *Cell* **23**, 561–571.
21. Rizki, M. T. M. (1957) *J. Morphol.* **100**, 437–458.
22. Whitten, J. M. (1964) *J. Insect Physiol.* **10**, 447–469.
23. Sparrow, J. C. (1978) in *The Genetics and Biology of Drosophila*, eds. Ashburner, M. & Wright, T. R. F. (Academic, New York), Vol. 2b, pp. 277–313.
24. Rizki, T. M. & Rizki, R. M. (1980) *Wilhelm Roux's Arch. Dev. Biol.* **189**, 197–206.
25. Nappi, A. J. & Carton, Y. (1986) in *Immunity in Invertebrates*, ed. Brehelin, M. (Springer, Berlin), pp. 171–187.
26. Mager, W. H. (1988) *Biochim. Biophys. Acta* **949**, 1–15.
27. Perry, R. P. & Meyuhas, O. (1990) *Enzyme* **44**, 83–92.
28. Gross, T., Nischt, R., Gatermann, K., Swida, U. & Kaufer, N. F. (1988) *Curr. Genet.* **13**, 57–63.
29. O'Connell, P. & Rosbash, M. (1984) *Nucleic Acids Res.* **12**, 5495–5513.
30. Heinze, H., Arnold, H. H., Fischer, D. & Kruppa, J. (1988) *J. Biol. Chem.* **263**, 4139–4144.
31. Chan, Y.-L. & Wool, I. G. (1988) *J. Biol. Chem.* **263**, 2891–2896.
32. Pearson, W. R. & Lipman, D. J. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 2444–2448.
33. Wettenhall, R. E., Nick, H. P. & Lithgow, T. (1988) *Biochemistry* **27**, 170–177.
34. Ferrari, S., Bandi, H. R., Hofsteenge, J., Bussian, B. M. & Thomas, G. (1991) *J. Biol. Chem.* **266**, 22770–22775.
35. Flotow, H. & Thomas, G. (1992) *J. Biol. Chem.* **267**, 3074–3078.
36. Leer, R. J., Van Raamsdonk-Duin, M. M. C., Molenaar, C. M. T., Witsenboer, H. M. A., Mager, W. H. & Planta, R. J. (1985) *Nucleic Acids Res.* **13**, 5027–5039.
37. Andersson, S. & Lambertsson, A. (1990) *Heredity* **65**, 51–57.
38. Mermod, J. J. & Crippa, M. (1978) *Dev. Biol.* **66**, 586–592.
39. Kay, M. A. & Jacobs-Lorena, M. (1985) *Mol. Cell. Biol.* **5**, 3583–3592.
40. Barnard, G. F., Staniunas, R. J., Bao, S., Mafune, K.-i., Steele, G. D., Gollan, J. L. & Chen, L. B. (1992) *Cancer Res.* **52**, 3067–3072.
41. Traugh, J. A. & Pendergast, A. M. (1986) *Prog. Nucleic Acid Res. Mol. Biol.* **33**, 195–230.
42. Glover, C. V. C. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1781–1785.
43. Itoh, K., Ueno, K. & Natori, S. (1986) *J. Biochem. (Tokyo)* **100**, 493–498.
44. Itoh, K., Ueno, K. & Natori, S. (1987) *FEBS Lett.* **213**, 85–88.
45. Gilbert, L. I., Combest, W. L., Smith, W. A., Meller, V. H. & Rountree, D. B. (1988) *Bioessays* **8**, 153–157.
46. Bryant, P. J. & Sang, J. H. (1968) *Nature (London)* **220**, 393–394.
47. Madhavan, K. (1972) *Wilhelm Roux's Arch.* **169**, 345–349.