Nucleic Acids Research, 1993, Vol. 21, No. 16 ***3699–3704***

The developmentally-regulated *Drosophila* gene *rox8* encodes an RRM-type RNA binding protein structurally related to human TIA-1-type nucleolysins

Stéphanie Brand and Henri-Marc Bourbon*
Centre de Biologie du Développement, UMR 9925 CNRS/UPS, Bat. IVR3, Université Paul Sabatier, 118 Route de Narbonne, 31062 Toulouse, France

Received May 7, 1993; Revised and Accepted July 5, 1993 GenBank accession nos L13037 and L13038

ABSTRACT

We report the molecular analysis of a novel *Drosophila melanogaster* gene, *rox8*, isolated in a PCR-based screen for sequences encoding RRM-type RNA-binding polypeptides. The *rox8* gene is predicted to encode a 50-kilodalton protein displaying extensive amino acid sequence similarities (46% overall identity; 57 to 60% similarity) to the two recently described human TIA-1-type nucleolysins. These cytolytic granule associated proteins, which bind polyadenylated sequences *in vitro* and trigger DNA fragmentation in permeabilized target cells, are suspected to participate in the apoptotic cell death pathway induced by T-lymphocytes and natural killer cells. The structural relatedness of the three proteins includes three tandemly-repeated consensus RNA-recognition motifs at the N-terminal end and a putative membrane targeting signal at the C-terminal end. *rox8* cytologically maps to 95D5-9 on the right arm of the third chromosome. Two *rox8* transcripts of 3 and 3.3 kb in length, respectively, result from a developmentally-modulated alternative usage of different polyadenylation sites and are differentially accumulated throughout the fly life cycle. Molecular characterization of *rox8* represents the first step in a genetic analysis of the potential roles of a TIA-1-related protein in RNA metabolism and/or programmed cell death in *Drosophila*.

INTRODUCTION

Many cellular and developmental processes are known to be regulated at the post-transcriptional level of gene expression. Such controls require several types of *trans*-acting factors recognizing specific RNA sequences. A large number of these RNA-binding regulatory proteins share an 80–90 amino acid consensus RNA-recognition motif designated to as an RRM (1). Members of the RRM protein superfamily are involved in various aspects of RNA synthesis, metabolism or function (for a review see ref. 2). Among these RRM-containing proteins, two human TIA-1 type nucleolysins have been recently identified as part of the nucleolytic components of cytolytic granules present in cytotoxic T-lymphocytes (CTLs) and natural killer cells (3, 4). Both proteins have been proposed to participate in the apoptotic cell death process induced by activated CTLs. Most if not all cells carry information to commit a suicide process known as apoptosis, which is initiated either in response to a specific trigger or by the disappearance of a required trophic factor (5). In many instances apoptosis requires protein synthesis in the dying cell, which suggests that a specific genetic program is induced by the suicide trigger. However, even if its general morphological and biochemical hallmarks are well documented, the molecular basis underlying apoptosis remain largely unknown (6). It may involve a common intracellular pathway which is initiated in response to different types of signals depending upon cell context or cell differentiation state (7).

We report here the molecular characterization of *rox8*, a *Drosophila* RRM-protein gene isolated through a degenerate polymerase chain reaction screen (8). *rox8* is located at 95D5-9 on the right arm of the third chromosome and encodes two mRNA species differentially accumulated throughout the fly life cycle. The predicted 50-kDa Rox8 protein exhibits a remarkable amino acid sequence similarity to the human TIA-1-type nucleolysins. In *Drosophila*, programmed cell death has been described morphologically during metamorphosis and in adults (9–11). However, a recent study has shown that a large number of cells undergo programmed cell death during embryogenesis following quite a dynamic and fairly reproducible pattern (12). Further investigations, using classical *Drosophila* molecular genetic analysis of the Rox8-encoding gene, should allow to unravel the potential role of such a RRM-containing protein in the programmed cell death process.

MATERIALS AND METHODS

Drosophila stock and culture

The Oregon-R stock of *Drosophila melanogaster* was used for RNA extraction and *in situ* hybridization to polytene

* To whom correspondence should be addressed

chromosomes. Flies were grown at 25°C on standard cornmeal-glucose-yeast medium.

PCR

Oligonucleotides were synthesized on an Applied Biosystems DNA synthesizer and recovered by ethanol precipitation. PCR was done as described (8). The ROX8 RRM sequences were amplified from pBS8 plasmid DNA (8) using as primers the SK and KS oligonucleotides (Stratagene).

Library screen

cDNA clones were isolated from a 4- to 8-hr embryo cDNA library made in the pNB40 vector (13) using a 450-bp DNA probe generated by PCR from pBS8 plasmid DNA, as described above. 2 positives (designated to as pNB8L and pNB8C) out of 10^5 plasmid recombinants were obtained. The 8G4 genomic clone was isolated from a λEMBL3-cloned library (14) using as a probe the pNB8L cDNA insert. Probes were labeled with (α^{32}P)-dATP by random priming (Megaprime kit; Amersham).

Sequencing and computer analysis

NB8L cDNA and 8G4 genomic fragments subcloned into pBluescriptII-(KS+)(Stratagene) were sequenced on both strands by the dideoxy method using a Sequenase™ kit (US Biochemical). T3, T7 or specific oligonucleotides were used as primers on double-stranded DNA templates. Computer programs used for sequence compilation and analysis were from the PC/Gene package (IntelliGenetics Inc.). Data base searches were carried out using the FASTA or BLAST programs (15, 16). The multiple alignment program CLUSTAL (17) was used to align the 465 amino acids of DmTIAR with the 375 amino acids of HsTIA-1 and HsTIAR (3, 4).

In situ hybridization to polytene chromosomes

Salivary gland polytene chromosome squashes from third-instar larvae were prepared and hybridized as described (18). 8G4 phage DNA was labeled by random priming using (bio-16)-dUTP. Detection was with streptavidin-conjugated horseradish peroxidase and diamino-benzimidine (Enzo Biochem.).

Developmental Northern analysis

Developmental Northern-blot analysis was done as described (18). Briefly, 2 μg of poly(A)$^+$ RNA from each developmental stages were electrophoretically separated and transferred to a nylon membrane (HybondN; Amersham) before UV-crosslinking (Stratalinker; Stratagene Cloning Systems). The NB8L cDNA insert (probe A) was labeled by random priming using (α^{32}P)-dATP. The probe B was obtained from pNB8L DNA by PCR using T7 promoter oligonucleotide (5'-AATACGACTCAC-TATAG-3') and a 23-mer specific oligonucleotide (positions *2404–2427* in the cDNA sequence shown in Fig. 2).

RESULTS

Molecular cloning and characterization of the *rox8* gene

The PCR-amplified DNA clone predicted to encode the ROX8 RRM sequences (8) was used as a probe to isolate two *rox8* cDNA clones (designated to as NB8C and NB8L) from an embryo cDNA library (13). The cDNA inserts of 1.9 and 2.5 kb in length, respectively, differ only in the extent of their 5' and 3' ends, as no internal differences were detected in restriction analysis and partial sequencing data (Fig. 1B).

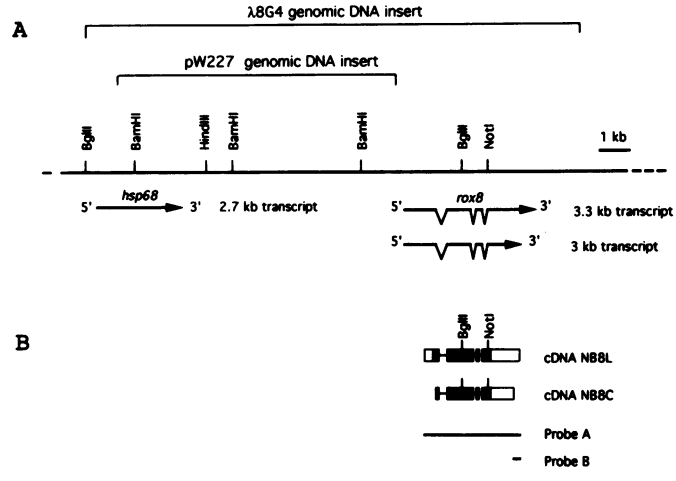

Figure 1. Physical map of the chromosomal region containing *rox8* and structural organization of genomic and cDNA clones. (**A**) A restriction map of 16.5 kb of genomic *Drosophila* DNA encompassing the *rox8* and *hsp68* genes. The DNA contained within the 8G4 phage, as well as within the previously reported pW227 genomic clone encompassing the hsp68-encoding sequences (26), are shown above the physical map. The orientation and structure of both transcription units are shown beneath the molecular map. Introns are indicated by 'v'-shaped lines. (**B**) The structures of the two *rox8* cDNA clones are diagrammed in alignment with the genomic map. Exons are represented by boxes: open boxes correspond to untranslated regions; solid boxes design coding sequences. Introns are represented by thin lines. Consensus polyadenylation signals are indicated by arrowheads. The cDNA NB8L is missing untranslated sequences at both the 5' and 3' ends of the 3.3 kb mRNA specie (data not shown). The poly(A) tracks at the 3' end of the cDNAs NB8L and NB8C preceding the *Eco*RI site (13) are 9 and 13 bp, respectively. The position of the PCR probe (referred to as probe B) used for specific detection of the 3.3 kb mRNA specie (see Fig. 4B) is also indicated.

The cDNA NB8L was in turn used as a probe to isolate a *rox8* genomic clone (8G4) from a phage library made from Oregon-R stock DNA (14). A restriction endonuclease map of the 16.5-kb-long 8G4 genomic DNA insert is shown in Figure 1A. Both NB8L cDNA and corresponding 8G4 genomic fragments were sequenced. The nucleotide (nt) sequence of the cDNA NB8L and its predicted protein amino acid (aa) sequence are shown in Figure 2, along with the entire corresponding genomic sequence including some 5'- and 3'- flanking sequences. Sequence analysis revealed that *rox8* has an overall size of about 3.5 kb and is made of four exons that are separated by introns of 171, 67 and 69 nt, respectively (Fig. 1B). All donor and acceptor splice sites conform to consensus splice sequences (19). RNase protection experiments with RNA from ovaries (data not shown) established that the entire 5'-untranslated sequence is likely to be contained within a single exon, extending slightly upstream of the 5' end of the cDNA NB8L. Interestingly, the cDNA clones NB8C and NB8L differ in the length of their 3'-untranslated regions; 642 versus 779 nt. The presence of an appropriately located consensus polyadenylation signal (AAUAAA; for a recent review see ref. 20) is only observed for the cDNA NB8C (Fig. 2). However, such a signal is found in the genomic sequence about 100 nt downstream the 3' end of the cDNA NB8L (at position *2378*, Fig. 2). As both cDNA clones ended at their 3' termini with short poly(A) sequences, the cDNA NB8L was likely generated by oligo(dT) priming to an internal A-rich sequence motif present in the corresponding genomic sequence (see Fig. 2).

The 2,569-nt-long cDNA NB8L contains a 469-codon open-

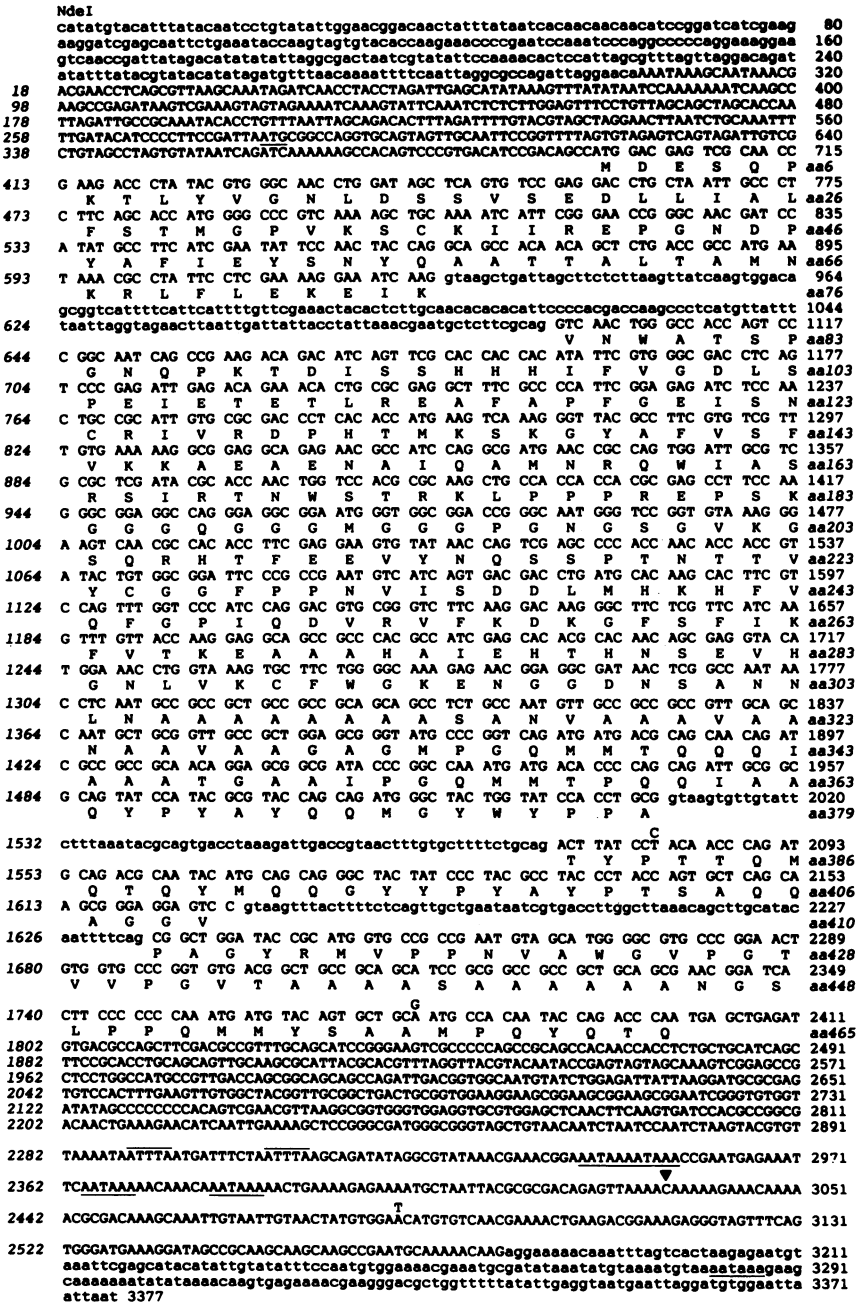

Figure 2. Nucleotide sequence of DNA from the *rox8* locus and the derived amino acid sequence of the encoded Rox8 protein. The nt sequence of *rox8* genomic DNA is shown, along with the NB8L cDNA nt sequence (in capital letters) and the inferred Rox8 aa sequence using the standard single letter code. The first nt of a genomic *Nde*I site is referred to as nt 1. The running tallies of nt and aa (italicized) are shown on the right, whereas the italicized numbers on the left refer to positions along the cDNA sequence with respect to the first nt of the cDNA NB8L. Introns and flanking sequences not present in this cDNA are in lowercase letters. The consensus polyadenylation signals (AAUAAA) are underlined. The nt at position *2427* which marks the 3′ end of the non-poly(A) part of the cDNA NB8L is indicated by an arrowhead. Two putative RNA-destabilizing elements (34) found in the 3′-untranslated region common to both mRNA species are overlined. Some polymorphisms between the 8G4 genomic and NB8L cDNA clones were observed. The nt recovered in the cDNA NB8L are indicated above the genomic sequence. None of these polymorphisms results in aa changes. These sequences have been assigned the GenBank accession numbers L13037 and L13038.

reading-frame (ORF) extending from nt *384* through *1791*. The first AUG codon in this long ORF (at position *396*) is embedded in a sequence (CAGCCAUGG) that is in good agreement with the consensus sequence for eukaryotic (21) and *D.melanogaster* (22) translation initiation sites [CC(A/G)CCAUGG and (C/A)A(C/A)(C/A)AUG, respectively). Another AUG is found upstream in the mRNA (at position *280*, Fig. 2) but does not conform to these consensus. Furthermore, initiation at this latter AUG would yield a 13 aa polypeptide which conceivably could have a role in regulating *rox8* mRNA translation, as has been found for some eukaryotic messages (23, 24). Finally, the position of the former AUG with respect to translation start in human TIA-1-type proteins (see Fig. 5) supports the notion that it is the *bona fide* ribosome initiation site.

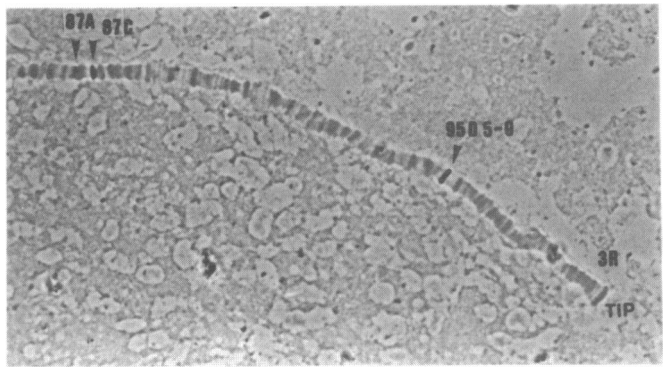

Figure 3. Cytological localization of *rox8*. Polytene chromosome squashes of third instar larvae salivary glands (Oregon-R stock) were hybridized to biotin-labeled 8G4 phage DNA. A strong signal (single arrowhead) was detected at position 95D5-9 on the right arm of the third chromosome. Two fainter signals (double arrowheads) were also readily apparent at positions 87A and 87C. These signals correspond to the location of the *hsp70* genes (25) which cross-hybridized with the closely-related *hsp68* gene presents in the 8G4 DNA probe (see Fig. 1A).

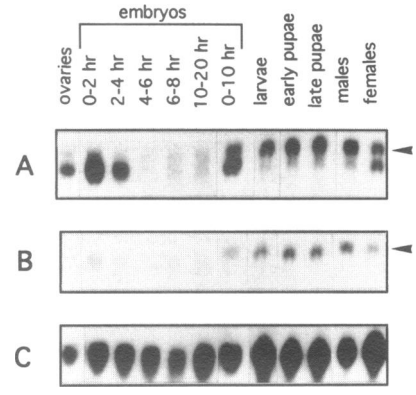

Figure 4. Structural relationships of two *rox8* transcripts and their temporal expression patterns during the fly life cycle. Approximately 2μg of poly(A)$^+$ RNA isolated from whole organisms of indicated stages (the three different larvae stages were collected as a pool; early and late pupae referred to as pre- and post-floating pupae, respectively) or ovaries of adult females were loaded per lane and separated by electrophoresis in a 1% agarose-formaldehyde gel. The size-fractionated RNA species were then blotted to a nylon membrane and probed with different DNA probes. (**A**) Using the cDNA NB8L as a probe (probe A; see Fig. 1B), two mRNA species of approximately 3 and 3.3 kb were detected at all examined stages albeit with different relative amounts. (**B**) Only the larger of the two transcripts (pointed out with an arrowhead) was detected with a PCR probe encompassing the 3'-terminal 180-pb of the cDNA NB8L (probe B; see Fig. 1B). (**C**) To correct for differences in the amount of RNA loaded on the gel, the blot was stripped and rehybridized with a probe for the rpL17A ribosomal protein gene (18), which is expressed at an approximately constant level throughout development. Sizes were determined by comparison with RNA size markers (Pharmacia).

rox8 is a single copy gene located on the third chromosome

Southern-blot analysis of genomic DNA probed with the cDNA NB8L under high stringency conditions, detected no restriction fragments other than those generated from 8G4 phage DNA, suggesting that *rox8* is present as a single copy gene in *D.melanogaster* (data not shown). *In situ* hybridization of the same probe to salivary gland polytene chromosomes yielded a single signal at the cytological interval 95D5-9 on the right arm of the third chromosome (not shown). However, hybridization with 8G4 phage DNA gave two weak signals at positions 87A and 87C, respectively, in addition to a strong signal at 95D5-9 (Fig. 3). Strikingly, 87A and 87C correspond precisely to the cytological location of the *hsp70* genes, that are closely sequence-related to the *hsp68* gene localized at 95D (25). Comparison of restriction maps of the 8G4 phage DNA insert and genomic clones containing *hsp68* (26, 27) established that *hsp68* and *rox8* are both present in the same recombinant phage, separated by about 9 kb of DNA.

Two differentially accumulated *rox8* mRNAs are generated by the alternative usage of distinct polyadenylation sites

To determine both the size and temporal expression pattern of *rox8* transcripts, we performed a developmental Northern-blot analysis using the cDNA NB8L as a probe (referred to as probe A; see Fig. 1A). Two mRNA species of approximately 3 and 3.3 kb were detected at all stages of the fly life cycle examined, albeit at variable relative levels (Fig. 4A). The 3 kb transcript is highly accumulated in ovaries of adult females and in very early embryos; its level is drastically lowered at or soon after the onset of gastrulation, about 4 hr after egg deposition. In contrast, the 3.3 kb transcript is abundant in larvae, pupae and adults, particularly in males. Since the relative sizes of the two detected *rox8* mRNA agree well with the structure of the two cDNAs NB8L and NB8C, these data suggested that these transcripts may arise from the alternative usage of two different polyadenylation sites. In order to test for this possibility, the same developmental Northern-blot was reprobed using a 180-pb PCR-amplified DNA fragment (referred to as probe B in Fig. 1B), corresponding to the 3'-terminal region of the cDNA NB8L not present in the cDNA NB8C (nt *2404–2569*, Fig. 2). As suspected, only the 3.3 kb mRNA was detected (Fig. 4B), demonstrating that this transcript results from utilization of a distal polyadenylation signal, possibly the one found at position 3382 in the genomic sequence (see Fig. 2 and above). It is noteworthy that the choice of 3'-processing site does not affect the encoded protein.

The *rox8*-encoded RRM-containing protein presents strong structural similarities to vertebrate TIA-1-type nucleolysins

The predicted *rox8* protein product (Rox8) is 465-aa long, with calculated molecular weight of 49,771 daltons and isoelectric point of 8.2. Data base searches for related sequences revealed strong similarities to the two known human TIA-1-type nucleolysins (3, 4). Rox8 displays about 46% overall aa sequence identity (57 to 60% conservative aa) with either human protein (herein designated as HsTIA-1 and HsTIAR) (see Fig. 5). Based on the level of aa sequence conservation with these nucleolysins, Rox8 can be divided into two distinct regions.

The first region includes three N-terminal RRMs in tandem which are highly conserved: 140 of the first 295 amino acids (51%) are identical in human and fly sequences and 65% correspond to conservative aa (Fig. 5). It is noteworthy that the similarity over the third RRM includes a conserved potential serine protease-cleavage site (Phe-Ile-Lys, aa *261–263*), that may be functionally relevant (3). An additional glycine-rich region in between the second and the third RRM resembles aa sequences encoded by *pen* repeats that might correspond to a flexible hinge between highly structured domains (28).

The sequence of the remaining C-terminal region (aa *296–465*)

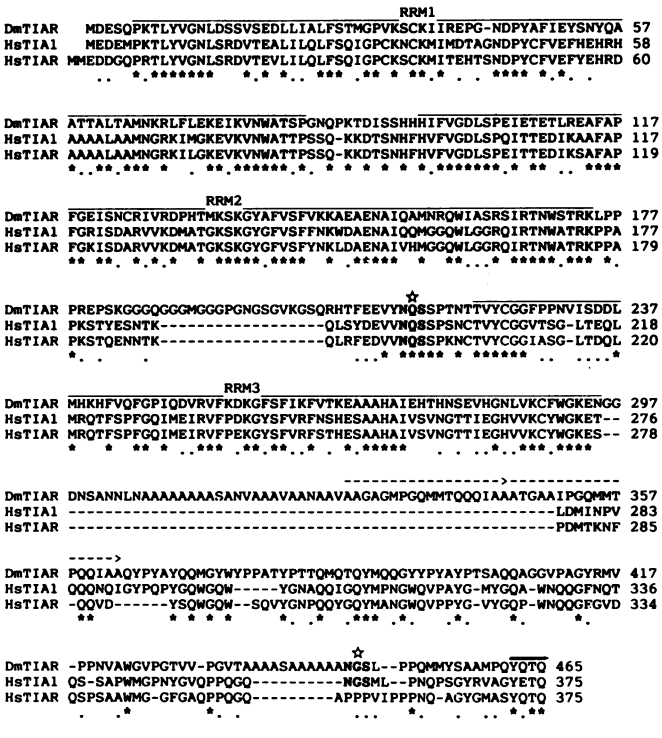

Figure 5. Amino acid sequence comparison of the *Drosophila* and human TIA-1-type proteins. An overall alignment of the Rox8 (herein designated to as DmTIAR), HsTIA-1 (3), and HsTIAR (4) aa sequences was achieved using the CLUSTAL program (17). The right column lists the running tally of aa in each protein. Dm TIAR is 46% identical to either human TIA-1-type nucleolysin. The aa invariant among the three sequences are marked with underlying asterisks; allowed conservative substitutions (35) with dots. Conserved potential N-glycosylation sites (36) are indicated by white stars. The location of the conserved C-terminal putative membrane-targeting signal (29) is marked by an over-bar. The horizontal arrows denote a tandemly-repeated 18-aa sequence only present in DmTIAR. The three RRM sequences (designated to as RRM1 through RRM3) are overlined.

is markedly less conserved, including when both human proteins are compared (see Fig. 5). However, the overall aa composition of the homologous parts is quite similar, with a high content of glutamines (16%), tyrosines (14%), and prolines (15%). Strikingly, a single charged aa can be found in this latter portion of the fly and human proteins (Arg at position *415* in Rox8). Moreover, a potential N-linked glycosylation site (which is only present in HsTIA-1) and a C-terminal tyrosine-containing sequence (Tyr-Gln-Thr-Gln-COOH) are recovered at the same positions. This signal sequence, which has been found in glycoproteins targeted to lysosomal membranes (29), is likely to promote membrane association for granule-exocytosis, as well as clustering into coated pits for endocytosis of various cell surface receptors (for a review see ref. 30). Finally, the larger size of Rox8 (90 aa) is essentially due to the presence in this portion of a long alanine-rich sequence (of the 55 additional aa, 25 are alanines) including a nearly perfect tandem repeat of 18 aa (beginning at aa *328*, see Fig. 5).

On the basis of the overall architecture and sequence conservation between Rox8 and the CTL-secreted nucleolysins, we conclude that the *Drosophila* gene *rox8* probably encodes an insect homolog of the vertebrate TIA-1-type nucleolysins. Consequently, hereafter we refer to Rox8 as DmTIAR.

DISCUSSION

We report here the structure and expression of *rox8*, a *Drosophila* gene which predicted encoded protein presents strong structural similarities to the two known human TIA-1-type nucleolysins. It is noteworthy that the three RRM domains of DmTIAR, HsTIA-1 and HsTIAR are highly conserved in their primary sequences (65% similarity) and relative positions. Considering that the RRM consensus sequence is a weakly conserved RNA-binding domain (1, 2), the high degree of aa sequence conservation of all three RRMs between such evolutionarily divergent phyla as arthropods and chordates suggests that each RRM may contribute to different RNA-binding specificities. Furthermore, these three RRMs exhibit a striking aa sequence similarity to the three RRMs of a 48-kDa *Saccharomyces cerevisiae* RNA-binding protein of undescribed function (31); about 38% aa are identical and 19% additional aa are conservative replacements (data not shown). It remains to determine whether these structural similarities are relevant to common RNA-binding specificities of TIA-1-type proteins. The human nucleolysins have been reported to bind *in vitro* to poly(A) sequences, though with much weaker avidity than the ubiquitous mRNA poly(A)-binding protein (PABP) (32), leaving open the *in vivo* relevance of this observation. The C-terminal part of the TIA-1 type proteins appears not to be subject to strong evolutionary constraints since in spite of scattered similarities between the human and fly proteins, their overall primary sequences have been poorly conserved. By analogy to what is known for other RNA-binding proteins, the non-RRM part of the TIA-1-type proteins could provide surfaces for specific protein-protein interactions. Therefore, the presence of three strongly conserved RRMs in the TIA-1-type proteins suggests that they may function in metabolism aspects of related RNAs; however, their particular roles might be modulated through their auxiliary C-terminal domains.

The ability of recombinant human TIA-1-type proteins to induce DNA fragmentation in permeabilized cells lends some credence to the hypothesis that they are involved in the induced apoptotic cell death pathway in CTL-target cells (3, 4). A 15-kDa recombinant C-terminal form of HsTIA-1 displays an *in vitro* nucleolytic activity similar to that of the complete protein, suggesting that the otherwise poorly-conserved glutamine-rich C-terminal domain is sufficient for this function. It remains to establish whether the implication of TIA-1-type proteins in developmentally programmed cell death is evolutionarily conserved. Isolation of the gene coding for a TIA-1-related *Drosophila* protein will permit to address this question *in vivo*, combining molecular and genetic approaches. We have examined the temporal expression pattern of *rox8* transcripts: two mRNAs, different in length, are both present throughout the fly life cycle but are differentially accumulated during development. Both are predicted to encode the same protein and are generated by a developmentally-regulated alternative usage of two different polyadenylation sites. Whether these differential mRNA abundance and usage of 3′ processing sites have some functional significance during development remains to be assessed.

Concerning the genetic analysis of the *rox8* locus, only seven lethals have been genetically mapped to the relevant chromosomal region (25). As a first step towards a molecular genetic evaluation of the *rox8* function, we are currently addressing the question of whether some of these lethals may actually correspond to the herein identified gene using P-element rescue experiments.

ADDENDUM

A *Drosophila* RRM sequence (RRM12) recently identified using a similar PCR screening strategy appears almost identical to the amplified ROX8-RRM sequence (33). Available data about the corresponding *rbp12* gene indicate that *rox8* and *rpb12* are closely related if not identical.

ACKNOWLEDGEMENTS

We wish to thank N.Brown and N.Randsholt for gifts of *Drosophila* cDNA and genomic libraries, respectively. We are grateful to Michèle Sauviat for help with DNA sequencing and plasmid DNA preparation, and to Stéphane Noselli for performing *in situ* hybridization to polytene chromosomes. We acknowledge Alain Vincent, David Cribbs and Stéphane Noselli for encouragement, comments on the manuscript, and many stimulating discussions. We also thank members of the *Drosophila* community of Toulouse for encouragement. We are also grateful to François Amalric and Alain Vincent for hospitality during the initial part of this work. Finally, we thank A.-M. Duprat for her support.

This work was supported by an ATIPE grant from the Centre National de la Recherche Scientifique, and S.B. was supported by a student fellowship from the Ministère français de la Recherche et de la Technologie.

REFERENCES

1. Query, C.C., Bentley, R.C. and Keene, J.D. (1989). *Cell* **57**, 89–101.
2. Kenan, D.J., Query, C.C. and Keene, J.D. (1991). *Trends Biochem. Sci.* 16, 214–220.
3. Tian, Q., Streuli, M., Saito, H., Schlossman, S. and Anderson, P. (1991). *Cell* **67**, 629–639.
4. Kawakami, A., Tian, Q., Duan, X., Streuli, M., Schlossman, S. and Anderson, P. (1992). *Proc. Natl. Acad. Sci. USA* **89**, 8681–8685.
5. Ellis, R.E., Yan, J. and Horvitz, H.R. (1991). *Annu. Rev. Cell. Biol.* **7**, 663–698.
6. Duke, R.C. (1991). In *Apoptosis: The Molecular Basis of Cell Death.* (eds, L.D. Tomei and F.O. Cope), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp 209–226.
7. Raff, M.C. (1992). *Nature* **356**, 397–400.
8. Brand, S. and Bourbon, H.-M. (1993). *Febs Letters* (submitted).
9. Lockshin, R.A. (1981). In *Cell Death in Biology and Pathology* (eds. I.D. Bowen and R.A. Lockshin), Chapman and Hall, London and New York, pp 79–121.
10. Kimura, K. and Truman, J.W. (1990). *J. Neurosci.* 10, 403–411.
11. Giorgi, F. and Deri, P. (1976). *J. Embryol. Exp. Morph.* **35**, 521–533.
12. Abrams, J.M., White, K., Fessler, L.I. and Steller, H. (1993). *Development* **117**, 29–43.
13. Brown, N.H. and Kafatos, F.C. (1988). *J. Mol. Biol.* **203**, 425–437.
14. Dura, J.M., Randsholt, N.B., Deatrick, J., Erk, I., Santamaria, P., Freeman, J.D., Freeman, S.J., Weddell, D. and Brock, H.W. (1987). *Cell* **51**, 829–839.
15. Pearson, W.R. and Lipman, D.J. (1988). *Proc. Natl. Acad. Sci. USA* **85**, 2444–2448.
16. Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990). *J. Mol. Biol.* **215**, 403–410.
17. Higgins, D.G. and Sharp, P.M. (1988). *Gene* 73, 237–244.
18. Noselli, S. and Vincent, A. (1992). *Gene* **118**, 273–278.
19. Mount, S.M., Burks, C., Hertz, G., Stormo, G.D., White, O. and Fields, C. (1992). *Nucl. Acids Res.* **20**, 4255–4262.
20. Proudfoot, N. (1991). *Cell* **64**, 671–674.
21. Kozak, M. (1984). *Nucl. Acids Res.* **12**, 857–872.
22. Cavener, D.R. (1987). *Nucl. Acids Res.* **15**, 1353–1361.
23. Miller, P.F. and Hinnebusch, A.G. (1989). *Genes Dev.* **3**, 1217–1225.
24. Oh, S.-K., Scott, M.P. and Sarnow, P. (1992). *Genes Dev.* **6**, 1643–1653.
25. Lindsley, D.L. and Zimm, G.G. (1992). *The genome of Drosophila melanogaster*, San Diego Academic Press, San Diego.
26. Holmgren, R., Livak, K., Morimoto, R., Freund, R. and Meselson, M. (1979). *Cell* **18**, 1359–1370.
27. Holmgren, R., Corces, V., Morimoto, R., Blackman, R. and Meselson, M. (1981). *Proc. Natl. Acad. Sci. USA* **78**, 3775–3778.
28. Haynes, S.R., Rebbert, M.L., Mozer, B.A., Forquignon, F. and Dawid, I.B. (1987). *Proc. Natl. Acad. Sci. USA* **84**, 1819–1823.
29. Williams, M.A. and Fukuda, M. (1990). *J. Cell. Biol.* **111**, 955–966.
30. Vaux, D. (1992) *Trends Cell. Biol.* **2**, 189–192.
31. Ripmaster, T.L. (unpublished). L01797.
32. Grange, T., Martins de Sa, C., Oddos, J. and Pictet, R. (1987). *Nucl. Acids Res.* **15**, 4771–4787.
33. Kim, Y.-J. and Baker, B.S. (1993). *Mol. Cell. Biol.* **13**, 174–183.
34. Shaw, G. and Kamen, R. (1986). *Cell* **46**, 659–667.
35. Dayhoff, M.O., ed. (1978). *Atlas of Potein Sequence and Structure*, Vol. 5, Suppl. 3. (Washington, D.C.: National Biochemical Research Foundation) p 345.
36. Hubbard, S.C. and Ivatt, R.J. (1981). *Annu. Rev. Biochem.* **50**, 555–583.