Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 1195–1199, February 1996
Cell Biology

HLH106, a *Drosophila* transcription factor with similarity to the vertebrate sterol responsive element binding protein

ULRICH THEOPOLD†, SOPHIA EKENGREN, AND DAN HULTMARK

Departments of Molecular Biology and Developmental Biology, Stockholm University, S-106 91 Stockholm, Sweden

Communicated by Joseph L. Goldstein, University of Texas Southwestern Medical Center, Dallas, TX, October 27, 1995

ABSTRACT **We cloned a *Drosophila* homolog to the sterol responsive element binding proteins (SREBPs). In vertebrates, the SREBPs are regulated by a mechanism that involves cleavage of the protein that normally resides in the cellular membranes and translocation of the released transcription factor into the nucleus. Regulation of the *Drosophila* factor HLH106 apparently follows the same mechanism, and we find the full-length gene product in the membrane fraction and a shorter cross-reacting form in the nuclear fraction. This nuclear form, which may correspond to proteolytically activated HLH106, is abundant in the blood cell line mbn-2. The general domain structure of HLH106 is very similar to that in SREBP. HLH106 is expressed throughout development, and it is present at high levels in *Drosophila* cell lines. In contrast to the rat homolog, HLH106 transcripts are not more abundant in adipose tissue than in other tissues.**

Recently, a class of transcription factors has been described that are activated by a proteolytic mechanism. They include the human and hamster sterol responsive element binding proteins SREBP-1 and SREBP-2 (1–4) and the SREBP-1 homolog from rat, also called adipocyte determination- and differentiation-dependent factor 1 (ADD1) (5). SREBP is normally anchored to cellular membranes in a transcriptionally inactive form. However, when the cells are starved for sterols, the protein is proteolytically cleaved to release the mature transcription factor, which translocates to the nucleus (6). These transcription factors contain a basic helix–loop–helix (bHLH) domain with an unusual DNA binding specificity. As shown for ADD1 (7), they bind specifically to two types of DNA sequences: the E-box sequences, which are typical targets of bHLH proteins, and the sterol regulatory element 1.

Through its interaction with sterol regulatory element 1, SREBP can regulate the expression of genes involved in cholesterol biosynthesis and uptake, such as the receptor for low density lipoprotein (LDL) and the hydroxymethylglutaryl CoA synthase (6, 8, 9). A common feature of these genes is that they are activated when the sterol content of the cell drops below a certain level and that their regulatory activity results in a rise in intracellular sterol concentration, either by increased uptake or by an increase of *de novo* synthesis of sterols. The net result of the activity of SREBP is to keep the intracellular cholesterol content within a physiological range. If the cholesterol content of the cell is high enough, SREBP precursor will not be activated, keeping all excess sterol outside the cell, one factor that may increase the risk of arteriosclerosis.

Given their dual DNA binding specificity, the SREBP/ADD1 factors may mediate the regulation of a wide range of genes, and it is possible that they have other functions in addition to sterol-regulated gene expression. ADD1 is strongly expressed in adipose cells, and this factor was suggested to participate in the differentiation of these cells (5) or in the control of general lipid metabolism (7).

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.

We describe here the isolation of HLH106,‡ a SREBP homolog from *Drosophila*, that might add to our understanding of the evolution of regulatory mechanisms relevant to lipid metabolism and the abnormal developments leading to arteriosclerosis.

MATERIALS AND METHODS

Flies and Cells. Canton S flies were kept on cornmeal/yeast food at 25°C with a 10-h light/14-h dark cycle. The cell line mbn-2 (10) was a gift from E. Gateff (Johannes Gutenberg University, Mainz) and $Kc_{(167)}$ (11) was from L. Søndergaard (University of Copenhagen, Copenhagen). Schneider's SL2 and SL2* (12) sublines are described in ref. 13. Cells were grown in Schneider's medium (Nord Cell) supplemented with 5% (vol/vol) heat-inactivated fetal calf serum, 1× Glutamax I (GIBCO), penicillin (50 units/ml), streptomycin (50 μg/ml), and gentamicin (50 μg/ml).

Immunoscreening Procedure. We screened 6×10^5 plaques of a cDNA library from Canton S flies that had been immunostimulated by the injection of bacteria (14). The antibody used for screening was an antiserum that was raised in rabbits against a purified glycoprotein from an mbn-2 cell membrane fraction. The secondary antibody (goat anti-rabbit immunoglobulin, Sigma) was used at a dilution of 1:5000. Plaques were screened as described (15). Out of 15 positive clones from the primary screen, three contained HLH106 sequence. The cDNA library was constructed with the λ ZAP II vector (16), and positive clones were isolated as pBluescript SK^- plasmids after *in vivo* excision from the parent phagemid clones.

DNA and RNA Blots. Procedures were performed according to conventional protocols (17). Genomic DNA was separated on a 1% agarose gel, blotted to a Hybond N filter, and hybridized under stringent conditions. Samples of total RNA (10–15 μg) were separated on a formaldehyde-containing 1% agarose gel. To standardize for unequal loading in the Northern blots, the filters were rehybridized as described (15) with a probe for the ribosomal protein gene *Rp49* (18), and the signals were normalized after quantitation with a PhosphoImager (Molecular Dynamics). rRNA and a 0.24- to 9.5-kb ladder (BRL) were used as molecular mass standards.

DNA Sequencing. The inserts of the cDNA clones were sequenced by using the chain-termination method and the Sequenase kit (United States Biochemical) on double-stranded templates. Unidirectional deletions were made from the *Sal* I–*Hin*dIII and *Xba* I–*Not* I sites in the pBluescript SK^- vector by digestion with exonuclease III and S1 nuclease using the Erase-a-Base deletion kit (Promega) as recommended by the supplier. After digestion with *Sal* I and *Not* I, the resulting cleavage

Abbreviations: SREBP, sterol responsive element binding protein; ADD1, adipocyte determination- and differentiation-dependent factor 1; bHLH, basic helix–loop–helix; LDL, low density lipoprotein.

†Present address: Department of Crop Protection, University of Adelaide, Waite Campus, Glen Osmond, South Australia 5064, Australia.

‡The sequence reported in this paper has been deposited in the Genbank data base (accession no. U38238).

products had to be protected with thionucleotides before the second digestion could be performed. There was full sequence agreement between all clones within overlapping segments.

Cell Fractionations. Fractionation of mbn-2 cells was performed as described by Wang *et al.* (6). Briefly, mbn-2 cells were pelleted, suspended in resuspension buffer, disrupted by Dounce homogenization, and centrifuged for 20 min at 16,000 $\times$ *g* in a microcentrifuge. The supernatant and the pellet were then treated separately. The supernatant was further centrifuged at $10^5 \times g$ for 1 h to obtain a cytosolic fraction (fraction E) and a membrane pellet. The membrane pellet was resuspended and washed in a high salt buffer and centrifuged to obtain a washed membrane pellet (fraction G). The supernatant (fraction F) contains the membrane wash.

The crude nuclear pellet from the microcentrifugation above was centrifuged through a sucrose cushion. Fraction A contains the supernatant from this first nuclear wash. The nuclear pellet was resuspended in a buffer supplemented with Nonidet P-40 before centrifugation. The resulting supernatant is a second nuclear wash (fraction B). Finally, chromatin-associated proteins in the washed nuclear pellet were solubilized in a high salt buffer, resulting in the nuclear fraction (fraction C) after removal of insoluble nuclear debris by centrifugation.

Whole-cell protein extracts were made by centrifugation of 1 ml of a densely growing culture of mbn-2 cells for 5 min at 4000 $\times$ *g*. The cells were resuspended in 1 $\times$ SDS/PAGE loading buffer containing 2% SDS (20% of initial volume) and sonicated for three 30-sec periods. Whole flies were homogenized in 1 $\times$ SDS/PAGE loading buffer (30 μl of buffer per fly) containing 3% SDS. The sample was boiled for 5 min and centrifuged at 16,000 $\times$ *g* in a microcentrifuge for 5 min before the supernatant was collected. All samples were denatured for 3 min at 95°C before loading on a polyacrylamide gel.

Western Blot Analysis. Electrophoresis of protein extracts was done in a 7.5% polyacrylamide gel on a Hoefer Mighty Small electrophoresis unit at 20 mA, essentially according to ref. 19. Pharmacia high-range molecular weight markers were used for reference. The proteins were blotted to a nitrocellulose membrane (Amersham) and immunostained as described (20), except for the use of 0.05% Tween 20 as a blocking agent in the solution throughout the procedure. The blotting efficiency was determined by staining the blot with Ponceau S (21). HLH106 protein was detected with a monoclonal antibody raised against amino acid residues 301–407 in human SREBP-1 (a gift from Joseph Goldstein and Michael Brown, University of Texas Southwestern Medical Center). The antibody was used at 0.5 μg/ml. An alkaline phosphatase-conjugated goat anti-mouse immunoglobulin (Sigma, 2.5 μg/ml) was used as secondary antibody.

RESULTS

Isolation and Sequence of HLH106 Clones. Three cDNA clones for a *Drosophila* SREBP homolog were found among the positive clones in an immunoscreen, by using an antiserum directed against a membrane glycoprotein isolated from the

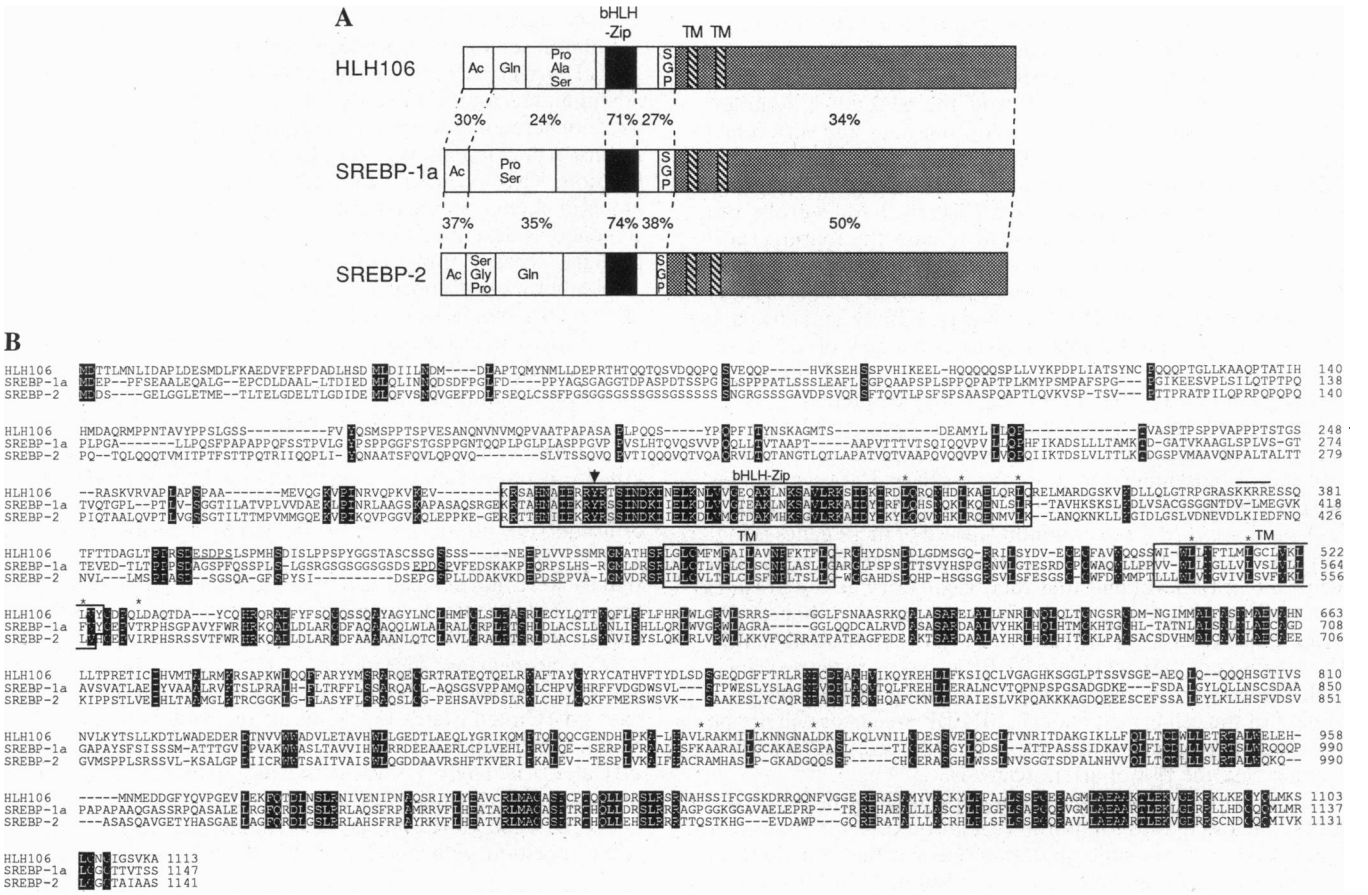

FIG. 1. Comparison between the amino acid sequences of *Drosophila* HLH106 and the two human SREBPs. (*A*) Schematic overview of the domain structure of the SREBP homologs. The bHLH domain, predicted transmembrane (TM) regions, and an N-terminal acidic domain (Ac) common to all three proteins are indicated. Additional domains enriched in specific amino acids are also indicated. (*B*) Alignment of the sequences. The sequences were aligned with the GENEJOCKEY II program (Biosoft) and dashes indicate gaps inserted to maintain alignment. Conserved residues are in white type on a black background. Boxes enclose the bHLH domain and the two presumptive transmembrane domains. A conserved tyrosine residue is marked with an arrow, and potential leucine zippers are marked with asterisks. Potential cleavage sites for the interleukin 1β-converting enzyme-like protease are underlined.

Drosophila blood-cell line mbn-2 (10, 13). This SREBP homolog is unrelated to the glycoprotein (U.T., C. Samakovlis, H. Erdjument-Bromage, N. Dillon, B. Axelsson, O. Schmidt, P. Tempst, and D.H., unpublished results), but its pattern of expression and its relationship to an important class of regulatory proteins prompted us to investigate these cDNAs further.

The longest cDNA clone, p106, contains a 3768-bp insert with a single long open reading frame of 1113 amino acid residues, preceded by stop codons in all reading frames. A high degree of similarity was found to SREBP-1/ADD1 and to SREBP-2. Significant similarity is also seen to the bHLH domains of other DNA binding proteins, with TFE3, TFEb, and USF giving the highest scores.

Fig. 1*B* shows an alignment of HLH106 to the human SREBPs, and Fig. 1*A* shows a schematic representation of the conserved regions. The sequences can be aligned over much of their length. The most conserved regions include the bHLH domain, with 71% sequence identity between HLH106 and SREBP-1, and two blocks of conserved sequence near the C terminus, each with 57% sequence identity. Notably, a tyrosine residue in the bHLH domain, which is critical for the dual DNA binding specificity of ADD1 (7), is also conserved in HLH106 (Fig. 1*B*, arrow). Other conserved features include two blocks of hydrophobic amino acids that are predicted to form transmembrane segments in SREBP-1 (6). The corresponding regions in HLH106 score as "certain" transmembrane regions according to the algorithm of Sipos and von Heijne (22). Furthermore, between the bHLH domain and the first hydrophobic segment is a region that is rich in serine, glycine, and proline residues. For SREBP-1, the proteolytic cleavage of the precursor occurs in the vicinity of this region (3, 23). The sequences of the N-terminal one-third of HLH106 and SREBP are poorly conserved and they cannot be uniquely aligned. However, they have a similar bias in their amino acid compositions. Like SREBP, HLH106 has an acidic region at the N-terminal end, followed by long blocks that are rich in glutamine, proline, and serine, but the detailed organization of these blocks differs.

Although HLH106 is somewhat more similar to SREBP-1 than to SREBP-2, the latter two proteins are more closely related to each other than either of them is to HLH106 (Fig. 1*A*). It is therefore likely that the the two mammalian forms separated after the divergence of insects and vertebrates. Unique features in HLH106 include a strongly basic region at positions 368–377, including the tetrapeptide KKRR (overlined in Fig. 1*B*) that may serve as a nuclear localization signal.

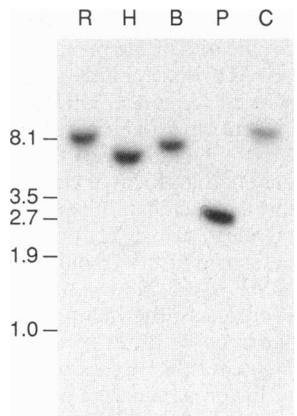

FIG. 2. Genomic Southern blot showing that HLH106 is encoded by a unique gene. Genomic DNA (10 μg) was cleaved with the indicated restriction enzymes (R, *Eco*RI; H, *Hin*dIII; B, *Bam*HI; P, *Pst* I; C, *Cla* I) and probed with an internal *Bam*HI–*Hin*dIII fragment of p106. Sizes in kb are indicated.

Furthermore, besides a short leucine zipper with only three conserved leucines that follows the bHLH domain, two additional potential leucine zipper domains can be found in the *Drosophila* protein; one is overlapping with one of the predicted transmembrane regions and one is located in a region that is unique to the *Drosophila* protein (Fig. 1*B*).

One of the three cDNA clones differs in sequence from the two others at the 5′ end, suggesting that alternatively spliced forms may exist, as for SREBP-1 (1). However for HLH106, only noncoding sequences are affected and all clones contain the same open reading frame.

As shown in Fig. 2, the HLH106 probe hybridizes to single fragments on a Southern blot when genomic DNA is cleaved with any one of several restriction enzymes. This indicates that HLH106 corresponds to a single unique gene in the *Drosophila* genome.

Tissue and Developmental Specificity of Expression. The expression of HLH106 was studied in a series of Northern blots. HLH106 is expressed as a single 3.6- to 3.8-kb transcript, in good agreement with the size of the longest cDNA insert, 3768 bp. Fig. 3*A* shows that the gene is expressed throughout development, with an increase in expression from early embryonic stages until adulthood, as seen after normalization to

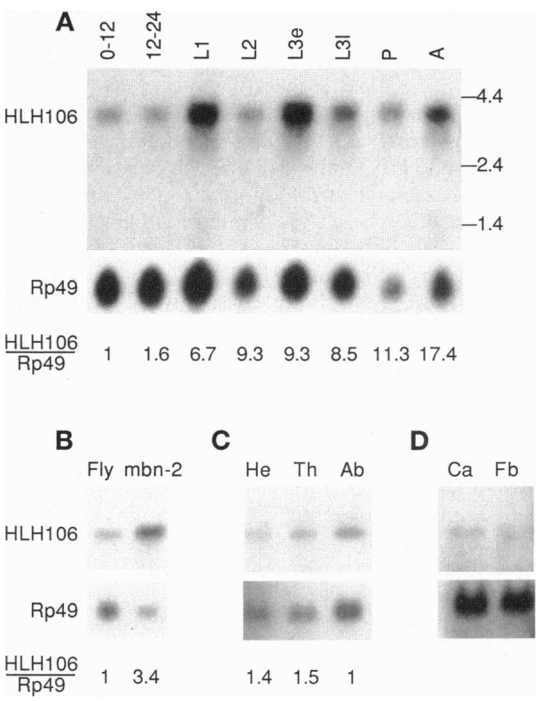

FIG. 3. Expression of HLH106 mRNA based on Northern blot analysis. (*A*) HLH106 is expressed throughout development. Each lane contains total RNA from the indicated developmental stages (0–12, embryos between 0 and 12 h after egg laying; 12–24, embryos between 12 and 24 h after egg laying; L1, first larval instar; L2, second larval instar; L3e, early third larval instar; L3l, late third larval instar; P, early pupa; A, adult stage). (*B*) Comparison of the expression in mbn-2 cells and whole flies. The lanes contain total RNA from whole animals or mbn-2 cells. (*C*) Comparison of the expression in head (He), thorax (Th), and abdomen (Ab). (*D*) Comparison of the expression in fat bodies (Fb), dissected from third instar larvae, and in the remaining carcasses (Ca). The filters in *A*, *C*, and *D* were probed with a *Hin*dIII–*Xba* I fragment containing the complete insert of p106, and filters in *B* were probed with an internal *Bam*HI–*Hin*dIII fragment. The lower part of each panel shows the same filter reprobed for the ribosomal protein gene *Rp49* (18), as an internal control. The figures below indicate the relative level of HLH106 expression. These values were corrected for unequal loading by division with the *Rp49* control and normalized to the value in one lane that was arbitrarily set to 1. The bands in *D* were too weak to be accurately quantitated. Sizes in kb are indicated in *A*.

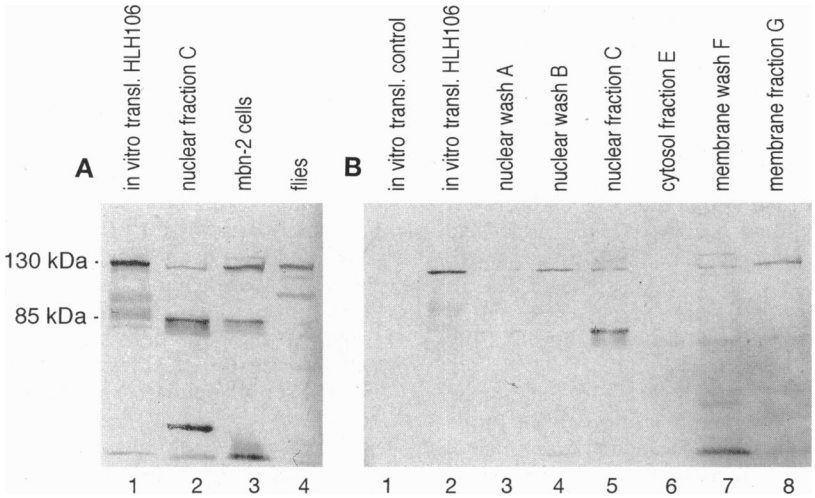

FIG. 4. Western blot showing the 130- and 85-kDa forms of the HLH106 gene product. (*A*) Presence of the 130-kDa form in whole flies and of both forms in mbn-2 cells. The *in vitro* translation product of HLH106 cDNA (130-kDa form) and the nuclear fraction C (85-kDa form) are included for comparison. (*B*) Distribution of the two forms in subcellular fractions. Lanes 1 and 2 show the *in vitro* translation product without or with HLH106 cDNA, respectively.

the expression of the ribosomal protein Rp49 (18). In adult flies, similar levels were expressed in the head, thorax, and abdomen (Fig. 3*C*). In larvae, similar levels are found in the fat body and in the remaining part of the body (Fig. 3*D*). Since HLH106 was isolated with an antiserum directed against a membrane protein from the mbn-2 cell line, we tested the expression of HLH106 in this cell line. As shown in Fig. 3*B*, the gene was approximately 3-fold more strongly expressed in the mbn-2 cells than in whole flies. Similar high levels of HLH106 mRNA were also seen in three other *Drosophila* cell lines: $Kc_{(156)}$, Schneider's SL2, and SL2* (data not shown).

Processing and Subcellular Distribution of the HLH106 Protein. To detect the protein product of the HLH106 gene, we used a monoclonal antibody directed against the bHLH domain in human SREBP-1. These antibodies were found to crossreact to *Drosophila* HLH106 protein, produced by *in vitro* translation from the p106 cDNA clone. As shown in Fig. 4*B*, the antibody detects a single major band in an immunoblot of the translation reaction, and this band is only seen when the HLH106 template is present (Fig. 4*B*, compare lanes 1 and 2). The apparent size of the protein is 130 kDa, in good agreement with the calculated molecular mass of 125 kDa. A band of the same size could also be detected in mbn-2 cells and in whole flies (Fig. 4*A*). In addition, a strongly crossreacting 85-kDa band is seen in extracts from the mbn-2 cells. This band is weak or absent in whole fly extracts. Since it appeared likely that this smaller product may correspond to the mature nuclear form of the *Drosophila* factor, we investigated the subcellular distribution of the two forms. When we fractionated an mbn-2 cell homogenate as described in ref. 6, the full-length form was strongly enriched in the cellular membrane fraction (Fig. 4*B*, lane 8), as expected for a membrane-bound factor, although traces of this band can also be seen in other fractions. In contrast, the 85-kDa form was almost exclusively found in the nuclear fraction (Fig. 4*B*, lane 5). Thus, this band shows the same subcellular distribution as the 68-kDa mature form of human SREBP, and it is likely that it corresponds to a similarly processed form of HLH106. However, we cannot exclude the possibility that other nuclear bHLH proteins may also crossreact with the antiserum. Variable quantities of crosshybridizing material of low molecular weight are likely to be degradation products.

Since SREBP processing is regulated by cholesterol availability, we tested whether HLH106 processing is similarly affected. However, in several experiments with cells or flies that were starved for cholesterol or were given additional cholesterol, we could not see any consistent effect on the levels of the two HLH106 forms on Western blots (data not shown).

DISCUSSION

The similarity in the domain structure of HLH106 to that of the vertebrate SREBPs is a strong argument that the proteins have related functions. The best conserved domain is the DNA binding bHLH motif, but sequence similarity is well conserved all the way to the C terminus of the protein. The conservation of two potential transmembrane regions supports the proposal that these regions are important for anchoring SREBP to the membrane (6). The acidic region at the N-terminal end of SREBP-1 has been shown to act as a transcriptional activation domain, and the similar region in HLH106 is likely to have the same function. HLH106 and ADD1/SREBP share an atypical tyrosine residue in the bHLH domain that has been shown to determine the dual DNA binding specificity (7). We therefore strongly expect that HLH106 has a similar dual specificity.

Like the vertebrate members of this family, HLH106 exists as a membrane-bound precursor and as a shorter crossreacting form that is bound to chromatin. A conserved glycine/serine-rich stretch is found in HLH106 at the site where SREBP-1 is processed by proteolytic cleavage (3, 23). It is likely that this is also the site where HLH106 is processed. A cysteine protease related to the interleukin 1β converting enzyme has been implicated in the processing of SREBP (23). This enzyme cleaves SREBP after an aspartic acid residue that is part of the conserved sequence EPD|SP. A similar motif, ESD|PS, can be found in HLH106 (underlined in Fig. 1), although it is situated N terminally of the glycine/serine-rich stretch. The predicted mature form of HLH106 would be about 47 kDa, considerably smaller than the apparent size of 85 kDa observed for the nuclear crossreacting species. However, a similar abberrant electrophoretic mobility was observed for the mature form of SREBP-1, at least partly due to phosphorylation (3, 23). Nevertheless, the identity of the 85-kDa band will have to be confirmed by other means.

The human and hamster SREBPs were isolated by virtue of their interaction with sterol-regulated genes, and their activation is controlled by the availability of cholesterol. However, so far we find no evidence that the processing of HLH106 is regulated by cholesterol. Insects differ from vertebrates in that they are not capable of *de novo* synthesis of sterols. These substances are taken up with the food and transported in the hemolymph by lipophorin, a functional equivalent of mamma-

lian LDL (24, 25), together with diacylglycerol and other lipids. A lipoprotein receptor has been isolated from the surface of the fat body (26), and an LDL receptor homolog has been implicated in the uptake of yolk proteins in *Drosophila* oocytes (27). The genes for such receptors are possible targets for HLH106, even though details in the regulation of the metabolism and transport of cholesterol and other lipids are likely to differ in insects and vertebrates.

In contrast to the situation in vertebrates (5), the overall expression of HLH106 is not higher in adipose tissue than in other tissues. However, this does not exclude a function of the transcription factor in the regulation of lipid uptake or metabolism, as lipids are used as an energy source in various tissues, distributed by a shuttle mechanism involving lipophorin, the insect equivalent of LDL (28). Our observation of a high level of expression of HLH106 in mbn-2 cells could be related to the fact that hemocytes in some insects tend to accumulate lipids (29). Alternatively, since high levels were also seen in other cell lines, they may simply reflect the needs of rapidly dividing cell lines.

In conclusion, we describe a *Drosophila* transcription factor with similarity to the family of vertebrate SREBPs. Although the function of HLH106 remains an open question, it appears to have a proteolytic mechanism of regulation similar to its vertebrate counterpart. The identification of *Drosophila* mutants with aberrant or absent expression of HLH106 should help to elucidate its exact function in the physiology of insects and may also help in understanding the function of vertebrate SREBPs.

We are grateful to Joseph Goldstein and Michael Brown for the gift of SREBP antibodies and for stimulating discussions. This work was supported by a grant from the Swedish Natural Science Research Council (to D.H.). U.T. was supported by fellowships from the Human Frontier Science Program Organization and the Swedish Medical Research Council.

1. Yokoyama, C., Wang, X., Briggs, M. R., Admon, A., Wu, J., Hua, X., Goldstein, J. L. & Brown, M. S. (1993) *Cell* **75,** 187–197.
2. Hua, X. X., Yokoyama, C., Wu, J., Briggs, M. R., Brown, M. S., Goldstein, J. L. & Wang, X. D. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 11603–11607.
3. Sato, R., Yang, J. X., Wang, X. D., Evans, M. J., Ho, Y. K., Goldstein, J. L. & Brown, M. S. (1994) *J. Biol. Chem.* **269,** 17267–17273.
4. Yang, J. X., Sato, R., Goldstein, J. L. & Brown, M. S. (1994) *Genes Dev.* **8,** 1910–1919.
5. Tontonoz, P., Kim, J. B., Graves, R. A. & Spiegelman, B. M. (1993) *Mol. Cell. Biol.* **13,** 4753–4759.
6. Wang, X., Sat, R., Brown, M. S., Hua, X. & Goldstein, J. L. (1994) *Cell* **77,** 53–62.
7. Kim, J. B., Spotts, G. D., Halvorsen, Y. D., Shih, H. M., Ellenberger, T., Towle, H. C. & Spiegelman, B. M. (1995) *Mol. Cell. Biol.* **15,** 2582–2588.
8. Smith, J. R., Osborne, T. F., Goldstein, J. L. & Brown, M. S. (1990) *J. Biol. Chem.* **265,** 2306–2310.
9. Gasic, G. P. (1994) *Cell* **77,** 17–19.
10. Gateff, E. (1978) *Science* **200,** 1448–1459.
11. Echalier, G. & Ohanessian, A. (1969) *C. R. Seances Acad. Sci. Ser. D* **268,** 1771–1773.
12. Schneider, I. (1972) *J. Embryol. Exp. Morphol.* **27,** 353–365.
13. Samakovlis, C., Åsling, B., Boman, H. G., Gateff, E. & Hultmark, D. (1992) *Biochem. Biophys. Res. Commun.* **188,** 1169–1175.
14. Kylsten, P., Samakovlis, C. & Hultmark, D. (1990) *EMBO J.* **9,** 217–224.
15. Theopold, U., Pintér, M., Daffre, S., Tryselius, Y., Friedrich, P., Nässel, D. R. & Hultmark, D. (1995) *Mol. Cell. Biol.* **15,** 824–834.
16. Short, J. M., Fernandez, J. M., Sorge, J. A. & Huse, W. D. (1988) *Nucleic Acids Res.* **16,** 7583–7600.
17. Davis, L. G., Dibner, M. D. & Battey, J. F. (1986) *Basic Methods in Molecular Biology* (Elsevier, New York).
18. O'Connell, P. O. & Rosbash, M. (1984) *Nucleic Acids Res.* **12,** 5495–5513.
19. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
20. Titus, D. E. (1991) *Promega Protocols and Applications Guide* (Promega, Madison, WI).
21. Tempst, P., Link, A. J., Riviere, L. R., Fleming, M. & Elicone, C. (1990) *Electrophoresis* **11,** 537–553.
22. Sipos, L. & von Heijne, G. (1993) *Eur. J. Biochem.* **213,** 1333–1340.
23. Wang, X. D., Pai, J. T., Wiedenfeld, E. A., Medina, J. C., Slaughter, C. A., Goldstein, J. L. & Brown, M. S. (1995) *J. Biol. Chem.* **270,** 18044–18050.
24. Law, J. H. & Wells, M. A. (1989) *J. Biol. Chem.* **264,** 16335–16338.
25. Soulages, J. L. & Wells, M. A. (1994) *Adv. Protein Chem.* **45,** 371–415.
26. Tsuchida, K. & Wells, M. A. (1990) *J. Biol. Chem.* **265,** 5761–5767.
27. Schonbaum, C. P., Lee, S. & Mahowald, A. P. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 1485–1489.
28. Law, J. H., Ribeiro, J. M. & Wells, M. A. (1992) *Annu. Rev. Biochem.* **61,** 87–111.
29. Gupta, A. P. (1985) in *Comprehensive Insect Physiology Biochemistry and Pharmacology*, eds. Kerkut, G. A. & Gilbert, L. I. (Pergamon, Oxford), Vol. 3, pp. 401–451.