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EXPRESSION OF THE TRANSFERRIN GENE DURING DEVELOPMENT OF NON-HEPATIC TISSUES : High level of transferrin mRNA in fetal muscle and adult brain

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Using a cloned rat transferrin cDNA probe, we looked for transferrin mRNA in the various rat tissues during development. In all the cases the mRNA detected seemed to be the same and to be product of a single gene. The transferrin gene is early expressed at a high level during liver differentiation. In the muscle and other non-hepatic and non-nervous tissues, the gene expression is maximal just before birth (19-20th day of gestational age), then markedly decreases during the postnatal development, the mRNA level being very low in the adult tissues. In brain, by contrast, transferrin mRNA level is very low before birth, then gradually increases during the postnatal development and reaches a plateau in the adult. Maximal mRNA concentration in fetal muscle (2 days before birth) and adult brain is about 1:7 to 1:10 of that obtained in adult liver. These results are analyzed in the light of the evidence that transferrin is not only an iron-binding protein, but also a factor involved in cell proliferation and differentiation, and particularly in nerve control of muscle differentiation.

The iron-binding serum protein transferrin has as essential function to bring iron to hematopoietic cells. More recently, however, transferrin has been related to proliferative processes by two lines of evidence (1,2). One line based on "in vitro" cell culture experiments clearly shows that transferrin is an essential requirement for "in vitro" cell growth (2,3) . Recently transferrin was identified as the neurotropic factor capable of promoting growth or differentiation of chicken muscle cells in culture (4). At the same time, Ekblom et al (5) established that transferrin is an essential growth factor for the nephrogenic mesenchyme, affecting differentiation by stimulating cell proliferation.

The second line of evidence relates transferrin to genes active in cancer cells. It has been shown that one onc-gene detected in chicken B-cell

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lymphomas and in human Burkitt lymphomas shares N-terminal sequence homology with transferrin (6,7) and that p. 97, a cell surface glycoprotein present in most human melanomas, is structurally and functionnaly related to transferrin (8).

The question then arises as to whether transferrin is involved in "in vivo" development of the tissues, and if so, whether the transferrin gene is active in each developing tissue or is only active in liver, transferrin being then transported to the different organs by the bloodstream.

Some data have been presented which suggest extrahepatic sites for transferrin synthesis (1). Except for the lactating mammary gland, the level of synthesis appears to be low. In addition, in the absence of specific DNA probes able to demonstrate that the gene is actively transcribed in a given tissue, this type of result is often difficult to interpret for a protein abundant in the serum.

We have prepared a cDNA probe for rat transferrin. Using this probe, we show in this paper that the transferrin gene is actively transcribed in all the tissues we have studied, except brain, before birth, and in brain after birth. The maximum expression in non-hepatic tissues reaches about one tenth of that in adult liver.

MATERIALS AND METHODS

Cloning and characterization of a human transferrin cDNA clone has been recently reported (9) ; it was used to detect several rat clones from a rat liver cDNA library (9,10). One of them, of about 1400 base long, was used in this study. The methods of RNA purification, Northern and dot blot analysis were as previously described (9,10,11).

RESULTS

. Detection of transferrin mRNA in the various rat tissues.

The cDNA probe hybridized with a single mRNA species of 2400 base pairs which was observed in rat liver and, at a lower concentration, in various other fetal and adult tissues (Fig. 1). In muscle, brain and liver, transferrin mRNA was polyadenylated and found in the polysomal fraction (not shown). Dot blot hybridization experiments showed that the liver transferrin mRNA content increases about 2.2 times between the 17th day of gestation

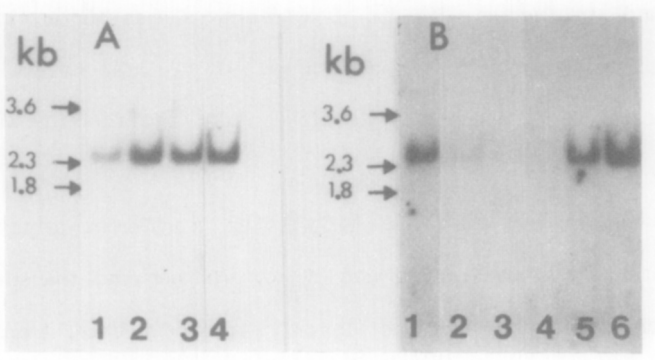


Figure 1.
Transferrin mRNA Northern blot analysis.

Electrophoresis was performed on 1.5 % (w/v) agarose gels in 10 mM Phosphate buffer, 2.2 M formaldehyde. 20 µg total RNA were deposited per slot. Hybridization with 2 x 10^6 cpm/ml of the labeled purified insert, washing at 65° C in excess 1 x SSC.

A. Liver RNA samples
1 : Fetal liver (17th day of gestation)
2 : 1 day after birth
3 : 6 days after birth
4 : Adult liver (60th day of life)
Exposure time : 2 hours at -80° C with intensifying screens.

B. Muscle and brain RNA samples
1 : Fetal muscle (19th day of gestation)
2 : Muscle, 10 days after birth
3 : Adult muscle (60th day of life)
4 : Fetal brain (21th day of gestation)
5 : Brain 20 days after birth
6 : Adult brain (60th day of life)
Exposure time : 36 hours at -80° C with intensifying screens.
Adult animals were males.

and the first postnatal day, then remains almost constant during the animal life. This increase approximately parallels the development of the hepatocyte mass of the liver (12), thus differing from albumin mRNA synthesis which increases more than 10 times during the last days of gestation (Guillouzo, C., personal communication). We also found that, in contrast to transferrin, the synthesis of aldolase B and L-type pyruvate kinase (10) (two other strong markers of hepatocytic differentiation) increases strongly after birth (Marie, J., Kahn, A., unpublished data).

The evolution of transferrin mRNA content is very different in lung, heart, spleen, kidney and especially muscle : here the specific mRNA content increases during fetal development to reach a maximum between day 3 and 1 before birth, then drops quickly after birth and remains stable at a very low

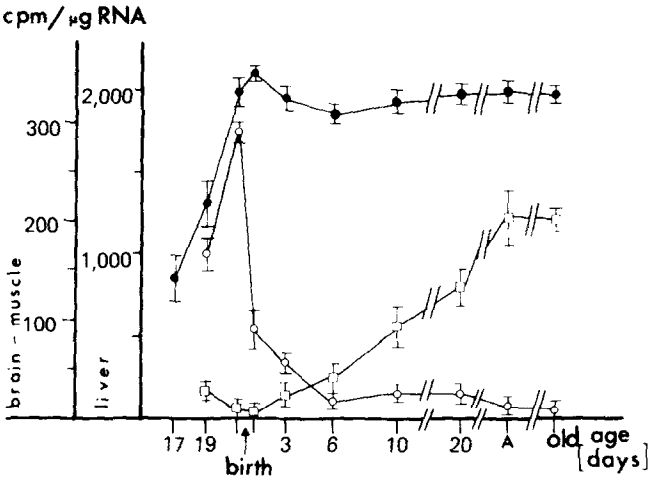
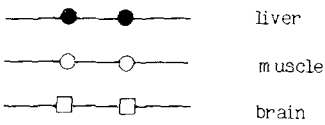


Figure 2.

Transferrin mRNA during development.

For measuring specific mRNA concentration by dot blot analysis, 10 µg and 5 subsequent 1 : 2 dilutions of total RNA (and poly A^+ mRNA for control) were dotted onto nitrocellulose filters, the hybridization being performed in the presence of 2 x 10^6 cpm/ml of labeled purified inserts (0.5 ml of hybridization mixture per 10 cm^2 of filter surface). The radioactivity was a linear function of the RNA amounts deposited on the filters. The results are expressed in cpm of radiolabeled probe per µg of RNA. Poly A^+ mRNA concentration of the different cellular RNA preparations was measured by hybridization with (^{3}H) polyuridilic acid (11). The ratio poly A^+ mRNA/total RNA proved to be similar for all the tissues studied. The values were extrapolated from the linear part of the curve of cpm versus RNA amount deposited on the filters.

2 scales are designated, one corresponding to brain and muscle, the other corresponding to liver,



Each point represents the mean of at least 6 values (2 measurements in at least 3 different animals), the length of the bars represents SEM. The age of fetuses are expressed in gestational days ; A : 60-day-old animal Old : 16-months-old animals. Adult and old animals were males.

level during adult life (Fig. 2 and Table I). In muscle the maximum mRNA concentration (at one day before birth) represents 1 : 7 of that in adult liver.

The evolution of transferrin gene expression during brain development exhibits a third pattern : the amount of mRNA is very low during fetal life, then increases after birth to reach a maximum in the adult (60th day of life) and then remains constant at 1 : 10 of the value found in adult liver.

TABLE I : Dot blot analysis of transferrin mRNA content of different rat tissues at different developmental stages

		Transferrin mRNA cpm/µg RNA
Small Intestine	Fetal	200 ± 15
	Newborn	80 ± 3
	Adult	- (not detectable)
Kidney	Fetal	110 ± 10
	Newborn	20 ± 2
	Adult	40 ± 5
Lung	Fetal	115 ± 15
	Newborn	25 ± 2
	Adult	30 ± 5
Heart	Fetal	50 ± 10
	Newborn	5 ± 1
	Adult	- (not detectable)
Spleen	Newborn	130 ± 18
	Adult	17 ± 2

Dots were performed as described in figure 2. Fetal samples were from 19-day-old fetuses, newborn animals were killed at the first day of life and adult males at 2nd month. Each measurement is given ± SEM.

DISCUSSION

In all the tissues studied transferrin mRNA exhibited the same length as judged by Northern blot analysis while Southern blots of genomic DNA hybridized with 3' transferrin cDNA probes (9 and unpublished data) showed a single hybridizing restriction fragment with most of the restriction enzymes used. It seems therefore that only one transferrin gene exists per haploïd genome and that this single gene is transcribed in different tissues into the same mRNA species.

The high level of expression of transferrin mRNA in liver can be explained by the recognized fact that this organ is the major site of transferrin production covering the physiological needs of the whole animal (1).

The temporary transferrin gene expression observed before birth for several tissues, in particular muscle, is reminiscent of the function of transferrin as a growth factor (1,2,3) involved in tissue differentiation (5) and could therefore suggest that transferrin plays a role in "in vivo" development.

The results obtained in brain are especially interesting. It was previously accepted that transferrin is essentially produced in the liver and delivered to other tissues via the circulating transferrin and local receptors. It appears now that the brain is able to synthesize transferrin and that this ability increases during postnatal development. The reported effect of transferrin as a neurotropic factor, transported by the nerve to the muscle, and acting as a mediator of nerve action on muscle (4), would then be under the direct control of the brain. More detailed studies will be necessary to establish this fact, and methods are presently being devised to precise which parts of the brain tissue are able to synthesize transferrin.

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