Analysis and Inactivation of vha55, the Gene Encoding the Vacuolar ATPase B-subunit in Drosophila melanogaster Reveals a Larval Lethal Phenotype*

(Received for publication, March 11, 1996, and in revised form, July 3, 1996)

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Vacular ATPases play major roles in endomembrane and plasma membrane proton transport in eukaryotes. A Drosophila melanogaster cDNA encoding vha55, the 55-kDa vacuolar ATPase (V-ATPase) regulatory B-subunit, was characterized and mapped to 87C2–4 on chromosome 3R. A fly line was identified that carried a single lethal P-element insertion within the coding portion of gene, and its LacZ reporter gene revealed elevated expression in Malpighian tubules, rectum, antennal palps, and oviduct, regions where V-ATPases are believed to play a plasma membrane, rather than an endomembrane, role. The P-element vha55 insertion was shown to be allelic to a known lethal complementation group l(3)SzA (= l(3)87Ca) at 87C, for which many alleles have been described previously. Deletions of the locus have been shown to be larval lethal, whereas point mutations show a range of phenotypes from subvital to embryonic lethal, and its LacZ reporter gene revealed elevated expression in Malpighian tubules, rectum, antennal palps, and oviduct, regions where V-ATPases are believed to play a plasma membrane, rather than an endomembrane, role. The P-element vha55 insertion was shown to be allelic to a known lethal complementation group l(3)SzA (= l(3)87Ca) at 87C, for which many alleles have been described previously. Deletions of the locus have been shown to be larval lethal, whereas point mutations show a range of phenotypes from subvital to embryonic lethal, implying that severe alleles confer a partial dominant negative phenotype. The P-element null allele of vha55 was shown also to suppress ectopic sex combs in Polycomb males, suggesting that transcriptional silencing may be modulated by genes other than those with known homoeotic or DNA binding functions.

The vacuolar ATPase is a multisubunit complex, related to the F$_{1}$/F$_{0}$ ATPase (1–3). The transmembrane protonophore is made of six copies of a 16-kDa proteolipid, linked by further subunits to a catalytic headgroup comprising three copies each of a 67-kDa A-subunit and a 57-kDa B-subunit. Traditionally, the A-subunit is described as catalytic, whereas the B-subunit is considered regulatory, although in reality the active sites for nucleotide binding and proton flux may lie on the interfaces between neighboring A- and B-subunits (4). Although there is known to be very high conservation within the V-ATPase family, the 57-kDa subunit is interesting as several transcripts are known, some of which are tissue-specific (5, 6). It has been argued further that choice of B-subunit transcript may affect the overall subunit composition of the holoenzyme by influencing the choice of other subunits during assembly of the V$_{1}$ headgroup (6), and the B-subunit is phosphorylated by a component of AP-2, the clathrin assembly complex (7), suggesting a role in control of vesicle trafficking.

V-ATPases, although originally defined as endosomal, are now known to energize plasma membrane transport in a variety of tissues, such as kidney, osteoclasts, and frog skin (8); and loss of kidney V-ATPase function in autoimmune disease is clinically significant (9). In an invertebrate model, a painstaking biochemical purification of particles on the goblet cell apical membranes of lepidopteran midgut (10) showed that the invertebrate K$^{+}$/H$^{+}$ antiporter (13) to produce a remarkably potent transport system (14, 15). Monospecific antibodies against the lepidopteran plasma membrane V-ATPase were used to demonstrate plasma membrane V-ATPases immunocytochemically in salivary glands, Malpighian (renal) tubules, recta, and cuticular sensillae, suggesting an energization of transport by proton, rather than sodium, motive force in probably all insects (16–18). Physiological evidence shows that Drosophila melanogaster Malpighian tubules, in common with those of other insects, are energized by a plasma membrane V-ATPase (19, 20). Accordingly, D. melanogaster not only contains a V-ATPase, but should embody the full range of plasma membrane and endomembrane roles for V-ATPase function, and our group has embarked on the characterization of V-ATPase genes with a view to dissecting the differing requirements of these roles using the genetic tools unique to Drosophila (21). The first steps in such a procedure, in D. melanogaster or any other species, are to clone and characterize the gene, obtain a chromosomal localization, and identify or produce a genetic null (a “knockout”), which may have an informative phenotype in its own right, but which provides a genetic background for subsequent genetic intervention. These are the steps reported here for vha55, the gene encoding the V-ATPase B-subunit in D. melanogaster; they represent the first knockout of a V-ATPase gene in an animal.

**EXPERIMENTAL PROCEDURES**

**Cloning and Sequencing of the vha55 Gene—Primers for polymerase chain reaction were based on two areas of particularly close homology in the then known sequences for the 57-kDa subunit (22), optimized for Drosophila codon preference (23).**

| Left 5’ primer | vha55 cDNA 5’ from 3’ end | Right 5’ primer |
|----------------|---------------------------|-----------------|
| C C A C G C G G A T C G A T C G A T C G A | G C T G C T G C T G C T G C T G C T | C C G A A G C C G C G G |
| T G T T T G G A A G A A G A G A G A G A G A G A | G A G A G A G A G A G A G A G A G A G A G A | C C T C G T A T C G C T A T C G C T A T C |

**SEQUENCES 1 AND 2**

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* This work was supported by Medical Research Council Grant G9120579CB, a Nuffield Foundation Research Fellowship, and a Royal Society Grant (to J. A. T. D.) and by general Funds of the University of Glasgow.

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‡ The abbreviations used are: ATPase, adenosine triphosphatase; V-ATPase, vacuolar ATPase; PCR, polymerase chain reaction; bp, base pairs; kb, kilobase(s) or 1000 bp; Pc, Polycomb; MOPS, 4-morpholinepropanesulfonic acid.

This paper is available on line at http://www-jbc.stanford.edu/jbc/ 30677
Two templates were used: 100 ng of first strand cDNA from Manduca sexta midgut and 1 µl of plate lysate from a D. melanogaster head cDNA library, constructed in λZapII. PCR products were sequenced directly to establish their identity, and a D. melanogaster cDNA library, prepared from eya2 adult fly heads, was screened by plaque hybridization using the D. melanogaster PCR product as a probe. Positives were obtained at FIG. 1.

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1:1000 and were observed to fall into one of three distinct strengths of hybridization. The insert of a single recombinant phage from the strongest hybridizing class of plaque was excised as a pBluescript plasmid, and the cDNA was sequenced over its entire length on both strands. Double-stranded sequencing was performed according to the Sequenase® II protocol (U. S. Biochemical Corp.), with the aid of synthetic oligonucleotide primers.

Northern Analysis—Total RNA was extracted from whole flies or from hand-dissected body segments by the RNAzol B method (24). Electrophoresis of RNA was carried out in 1% formaldehyde-agarose gel in MOPS buffer and followed by transfer to a nylon filter. RNA was cross-linked to the filters by UV radiation. The Northern blot filters were probed with the vha55 cDNA or a control rp49 cDNA encoding a ribosomal protein, each labeled with 32P by random priming. Blots were prehybridized in Church buffer (7% SDS, 1% bovine serum albumin, 1 mM EDTA, Na2HPO4 0.25M, pH 7.2) at 55 °C for 2–3 h and hybridized in Church buffer overnight. The filters were then washed at 55 °C with 2 x SSC, 0.1% SDS for 30 min, then 0.5 x SSC, 0.1% SDS for 30 min, and finally 0.1 x SSC, 0.1% SDS for 30 min. Filters were then exposed to Fuji x-ray film for 1–3 days. Blots were prehybridized in Church buffer at 65 °C for 15 min, 2.0 x SSC, 0.1% SDS at 65 °C for 15 min. Bands were visualized by autoradiographic exposure to Fuji x-ray film for 3 days. Lane 1, RNA ladder (Life Technologies, Inc.); lanes 2–4, 0.1, 0.2, and 0.5 µg, respectively, of adult head + body mRNA. SSC is 0.15 M NaCl, 0.015 M Na3 citrate, pH 7.4. B, tissue-specific Northern blot of total RNA. Upper panel, blot probed with 32P random-primed 830-bp cDNA fragment corresponding to the original PCR product. Lower panel, same blot, stripped and reprobed with 32P random-primed cDNA for the ribosomal rp49 gene, to provide a control for different RNA loadings (approximately 5 µg/lane). Lane 1, head; lane 2, thorax; lane 3, abdomen; lane 4, adult males; lane 5, adult females.

For crosses, virgin females were collected at 4-h intervals, mated singly to males, and tapped into fresh tubes daily for 1 week before discarding. Particular care was taken with Pc crosses to allow the progeny to grow under uncrowded conditions, as the penetrance of the phenotype is particularly sensitive to environmental conditions (26). Emerging adults were collected for several days from each tube, to reduce the risk that certain classes of phenotype might be slower growing and thus underrepresented in early collections.

Reporter Gene Expression—Embryos were washed from staged egg plates, dechorionated, fixed, devitellinized, and stained with 4-bromo-3-indole-acetamide (Sigma Chemical Co.). Larvae or adults were dissected in Drosophila saline, pinned out, fixed, and stained similarly. Staining patterns were viewed either under a Wild Stereomicroscope or a Leitz Ortholux microscope and photographed using Kodak Ektar film.

RESULTS

Cloning of the vha55 Gene—An 830-bp PCR product was isolated from a D. melanogaster eye-head cDNA library by PCR with degenerate primers based on conserved domains in known V-ATPase B-subunits. The PCR product was sequenced directly to establish its identity and used to identify a cDNA from the same Drosophila head library. The 2.6-kb cDNA contained a long open reading frame of 1470 bp, encoding a polypeptide of 490 amino acids with deduced molecular mass close to 55 kDa (Fig. 1) Sequence identity at the amino acid level with known V-ATPase B-subunits is extraordinarily high (Fig. 2); only 11 of the 490 residues differed between the M. sexta (29) and D. melanogaster sequences, while the human brain sequence is 90% identical. Peptide sequence motifs were identified using the PROSITE data base. In common with all known
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F-ATPase (ATP synthase) A-subunits and V-ATPase B-subunits, the sequence shows the PPVNLPSLS motif at 370 (Fig. 1), which is highly conserved in the V-ATPase family (5). The vha55 gene falls within the 87C2–3 and 87C6–8 regions, and is located close to the predicted location of the vha55 gene in Df(3R)

Gene Expression Studies—Northern analysis of head + body mRNA (Fig. 3A) reveals the presence of at least four bands of 2.8, 2.5, 2.0, and 1.8 kb, suggesting transcriptional complexity. This is interesting, because in vertebrates the B-subunit is thought to be the most transcriptionally diverse subunit in this highly conserved proton pump (5). The vha55 cDNA has a 5′-untranslated region of 86 bp and a long 3′-untranslated region of 1019 bp (Fig. 1). Separate transcripts, differing in length of their 3′-untranslated regions, have been identified in kidney and brain, with brain cDNAs being 1 kb longer than those from kidney (5, 6), suggesting that, in humans, the B-subunit may be a useful target for selective therapeutic intervention against kidney or osteoclast isofoms (30).

Southern Blot Analysis and Chromosomal Localization—D. melanogaster (Oregon R) genomic DNA was cleaved with a range of restriction endonucleases, Southern blotted, and probed with vha55 cDNA. Hybridization and washing at either high (not shown) or low stringency suggests that this is a single-copy gene (Fig. 4). In accordance with this, salivary gland polytene chromosome squashes (25) probed with vha55 cDNA revealed a single band at 87C (not shown). The availability of physical mapping clones spanning most of the D. melanogaster genome allows genomic clones to be obtained rapidly when such localizations are known. In this case, six P elements clones spanning 87C (a kind gift of Dr. Stephen Russell) were screened by PCR with internal nondegenerate primers bracketing a 1.2-kb intron and so known to yield products of 430-bp off cDNA and 1600-bp off genomic DNA templates. Two positive clones (DS00602 and DS00681) were obtained, both previously mapped to 87C2, and their identities confirmed by Southern blotting of P1 DNA with vha55 cDNA (not shown).

To confirm the localization, Df3Rkar3J, a fly line carrying a deficiency spanning 87C1–87D1, was subjected to quantitative Southern analysis using a fragment of the vha55 cDNA as a probe. Fig. 5 confirms that the vha55 gene falls within the Df3Rkar3J deficiency.

Identification of a Lethal P-element Insertion within vha55—A powerful tool in the analysis of any gene is the identification of mutant or null alleles. Two attempts to mutate vha55 by site-selected P-element mutagenesis (32) were unsuccessful. However, two lethal P-element insertions, l(3)2E9 and l(3)5043 mapping to 87C2–3 and 87C6–8 respectively, were investigated on the basis that they might lie sufficiently close to the predicted location of vha55 (87C2–3) to permit mutagenesis by "local jumping," a strategy that relies on the relatively common reinsertion of excised P-elements into chromosomal DNA (30). These positions were verified by cloning and sequencing the PCR products and place the intron between bases 160 and 161 of the sequence reported in Fig. 1.

vha55 Corresponds to SzA, a Documented Lethal Locus within 87C—The region covered by Df3Rkar3J has been subjected to saturation mutagenic analysis, in which a total of just four lethal complementation groups have been identified (33).
The existence of a large number of small deficiencies spanning 87C (33) permitted an accurate localization of l(3)2E9 by complementation. l(3)2E9 virgins, balanced over TM3 (carrying Stubble, a dominant marker), were crossed singly to males carrying each of a number of deficiencies (also balanced over TM3) with breakpoints in 87C. If any progeny had wild-type phenotype; whereas complete deletion of the gene produces later lethality, after the emergence of homozygous first-instar larvae (33). This suggests that subunits carrying point mutations may misincorporate into and disrupt maternally derived V-ATPases in Drosophila.

Fig. 7. The lethal effect of l(3)2E9 maps to SzA, a lethal locus at 87C4–5. Single virgin females of l(3)2E9/TM3 were crossed to males carrying a number of deficiencies with breakpoints in 87C, all balanced by TM3. Complementation between the lethal locus and the deficiency was assessed by scoring the progeny for wild-type or Stubble bristles. SzA, SzB, SzC, and SzD are the four lethal complementation groups identified in a saturational mutagenic analysis of the region defined by Df(3R)kar3J, kar is the karmoisin eye color marker. (Diagram based on Gausz et al. (33)).

### Table I

| Allelism between lethal P-element insertions within 87C and SzA | Sh+ | Sh- |
|---------------------------------------------------------------|-----|-----|
| l(3)2E9 × SzA1                                                | 0   | 77  |
| l(3)2E9 × SzA9                                                | 0   | 113 |
| l(3)2E9 × SzA12                                               | 0   | 137 |
| l(3)05043 × SzA1                                              | 21  | 50  |
| l(3)05043 × SzA9                                              | 42  | 72  |
| l(3)05043 × SzA12                                             | 8   | 13  |

Fig. 6. Identification of a lethal P-element insertion in vha55. A, genomic DNA, prepared as described in the text, was used to prime a PCR using either forward primer C653 (bases 29–51) or reverse primer G155 (bases 437–459) in conjunction with P31, a primer which matches the 31-base pair perfect inverted repeat flanking the P-element. Lane 1, 1-kb ladder (Life Technologies, Inc.); lanes 2, 3, 6, and 9, controls with C653 and G155 primers with cDNA template; lane 4, G155/P31 primers and l(3)2E9 genomic DNA template; lane 5, C653/P31 primers with the same template; lane 7, G155/P31 primers and l(3)05043 genomic DNA template; lane 5, C653/P31 primers with the same template. The bands in lanes 4 and 5 are approximately 1100 and 600 bp, respectively, and these sum to the size of the product expected from C653/G155 with wild-type genomic DNA. B, summary of the placement of P{LacW} within the vha55 gene. Numbers refer to the bases of the cDNA sequence in Fig. 2.
V-ATPase holoenzymes earlier than the enzyme would have become ineffective by dilution among the cells of the developing embryo, so producing an earlier lethal phase. As a V-ATPase presumably needs all three of its B-subunits to function, this could provide a "dominant negative" phenotype characteristic of multisubunit proteins. We found the lethal phase of the vha55j2E9 homozygotes to be around the time of hatching, as seen with Df(3R)karSz29, and of w+ Sb+ progeny for crosses involving Df(3R)karSz29, and of w+ Sb+ progeny for crosses involving l(3)j2E9(7CS).

Affected larval Malpighian tubules in SzA2 flies are also colorless, lacking characteristic luminal white concretion bodies (33). This can be interpreted as a failure of V-ATPases to acidify the tubule lumen and precipitate urates or mineral concretions. The apical membranes of tubule cells are packed arrays of V-ATPases (34); and D. melanogaster tubules are known to be highly sensitive to bafilomycin, a selective inhibitor of V-ATPases (19, 35), so tubules are natural sites to observe manifestations of defects in V-ATPase function. The tubule phenotype is cell autonomous in transplants of tubules to healthy flies, as would be expected for a V-ATPase mutant, and the failure of the transplanted cells to grow or thrive prompted Gausz to suggest that the SzA+ gene product was required for growth of most cell types (33).

**LacZ Reporter Element**—The P(LacW) element in vha55j2E9 contains a LacZ enhancer detector element, although PCR with P-element end-specific primers (not shown) revealed the orientation of the reporter to be opposite to that of transcription of vha55. Despite this, the LacZ pattern described below is plausible (Fig. 8) and is identical to those we have observed with P-element insertions into two other V-ATPase subunits. As would be expected of a housekeeping gene, expression was reported in most tissues; however, it was particularly strong in those epithelia where V-ATPases have been demonstrated immunocytochemically on plasma membranes of other insects (36): the Malpighian tubules (Fig. 8, B and C), the rectum (Fig. 8D) and the salivary glands and the cuticular sensillae (Fig. 8E). There is also staining of the uterus and female accessory glands, showing strong staining of uterus and accessory gland.

**FIG. 8.** Expression patterns of the vha55 gene reported by LacZ in the l(3)j2E9/TM3 P-element line. A, embryonic, showing a loop of the midgut stain; B, later embryonic, showing additional tubule expression; C, adult tubules, showing staining confined to nuclei of main segment; D, adult hindgut, showing intense staining of the rectal pads; E, adult head, showing staining of antennal bases and labial palps; F, adult female accessory glands, showing strong staining of uterus and accessory gland.

**TABLE II**

| Complementing/total progeny | ex1 | ex2 | ex3 | ex4 | Df(3R)karSz29 |
|-----------------------------|-----|-----|-----|-----|---------------|
| Df(3R)karSz29               | 50/147 | 28/96 | 37/123 | 10/29 | (0)           |
| l(3)j2E9(7CS)               | 49/196 | 5/53 | 5/44 | 8/25 | 0/51          |

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Y. Guo, unpublished observations.
by imprecise excision of the P-elements. Although the yeast model is ideal for the mutagenic analysis of endomembrane V-ATPases in unicellular organisms (42), it is interesting to note that disruption of V-ATPase subunits in yeast is not obligatorily lethal, but results in a pH-dependent phenotype (43). For multicellular animals, then, Drosophila may prove a more suitable model, which will allow an analysis in an organism which possesses the full spectrum of predicted endomembrane, epithelial, neuronal, and sensory phenotypes.

In summary, this work provides a first description of the expression patterns and lethal phenotypes associated with a range of alleles of a V-ATPase subunit in Drosophila. It suggests that mutations will be lethal recessive and that, for at least those subunits which are present in multiple copies in the holoenzyme, severe alleles will be amorphs. It also predicts that epithelial dysfunction may be detectable at an early stage in a number of tissues where V-ATPases play a plasma membrane role. As further V-ATPase mutations are identified, either in Drosophila or in other animals, it will be interesting to establish whether they match this prototype.

Acknowledgments—We are most grateful to S. Arkison for technical help, to Dr. A. Dornan for a preliminary chromosomal localization of the gene, and to Dr. F. Lyall for invaluable discussions. We are also very grateful to Prof. Janos Gausz, and the Bloomington and Umea Stock Centers, for the provision of useful stocks. We are also grateful to Drs. John Pitts and Malcolm Finbow for their critical reading of the manuscript.

REFERENCES

1. Nelson, H., and Nelson, N. (1989) FEBS Lett. 247, 147–153
2. Nelson, N., and Taiz, L. (1989) Trends Biochem. Sci. 14, 113–116
3. Stone, D. K., Crider, B. P., and Xie, X. (1990) Kidney Int. 38, 649–653
4. Linsley, P. K., Newman, P. R., and Fargue, M. (1996) J. Biol. Chem. 271, 2018–2022
5. Puopolo, K., Kumamoto, C., Adachi, I., Magner, R., and Fargue, M. (1992) J. Biol. Chem. 267, 3096–3706
6. Nelson, R. D., Guo, X. L., Masood, K., Brown, D., Kalkbrenner, M., and Gluck, S. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 3541–3545
7. Myers, M., and Fargue, M. (1993) J. Biol. Chem. 268, 9184–9186
8. Gluck, S. (1992) J. Exp. Biol. 179, 29–37
9. DeFranco, P. E., Haragusi, L., Schmitz, P. G., Bastani, B., and Li, J. P. (1995) J. Am. Soc. Nephrol. 6, 295–301
10. Cioffi, M., and Woltersberger, M. G. (1988) Tissue & Cell 15, 691–703
11. Anderson, E., and Harvey, W. R. (1966) J. Cell Biol. 31, 107–134
12. Schwech, H., Klein, U., Schindlebeck, M., and Wieczorek, H. (1989) J. Biol. Chem. 264, 11316–11142
13. Wieczorek, H., Putzenlechner, M., Zeiske, W., and Klein, U. (1991) J. Biol. Chem. 266, 15340–15347
14. Dow, J. A. T. (1984) Am. J. Physiol. 246, R633–R635
15. Dow, J. A. T. (1992) J. Exp. Biol. 174, 353–373
16. Harvey, R. W., Cioffi, M., and Woltersberger, M. G. (1983) Am. J. Physiol. 244, R163–R175
17. Wieczorek, H. (1992) J. Exp. Biol. 172, 335–343
18. Dow, J. A. T. (1994) in Organelar Proton-ATPases (Nelson, N., ed.) pp. 75–102, R. G. Landes Company, Austin, TX
19. Dow, J. A. T., Maddrell, S. H. P., Gerta, A., Skauer, N. V., Brogan, S., and Kaiser, K. (1994) J. Exp. Biol. 197, 421–428
20. O'Donnell, M. J., Dow, J. A. T., Huesmann, G. R., Tublitz, N. J., and Maddrell, S. H. P. (1996) J. Exp. Biol. 199, 1163–1175
21. Rubin, G. M. (1988) Science 240, 1453–1459
22. Bernasconi, P., Rausch, T., Jeggo, H., and Taiz, L. (1989) FEBS Lett. 251, 132–136
23. Arta, S., Gajbhiy, T., Ishishita, F., Maruyama, T., and Ikemura, T. (1988) Nucleic Acids Res. 16, suppl. r315–r391
24. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
25. Ashburner, M. (1989) Drosophila: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
26. Guo, Y., Kaiser, K., Wieczorek, H., and Dow, J. A. T. (1996) Genes (Amst.) 172, 239–245
27. Gloor, G., and Engels, W. (1992) Drosophila Information Newsletter 71, 145–148
28. Kennison, J. A., and Tamkun, J. W. (1988) Proc. Natl. Acad. Sci. U.S.A. 85, 8136–8140
29. Novak, F. J., Graf, R., Waring, R., Woltersberger, M. G., Wieczorek, H., and Harvey, W. R. (1992) Biochim. Biophys. Acta 1132, 67–71
30. Nelson, N. (1991) Trends Pharmacol. Sci. 12, 71–75
31. Guo, Y., Wang, X., Carter, A., Kaiser, K., and Dow, J. A. T. (1996) Biochim. Biophys. Acta 1293, 4–9
32. Kaiser, K., and Goodwin, S. F. (1996) Proc. Natl. Acad. Sci. U.S.A. 93, 1656–1659
33. Gauz, J., Bence, G., Gyorkovics, H., Ashburner, M., Ish-Horowitz, D., and Holdén, J. J. (1979) Genetics 93, 917–934
34. Harvey, W. R., Cioffi, M., Dow, J. A. T., and Woltersberger, M. G. (1983) J. Exp.
30684

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35. Bertram, G., Shleithoff, L., Zimmermann, P., and Wessing, A. (1991) J. Insect Physiol. 37, 201–209
36. Klein, U. (1992) J. Exp. Biol. 172, 345–354
37. Dimitriadis, V. K. (1991) J. Insect Physiol. 37, 167–177
38. Moehrle, A., and Paro, R. (1994) Dev. Genet. 15, 478–484
39. Lewis, E. B. (1954) Am. Nat. 88, 225–239
40. Brown, D., Saholic, I., and Gluck, S. (1992) J. Exp. Biol. 172, 231–243
41. Cioffi, M. (1979) Tissue Cell 11, 467–479
42. Anraku, Y., Hirata, R., Wada, Y., and Ohya, Y. (1992) J. Exp. Biol. 172, 67–81
43. Nelson, H., and Nelson, N. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 3503–3507
44. Gu, H. H., Gallagher, M. J., Rupkey, S., and Dean, G. E. (1990) Nucleic Acids Res. 18, 7446
45. Nelson, H., Mandiyan, S., and Nelson, N. (1989) J. Biol. Chem. 264, 1775–1778
46. Bowman, E. J., Tenney, K., and Bowman, B. J. (1988) J. Biol. Chem. 263, 13994–14001
47. Ghislain, M., and Bowman, E. J. (1992) Yeast 8, 791–799
48. Gill, S. S., and Ross, L. S. (1991) Arch. Biochem. Biophys. 291, 92–99
49. Manolson, M. F., Ouellette, B. F. F., Filion, M., and Poole, R. J. (1988) J. Biol. Chem. 263, 17987–17994