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Rat lactate dehydrogenase A and B subunit concentrations are not regulated by mRNA abundance in liver and heart

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RNA was isolated from rat liver and heart tissues at various times up to 12 weeks after birth, and probed on slot blots with lactate dehydrogenase A and B cDNA probes. Although the relative abundances of LDH A in liver and LDH B in heart increased substantially in the 12 weeks after birth, mRNAs for both isoenzymes remained remarkably stable in both tissues over the same period. The implications of these observations for the regulation of constitutive gene expression are discussed.

Lactate dehydrogenase A; Lactate hydrogenase B; Developmental regulation

1. INTRODUCTION

Regulation of gene expression in higher eukaryotes is a remarkably complex process, with different stages having primary involvement in different situations (e.g. [1,2]). Thus, transcription is often the main regulatory step during terminal differentiation and the activation or repression of tissue-specific genes but post-transcriptional events such as RNA processing, transport, translation or protein degradation can be more important in other circumstances and in particular in regulating the expression levels of constitutive genes. Lactate dehydrogenases constitute interesting examples of both such gene classes; A and B isoenzymes are expressed constitutively, but at varying levels, in all mammalian tissues whereas LDH C is expressed only in testes following the onset of spermatogenesis (e.g. [3-6]). LDH C regulation may be at the transcriptional level; certainly there is evidence that LDH C mRNA increases in parallel with isoenzyme production in mouse [6]. The situation with the constitutive LDHs is less clear, though LDH A poly A+ mRNA levels were found to be remarkably constant between tissues expressing widely different amounts of the polypeptide [7]. In this study we have quantified LDH A and B mRNA levels during the first twelve weeks of life in tissues exhibiting marked changes in one or other proteins during the same period.

2. MATERIALS AND METHODS

2.1. *Materials*

Rats were of the Wistar strain and bred in the laboratory. Probe pRLD42 was a cDNA clone made in pBR322 from rat LDH A mRNA [8] and mb162 was a cDNA clone made in phage M13 from mouse LDH B mRNA [9]. Both were grown and DNA isolated from them by standard methods [10]. Genescreen plus membranes were from Du-Pont (UK) Ltd; [α-^{32}P]dCTP was from Amersham International (UK). Restriction enzymes and DNA polymerase (Klenow fragment) were from Gibco BRL (UK).

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2.2. *RNA isolation and blotting*

Tissues were excised, ground under liquid nitrogen, RNA isolated, electrophoresed and Northern blotted or slot-blotted (always in triplicate) directly onto Genescreen plus all as described elsewhere [11]. Aurintricarboxylic acid (ATA) was used as a ribonuclease inhibitor in all isolations and removed by Sephadex G-50 chromatography immediately prior to slot-blotting.

2.3. *DNA labelling and hybridisation analysis*

cDNA probes were labelled by random primer extension [12]. Hybridisation conditions included 50% (v/v) formamide at 42°C and most stringent washes were at 50°C for testes Northern blots; 30°C for pRLD42 and liver RNA slot blots; 42°C for pRLD42 and heart RNA slot blots; and 50°C for mb162 with all slot blots, always in 2 × SSC, 1% SDS [11]. Autoradiography followed at −70°C.

2.4. *Quantitation of mRNA*

Autoradiographs of slot-blots were scanned using an LKB Ultroscan XL. Integrated peak areas for each set of triplicates were averaged and plotted against μg RNA on the blot; gradients over areas of linear response were used as an arbitrary measure of relative LDH mRNA concentration at each age point. Since not all age points could be accomodated on the same membrane, overlapping age points were made such that at least one was common to all blots. Compensation was then made between blots to allow for variations in general autoradiograph intensities.

3. RESULTS

Cloned probes for rodent LDH A and B mRNAs were first tested by hybridisation to Northern blots as shown in Fig. 1. Testes were used as the RNA source because both isoenzymes are abundant in this tissue ([13], and unpublished observations) and because intact RNA is particularly easy to isolate from it. pRLD42 lit

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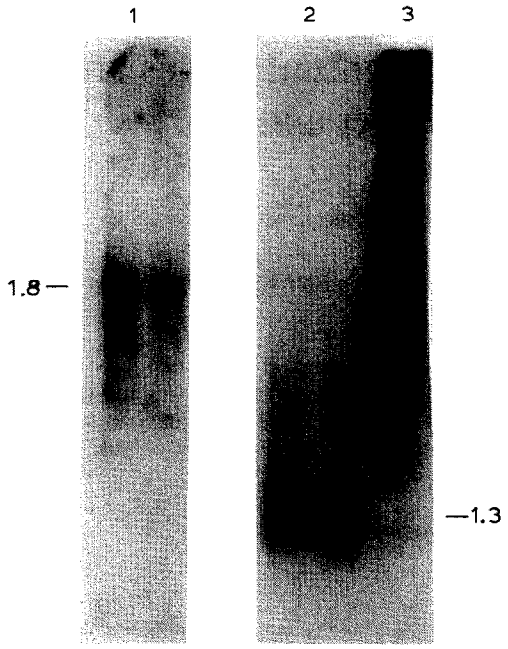

Fig. 1. Probe characterisation. Northern blots of testes RNA probed with pRLD42 (LDH A) in duplicate lanes 1, mb162 (LDH B) in duplicate lanes 2, and marker DNA (overexposed) in lane 3.

up a band of about 1.8 kb, the expected size for LDH A mRNA [8] and mb162 hybridised to a substantially smaller mRNA of the expected size for LDH B mRNA [9]. Thus the murine LDH B probe was apparently suitable for work with rat RNA. Wash stringencies for subsequent slot-blot analyses were determined empirically using Northern blot hybridisations. Maximal stringencies still leaving a unique and readily detectable mRNA band were surprisingly low; in liver, for example, pRLD42 picked out a strong signal with washes at 30°C in 2 × SSC, 1% SDS but only a very faint one at 40°C in the same buffer. By contrast, the signal from pRLD42 was stable with testes RNA up to 50°C but still only in relatively high ionic strength solutions [11]. RNA from all tissues was isolated in the presence of ATA and appeared to be of good quality as judged by 18s and 28s rRNA banding patterns on ethidium bromide-stained agarose gels [11]; it seems most likely that the requirement for low stringencies reflected very low abundances of the mRNAs in the tissues studied.

A typical slot blot of rat liver RNA samples from animals of different ages is shown in Fig. 2A. In some experiments a minimal load of 0.1 μg RNA was employed, and Fig. 2B shows an example of the relationship between hybridisation signal and RNA load over the lower end of the concentration range. In most cases relationships were linear up to at least 5 μg per slot [11]. For all ages, RNA was made from at least two rats and analysed separately.

Changes in LDH A and B protein and mRNA levels in liver and heart tissues during the first twelve weeks post-partum are shown in Fig. 3. Alterations in LDH polypeptides relative to total protein were determined from radioimmunoassay data described elsewhere [4]; the two tissues behaved very differently, with the minor isozyme (B in liver, A in heart) remaining fairly stable but with the dominant forms (A in liver, B in heart) increasingly substantially through the first 12 weeks of life. By contrast, both LDH A and LDH B mRNAs remained remarkably constant in both tissues over the same timespan.

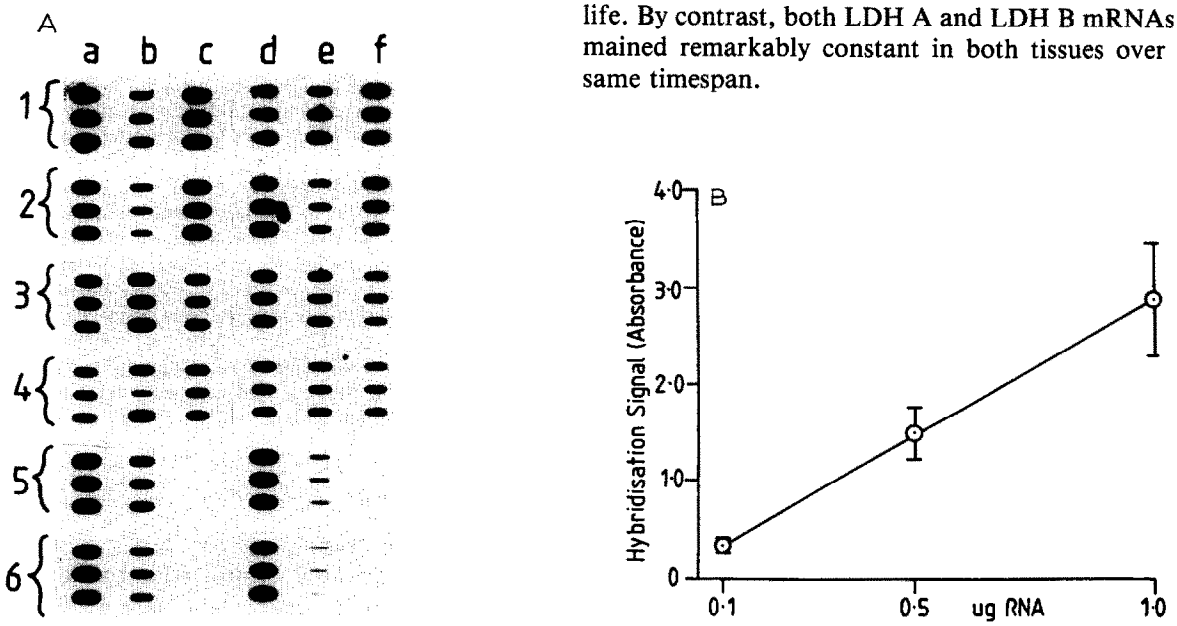

Fig. 2. Slot blot characterisation. (A) Example of slot blot using liver RNA probed with pRLD42. 1a–4a: 10, 5, 1 and 0.5 μg RNA triplicates from neonatal rat; 5–6a, 1–2b: same from 1-week-old rat; 3–6b: same from 2-week-old rat; 1c–4c: same from 3-week-old rat. Lanes d–f are the same as a–c using RNA from a second series of rats. (B) Linearity of hybridisation response. Hybridisation signals determined by densitometry tracing of a liver RNA/pRLD42 probe slot blot autoradiograph are shown together with standard deviations derived from the triplicate samples.

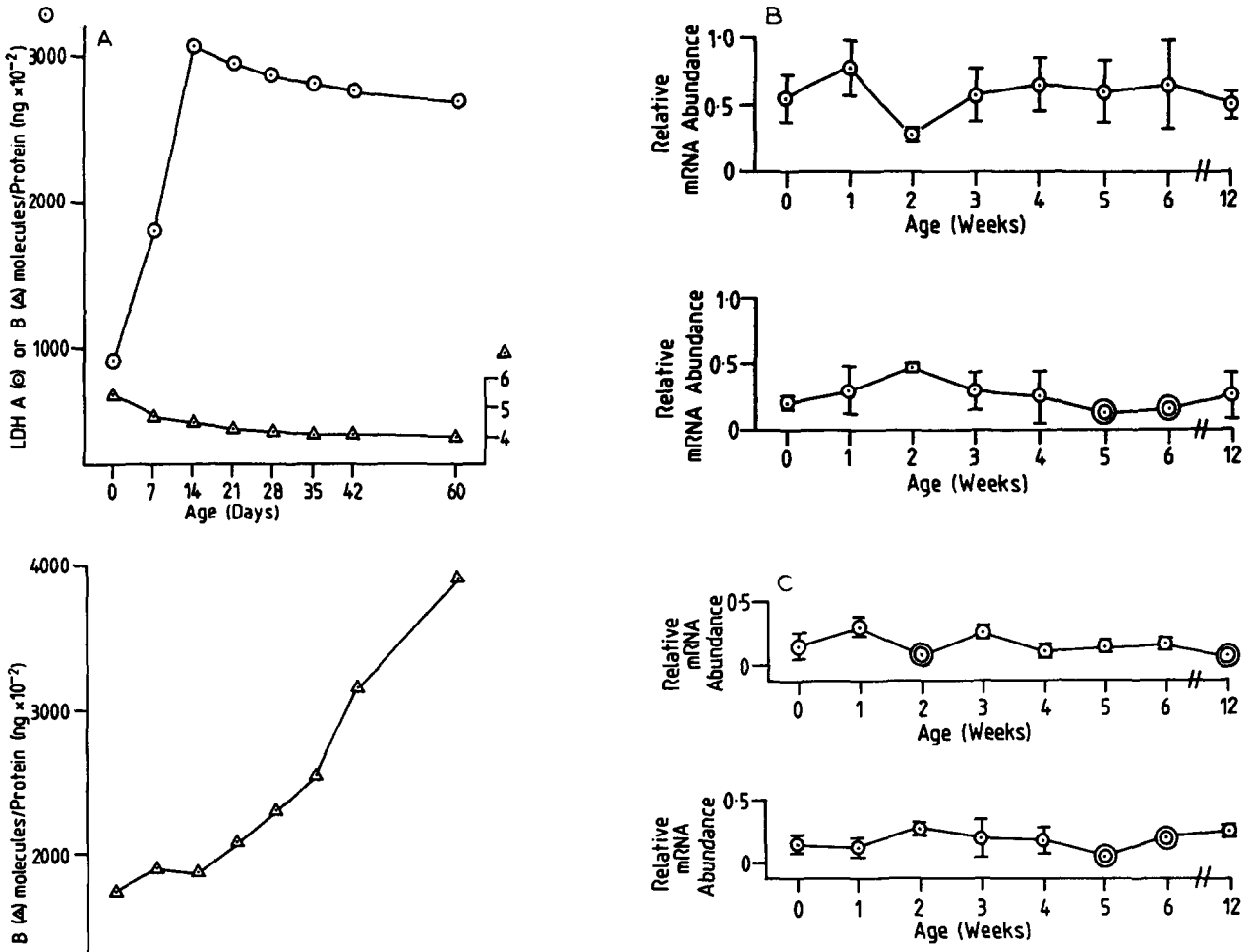

Fig. 3. Tissue-specific changes in LDH proteins and mRNAs during growth. (A) Relative changes in LDH A and B per ng protein in liver (upper panel) and heart (lower panel). (B) Relative changes in LDH A mRNA in liver (upper panel) and in heart (lower panel). (C) Relative changes in LDH B mRNA in liver (upper panel) and in heart (lower panel). For (B) and (C), standard deviations between duplicate rats are shown by error bars.

4. DISCUSSION

Hybridisation analysis of rat liver and heart RNA using probes against LDH A and B mRNAs led to two major conclusions. The first, as deduced from the need to use low stringency washes, is that all LDH mRNAs were probably of very low abundance in these tissues. This was not especially surprising since lactate dehydrogenases are among the most stable of enzymes with half-lives estimated in days or weeks in vivo (e.g. [3,4]). It may be that only a very few mRNA molecules are needed to maintain steady-state levels of these proteins. Other explanations of the need for low stringency washes are of course possible; mb162 was almost certainly not completely homologous with the rat mRNA and this must have contributed to poor hybrid stability. The results with pRLD42 cannot be explained in this way however, though one possibility is that more than one form of LDH A mRNA exists in mammalian cells. Such variety can be generated by alternative splicing events, examples of which include α-amylase and aldolase (e.g. [14,15]) where transcription may start from multiple promoters the use of which is regulated in a tissue-specific manner (e.g. [14,15]). Miles et al. [8] did obtain some results suggestive of more than one type of LDH A mRNA in glioma cells, but this has not been confirmed in any subsequent study. pRLD42 is complementary to the 3′ 630 nucleotides of LDH A mRNA and includes mainly non-translated tail sequences, where alternative splicing is clearly possible. However, we obtained similar results (not shown) with pLDH2, a full-length cDNA clone made against rat

fibroblast LDH A mRNA [16] in which changes in the proportionally much smaller non-coding regions should have had less consequence for hybrid stability.

The second conclusion is that LDH A and B polypeptide levels were not regulated by mRNA abundance in the situations we studied. As indicated above, it remains possible that alternative splicing could have generated different species of mRNAs and that such subspecies might have varied in relation to polypeptide abundance. However, it would be remarkable if changes of this type were masked as effectively as our data suggest such as to maintain an essentially invariant total amount of LDH mRNA without any evidence of mRNA size heterogeneity. It seems more probable that regulation was occurring at the translational level or subsequent to it in these tissues, a result in keeping with the preliminary observations of Piechaczyk et al. [7] on LDH A mRNA levels in a number of different rat tissues. The nature of this regulatory process remains quite unknown at the present time.

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