
DEVELOPMENTAL AND ORGAN-SPECIFIC EXPRESSION OF TRANSFERRIN IN *DROSOPHILA MELANOGASTER*

N. Harizanova¹, M. Tchorbadjieva², P. Ivanova¹, S. Dimov¹, K. Ralchev¹
Sofia University "St. Kliment Ohridski", Faculty of Biology,
Dept. of Genetics, Sofia, Bulgaria¹
Sofia University "St. Kliment Ohridski", Faculty of Biology,
Dept. of Biochemistry, Sofia, Bulgaria²

ABSTRACT

Polyclonal antibodies raised against recombinant D. melanogaster transferrin were used to study both developmental and organ-specific expression of D. melanogaster transferrin. It was shown that transferrin is present at all developmental stages except late embryos and early larvae. The organ distribution of transferrin reveals that it is abundant in the hemolymph and from all organs examined was detected only in the ovaries. The maternal origin of the transferrin in early embryos, the activation of its synthesis and its accumulation in the oocytes are discussed.

Introduction

Transferrins are a group of iron-binding proteins well characterized in vertebrates. They are single-chain glycoproteins of molecular weight near 80 kDa, bearing two structurally similar but functionally distinct iron-binding sites. The polypeptide chain is arranged in two lobes, respectively representing the N-terminal and C-terminal halves of the molecule.

Molecular cloning of insect transferrins from *Aedes aegypti* (1), *Bombix mori* (2), *Drosophila melanogaster* (3) *Sarcophaga peregrina* (4) and *Riptorus clavatus* (5) suggests an extensive deletion in the C-terminal lobe resulting in sizes around 66 kDa. As a result most insect transferrins have a functional iron-binding site only in the N-terminal lobe. The role of insect transferrin is less well defined. The evidences obtained so far showed that the insect transferrin is an iron transport protein (6), an antibiotic agent (1,

2, 3) and a vitelogenic protein (4).

In this paper we report the developmental and organ-specific expression *D. melanogaster* transferrin.

Materials and Methods

Drosophila Culture. The wild-type *D. melanogaster* Oregon R strain was maintained on a standard corn meal/agar medium at 25°C. Embryos (2 and 20 h), larvae (first, second and third instar), pupae and female adults were selected from mass cultures.

Insects were homogenized in 250ml PBS containing 4mM PMSF and centrifuged for 15 min at 12000 rpm. Protein concentration was determined by the method of Bradford (7).

Organs from third instar larvae and female adults were dissected in PBS. Organs from ten insects were pooled and homogenized in 30 ml PBS, containing PMSF. Larval hemolymph was collected in the drop of PBS used for dissection of larvae. Hemolymph

from adult females was collected by centrifugation according to Kambyseilis (8). Adult fat bodies were collected together with the hemolymph and the carcass.

Production of antibodies. Partially purified recombinant *D. melanogaster* transferrin kindly provided by Dr. J. Law, University of Arizona, was used for antibody production. The protein was mixed with 4 x SDS-PAGE sample buffer and subjected to 12,5% polyacrylamide gel electrophoresis. The gel was briefly stained in 0,1% Coomassie blue R-250 in 40% methanol and 10% CH₃COOH. The recombinant transferrin band was excised from the gel, the gel slice (containing approximately 200 mg recombinant protein) was washed in dd H₂O, homogenized in 1 ml RIBI Adjuvant (RIBI Immunochem. Research Inc., Hamilton, MT) and injected subcutaneously into a rabbit (9). Four weeks after the first injection a 200mg booster was injected. Two weeks after the boosting blood was collected and used for serum preparation. The immune serum was tested by immunodot assay (10).

Protein gel electrophoresis. SDS-PAGE was performed in 12,5% homogenous slab gel according to Laemmli (11). Aliquots of supernatants from each developmental stage containing 60mg of protein (approximately equivalent to the protein content of two flies) were mixed with 4x SDS-PAGE sample buffer, boiled for 5 min and loaded on the gel. Hemolymph and organs from ten animals prepared for SDS-PAGE as described above were loaded per lane.

Western blot. After electrophoresis polypeptides were transferred onto a Protran membrane (Schleicher & Schuell). Blots were blocked with 1% non-fat dry milk in 0,02M Tris-HCl, 0,01M NaCl, pH 7,5, incubated with rabbit anti-*D. melanogaster* transferrin

serum (1:500), washed and incubated with goat anti-rabbit IgG alkaline phosphatase conjugate (Bio-Rad Laboratories, Hercules, CA, USA). Transferrin was visualized using alkaline phosphatase stain (330mg/ml NBT and 165mg/ml BCIP in 100mM NaCl, 5mM MgCl₂, 100mM Tris-HCl, pH 9,5).

Results and Discussion

Fig.1 shows a Western blot of homogenates of whole animals taken at different life stages. A band with electrophoretic mobility just below the recombinant transferrin is observed at almost all developmental stages except late embryos and early larvae. We consider that it responds to the transferrin. The difference in the electrophoretic mobility of both proteins is due to the presence of His. Tag in the recombinant protein. Because no RNA messages were found in the embryos (3) we assume that transferrin in the early embryos is of maternal origin. Transferrin accumulated during oogenesis in the eggs disappears with time and no significant activation of the transferrin gene was detected until the third larval stage when both the messages (3) and the protein are observed. The Western blot on **Fig.1** shows the presence of additional bands with molecular weight lower than the transferrin. The one with the highest electrophoretic mobility is observed at all developmental stages even when no transferrin is present. Most probably it is a result of cross reaction of the anti-serum used for probing the blot. Cross reaction or proteolytic degradation could be the reason for the appearance of the other bands. The fact that a single band is observed in the hemolymph excludes to a great extent the possibility that transferrin is proteolytically degraded during the preparation of the protein homogenates. But we cannot exclude

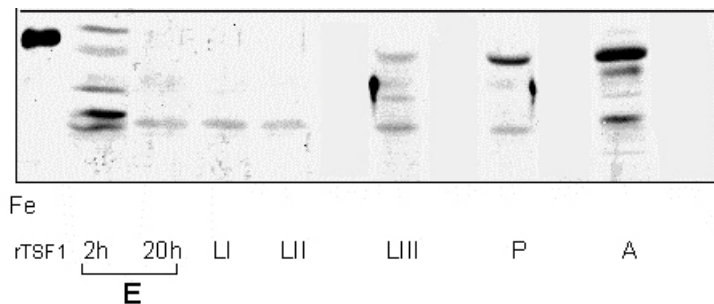


Fig.1 Developmental expression of *D. melanogaster* transferrin. Western blot of protein extracts prepared from individuals at different stages of development and probed with polyclonal antiserum raised against *D. melanogaster* transferrin. E—embryos, LI, LII, LIII—first, second and third instar larvae, P—pupae, A—adults, rTSF—recombinant transferrin.

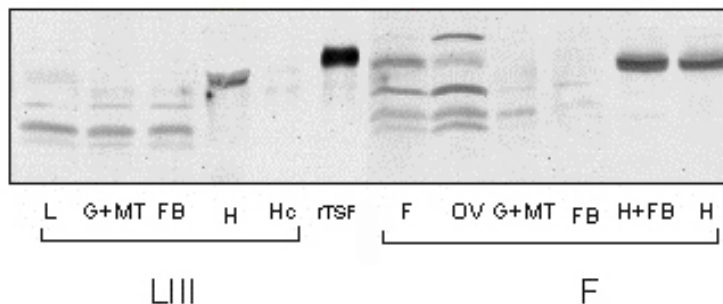


Fig. 2. Organ-specific expression of *D. melanogaster* transferrin Western blot of protein extracts from organs of third instar larvae and female adults probed with antibodies against *D.melanogaster* transferrin. L—larvae, F—female, G—gut, MT—Malpighian tubules, FB—fat body, H—hemolymph; Hc- hemocytes, rTSF—recombinant transferrin.

that for some reason transferrin is proteolytically cleaved in vivo.

The results presented on **Fig.2** reveal that transferrin is abundant in the hemolymph both in third instar larvae and adults. Preliminary results (not published) reveal that transferrin messages are synthesized in the fat body. Because we cannot detect the protein in the fat body we assume that immediately after its synthesis there, transferrin is secreted to the hemolymph.

The organ distribution of the protein in females shows that from all examined organs the ovaries are the only ones where transferrin can be detected. Most probably it is taken up by the developing oocyte and not

synthesized there. This is consistent with the fact that no messages have been found there (data not published). Nothing is known about the mechanism of transferrin uptake into the oocyte. Nurse and follicle cells surrounding the oocyte could be involved in this process. *D. melanogaster* genome contains several proteins with transferrin receptor-like features with low sequence identity with mammalian transferrin receptor. May be the accumulated transferrin is proteolytically degraded in the oocyte. But further experiments with affinity purified transferrin IgG are necessary in order to exclude the possibility that the lower molecular weight bands are a result of cross-reaction of the antiserum. The

function of the transferrin in the oocyte is still unclear. Either it supplies iron necessary for ovogenesis and embryogenesis or it prevents the developing embryo from bacterial infection. The second hypothesis is consistent with the observed up-regulation of the transferrin messages following bacterial challenge (3). Furthermore, the transferrin gene of *D. melanogaster* (12) contains promotor region sequences known to bind nuclear-kappa B-like transcription factors involved in the insect immune response. Proteolytic fragments of transferrin could participate in the immune defense as well. It has been recently shown that products of proteolytic cleavage of transferrin induce nitric oxide response of goldfish macrophages (13).

REFERENCES

1. **Yoshiga T., Hernandez V., Fallon A., Law J.** (1997) Proc. Natl. Acad. Sci .U.S.A., **94**, 12337-12342.
2. **Yun E., Kang S., Hwang J., Goo T., Kim S.** (1999) Biol. Chem., **380**, 1455-1459.
3. **Yoshiga, T., Georgieva T., Dunkov B., Harizanova N., Ralchev K., Law J.** (1999) Eur. J. Biochem., **260**, 414-420.
4. **Kurama T., Kurata S., Natori S.** (1995) Eur. J. Biochem., **228**, 229-235.
5. **Hirai M., Watanabe D., Chinzei Y.** (2000) Arch. Insect Biochem. Physiol., **44**, 17-26.
6. **Huebers H., Huebers E., Finch C., Webb B., Truman J.** (1988) J. Comp. Physiol. B., **158**, 291-300.
7. **Bradford M.** (1976) Anal. Biochem., **72**, 1976, 248-254.
8. **Kambysellis M.** (1984) Drosophila Inform. Serv., **60**, 219-220.
9. **Ivanova A., Eshkenazy M., van Onkelen.** (1994) Biotechnol. & Biotechnol. Eq., **2**, 7 – 11.
10. **Odjakova M. K., Getova T. G., Kojuharova M. S.,** (2001) Biotechnol. & Biotechnol. Eq., **15** (2), 62 – 66.
11. **Laemmli U.** (1970) Nature, **277**, 680-687.
12. **Adams M., Celniker S., Holt R., Evans C., Gocayne J.** (2000) Science, **287**, 2185-2189.
13. **Stafford J., Neumann N., Belosevic M.** (2001) Dev. Comp. Immunol., **25**, 101-115.