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Developmental changes in the expression of HMG 2a protein

Tatsuzo Oka[a], Tkayo Sasakawa[a], Naomi Komori[a], Ken-ichi Miyamoto[a], Isao Suzuki[b], Toshihiro Sassa[a] and Yasuo Natori[a]

[a]*Department of Nutrition, School of Medicine, University of Tokushima, Tokushima 770, Japan* and [b]*Department of Food and Nutrition, Kumamoto Women's University, Kengunmachi, Kumamoto 862, Japan*

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The levels of HMG 2a chromosomal protein and its mRNA change during the post-hatched development of chicks were investigated. The contents of both HMG 2a and 2b proteins of liver, heart, brain, muscle and gizzard were abundant in the newly hatched chicks but their contents decreased significantly in those tissues of the 70-day-old chicks. The HMG 2a mRNA levels of liver, heart and brain in 70-day-old chick decreased to about 40% of those mRNA in the newly hatched chicks while the HMG 2a mRNA levels of muscle and gizzard in the 70-day-old chicks increased 5- and 3-foid, respectively. These results suggest that the decrease in the HMG 2a protein contents of the muscle and gizzard in the 70-day-old chicks may be largely due to the stimulation of HMG 2a protein degradation or the reduction of HMG 2a mRNA translation.

HMG 2a protein: HMG 2a mRNA; Chick; Post-hatched development

1. INTRODUCTION

The high mobility group (HMG) chromosomal proteins are the most abundant class of nonhistone proteins found in the nuclei of higher eukaryotes [1]. There are four major HMG proteins (HMG 1, 2, 14 and 17) in all the eukaryotic cells examined to date [2]. In chicks, HMG 2 has been further resolved by chromatography into two subfractions, called HMG 2a and 2b [3].

Recently we have isolated the HMG 2a cDNA, which encodes a protein of 201 amino acids, from a λgt11 expression library of chick liver using polyclonal antibodies [4]. In Northern blotting, 2.0 kb and 1.2 kb mRNAs were found in the liver from newly hatched chicks and shown to decrease during post-hatched development of the chicks.

In this study, we report that the relative levels of HMG 2a proteins and its mRNA from various tissues change significantly during development of the animal.

2. MATERIALS AND METHODS

2.1. *Animals*

Newly hatched and 70-day-old white leghorn male chicks were obtained from a local hatchery (Ishii Hatchery, Tokushima).

2.2. *Western blotting*

The homogenate samples from various tissues treated with polytron homogenizer (Kinematica GmbH, Krens/Luzern, Switzerland) were analyzed on polyacrylamide gels (15%) according to the methods of Laemmli [5]. After electrophoresis, the gels were blotted onto a nitrocellulose membranes (Schleicher & Schuell, Germany). Western blot analysis was carried out using polyclonal antibody against HMG 2a as described previously [6].

Correspondence address: T. Oka, Department of Nutritional Chemistry, School of Medicine, The University of Tokushima, Tokushima 770, Japan. Fax: (81) (886) 31-9476.

2.3. *Northern blotting and slot blotting*

Total cellular RNA was isolated from the various tissues by the guanidium thiocyanate method [7] and poly(A)$^+$ RNA was fractionated using oligo(dT) cellulose column (Pharmacia, Sweden). Ten μg of poly(A)$^+$ RNA was electrophoresed in a 1.5% formaldehyde-aga-

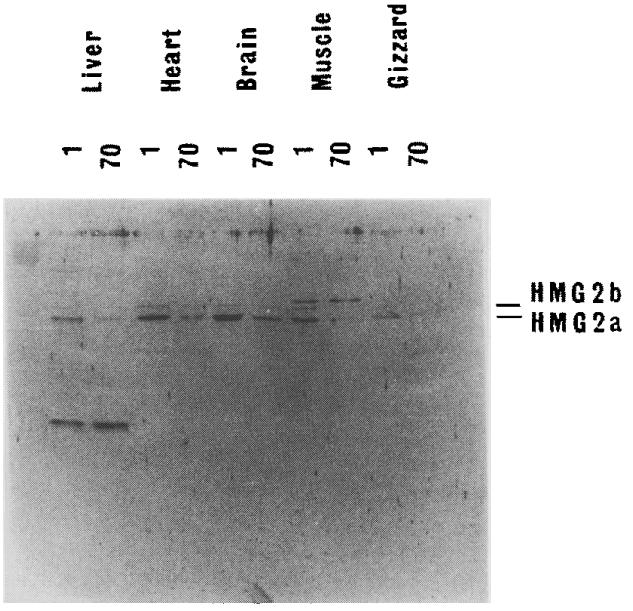

Fig. 1. Western blot analysis of HMG 2 proteins from various tissues in the newly hatched (1) and 70-day-old chicks (70). 20 μg of the homogenate protein from various tissues were electrophoresed on 15% polyacrylamide gel. Western blot analysis was carried out as described in Materials and Methods.

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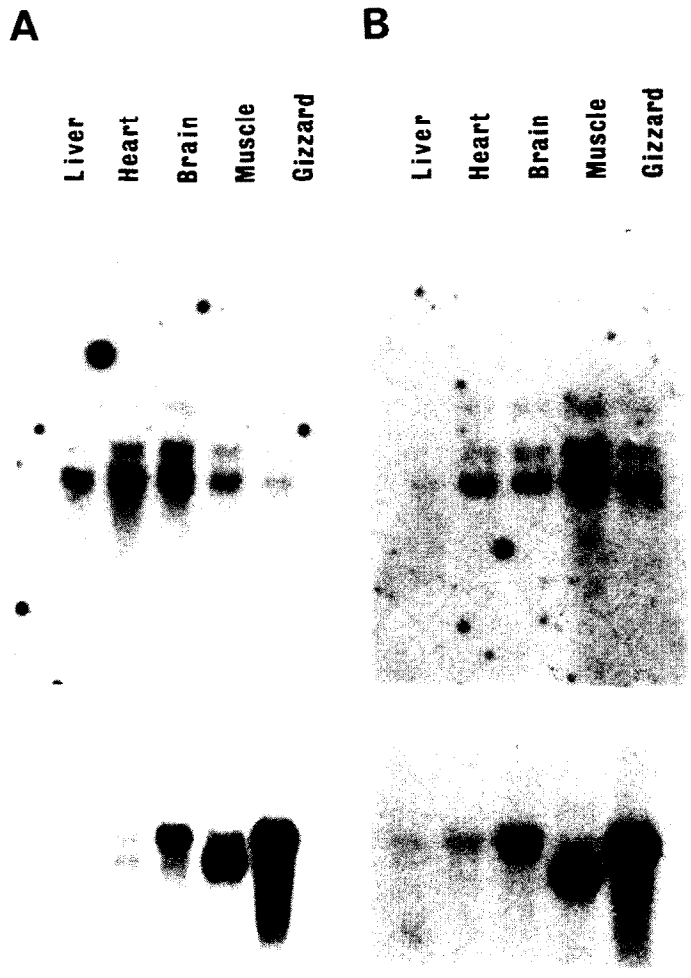

Fig. 2. Northern blot analysis of HMG 2a mRNA from various tissues in the newly hatched (A) and 70-day-old chicks (B). The top panel shows a Northern blot of HMG 2a mRNA and the bottom panel shows β-actin mRNA.

rose gel, blotted to a nitrocellulose membrane and hybridized to the HMG 2a cDNA as described previously [4]. The probes were labeled according to the Random Primed Labelling Kit (Takara Shuzo Co.) protocol. The blot was washed twice in 2 × SSC, 0.1% SDS at 65°C and once in 2 × SSC, 0.1% SDS at 65°C, after which exposed to Kodak X-Omat film at −70°C.

For the slot blotting, total RNA samples were serially diluted, applied with a manifold onto a nitrocellulose filter, hybridized to random primed chick HMG 2a cDNA and autoradiographed.

3. RESULTS AND DISCUSSION

Western blot analysis of the homogenate samples obtained from various tissues of the newly hatched and 70-day-old chicks are shown in Fig. 1. Anti-HMG 2 antibody used in this study cross-reacted with both HMG 2a and 2b proteins as described previously [6]. The levels of HMG 2a protein were higher than those of HMG 2b protein in all tissues and both the proteins levels of heart and brain were high compared with those of the liver, muscle and gizzard in the newly hatched chicks. However, the contents of both proteins in the newly hatched chicks decreased significantly in the 70-day-old chicks. We previously determined the HMG 2b contents in livers quantitatively using ELISA (2.56 ± 0.4 μg/ml DNA for the newly hatched chicks; 1.20 ± 0.2 μg/mg DNA for the 70-day-old chicks) [6]. It thus appears that the HMG 2 levels of all tissues in chicks are downregulated during post-hatched development.

Northern blot analysis of whole poly(A)$^+$ RNA isolated from various tissues of the newly hatched and 70-day-old chicks are shown in Fig. 2. As described previously [4], the 2.0 kb and 1.2 kb transcripts were clearly observed in all the tissues. The liver, heart, and brain of newly hatched chicks were abundant in both transcripts, but their contents of those tissues in 70-day-old chicks decreased. It thus appears that the suppressed expression of HMG 2a mRNA in the course of development leads to the decrease in the level of HMG 2a protein in the chick liver, heart and brain. In contrast, the levels of both transcripts in the muscle and gizzard from 70-day-old chicks increased compared

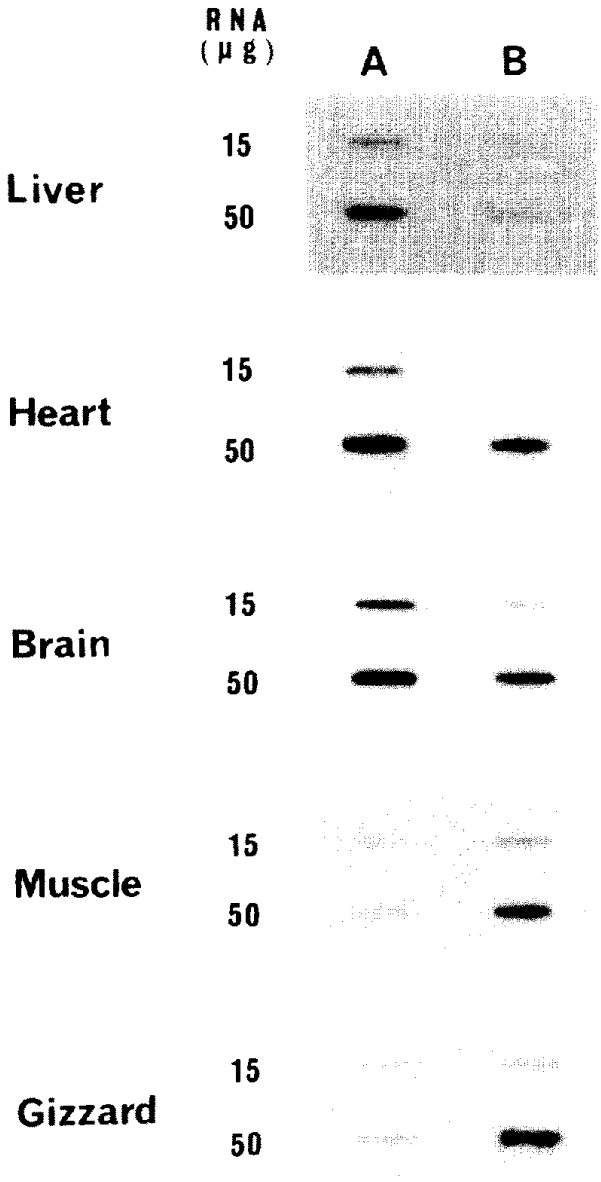

Fig. 3. Slot blot analysis of HMG 2a mRNA from various tissues in the newly hatched (A) and 70-day-old chicks (B). The relative contents of the HMG 2a mRNA in the tissues were estimated by scanning autoradiographs of the slot blot with LKB 2202 Ultro Scan Laser Densitometer.

with those in the newly hatched chicks, although the contents of HMG 2a proteins in the 70-day-old chicks decreased.

We next attempted to quantitate the changes occurring with development by employing a slot blot analysis. As shown in Fig. 3, the HMG 2a mRNA levels of liver, heart and brain in the 70-day-old chicks are remarkably decreased to about 40% of those in the newly hatched chicks while the HMG 2a mRNA levels in muscle and gizzard increased 5- and 3-fold, respectively, compared with those in the newly hatched chicks. The present results suggest that the decrease in the contents of HMG 2a proteins of the muscle and gizzard from 70-day-old chicks are largely attributed to the stimulation of the HMG 2a protein degradation or the reduction of the translation of HMG 2a mRNA.

It seems like that undifferentiated cells synthesize more HMG mRNA than differentiated cells, and the levels of HMG mRNAs are downregulated during cellular development and differentiation [8]. In this sense, the HMG 2a mRNA expression in the muscle and gizzard of 70-day-old chicks is unusual. Recently, Crippa et al. [9] reported that the levels of the mRNA extracted from the red blood cells of 14-day-old chicken embryo was significantly reduced compared with that obtained from 5-day-old embryos but their protein levels quantitatively remained. Crippa et al. [9] proposed that their results may be explained by the suppression of the degradation of the HMG proteins.

At present, it is not clear which regulation on a translation level or a protein turnover level causes a decrease in the contents of the HMG 2a protein in 70-day-old chicks.

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