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Multiple control level governing H1° mRNA and protein accumulation

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We have studied the variation of histone H1° and of its coding mRNA during rat liver regeneration after partial hepatectomy. Our data showed that while H1° decreased when cell proliferation was initiated, H1° mRNA accumulated in a proliferantion-dependent manner as did H3 mRNA. These results showed two interesting aspects of the regulation of H1° expression in vivo, confirming results we have obtained previously in vitro: first H1° mRNA accumulation is a proliferation-dependent event; second, H1° protein accumulation may be uncoupled from that of its coding mRNA.

H3; DNA replication; Hepatectomy; Translational control

1. INTRODUCTION

The H1 histone family is thought to be involved in the stabilisation of nucleosome, the condensation of nucleosomal fibers and the formation of a higher order structure in chromatin [1-2]. Among them, $H1^0$ appears during the terminal stage of differentiation [3-5]. In murine erythroleukemia (MEL) cells for example, the protein accumulates during the induced differentiation [6], and when studying the molecular basis of this induction, we have found that $H1^0$ mRNA accumulation is linked to the DNA replication [7], and also that the accumulation of the protein itself can be uncoupled from that of its coding mRNA [8]. In order to address the same question in a system which is able to differentiate in vivo, we have studied the accumulation of $H1^0$ mRNA and protein during rat liver regeneration after partial hepatectomy. It has been shown previously, that $H1^0$ decreases upon rat liver regeneration [3,9,10]. We have confirmed this observation and provided data showing that, although present in resting rat liver cells, $H1^0$ mRNA accumulated when cell proliferation was initiated after partial hepatectomy like H3 coding mRNA. However, during the same time where $H1^0$ mRNA accumulated, the protein itself decreased. These observations evidenced two interesting aspects of the mechanism controlling $H1^0$ regulation: $H1^0$ mRNA accumulation is a DNA replication-dependent event and moreover there exist a mechanism which uncouples $H1^0$ accumulation from that of its coding mRNA during the proliferation stage of cell differentiation.

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2. MATERIALS AND METHODS

2.1. *Rat partial hepatectomy*

Male Wistar rats were partially hepatectomized as described previously [3].

2.2. *RNA extraction and Northern-blot analysis*

RNA from hepatocyte was prepared from 0.5 to 0.8 g of rat liver as described elsewhere [7,11].

3. RESULTS

3.1. *$H1^0$ protein and mRNA accumulation during rat liver regeneration after partial hepatectomy.*

In order to investigate the variation of $H1^0$ protein and mRNA during rat liver regeneration, rats were partially hepatectomized and after various times, RNA was purified on the one hand and histones were analyzed on the other. Northern blot analysis showed that $H1^0$ mRNA was present in resting adult rat liver cells and surprisingly its amount increased drastically 24 h after partial hepatectomy and this accumulation continued up to 72 h. Histone H3 mRNA was undetectable in adult rat liver cells as well as 10 h after partial hepatectomy; its accumulation could be only observed 24 h after surgery, showing the absence of any cell proliferation at least before 10 h. Significant amounts of H3 mRNA had accumulated at 48 h and 72 h indicating the existence of important cell multiplication at those times. $H1^0$ mRNA accumulation occurred simultaneously with that of H3 mRNA, suggesting that, like H3 mRNA, $H1^0$ mRNA accumulation is linked to DNA

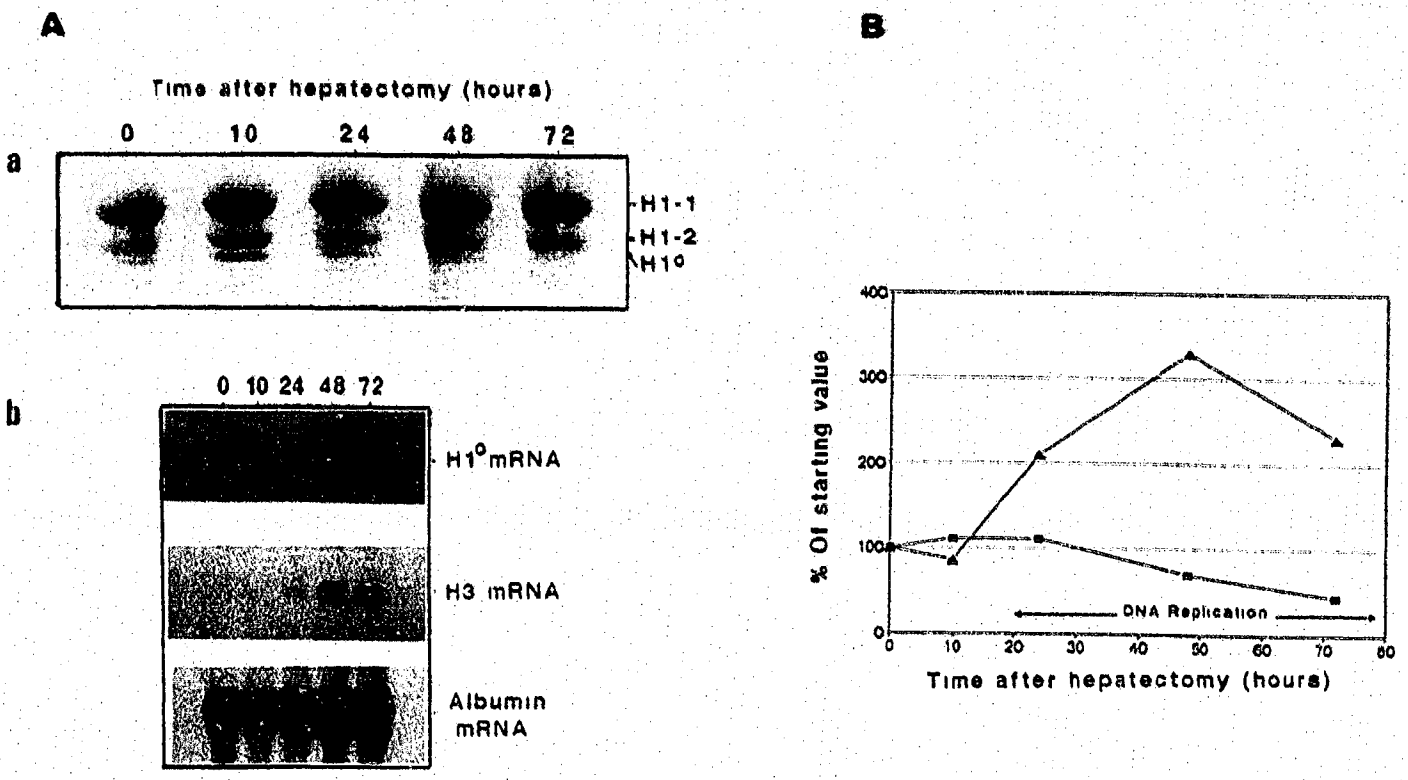

Fig. 1. Variation of the amount of $H1^0$ protein and mRNA during rat liver regeneration after partial hepatectomy. Rats were partially hepatectomized and the regeneration of liver was allowed to proceed for the indicated time. (A) Nuclear proteins were extracted from rat liver (time 0) or rat liver after surgery (10, 72 h), analyzed on 15% SDS-PAGE and staine with Amido black. The position of H1-1, H1-2 and $H1^0$ is indicated (a). RNA was purified from the above samples and 50 μg of purified RNA was used to obtain a Northern blot which was then probed with ^{32}P-labeled $H1^0$, H3 and albumin probes (b). (B) Densitometric analysis of $H1^0$ protein (■) and mRNA (▲) variation. $H1^0$ expressed as % of total H1 and values for $H1^0$ mRNA and protein are represented as % of the starting value. Arrows indicate the DNA replication period as judged by the expression of H3 mRNA between 24 and 72 h.

replication. During the period examined the amount of albumin mRNA increased slightly. In the above experiment we have confirmed the previously reported decrease of $H1^0$ [3,10] and found that the amount of $H1^0$ protein decreased after 24 h and continued to decrease up to 72 h after partial hepatectomy. The fact that the amount of $H1^0$ mRNA accumulated during this period demonstrates clearly that the accumulation of $H1^0$ mRNA and protein was uncoupled.

4. DISCUSSION

This paper provides two major facts about the regulation of $H1^0$ accumulation. First, $H1^0$ mRNA accumulation appears to be a proliferation-dependent event. When investigating the variation of $H1^0$ mRNA as a function of the position of cells in the cell cycle in MEL cells, we have shown previously, that $H1^0$ mRNA accumulated in a DNA replication-dependent manner [7]. Here we confirmed this phenomenon in vivo in rat hepatocytes. Although $H1^0$ mRNA was present in resting adult rat liver cells, it accumulated simultaneously with H3 mRNA after partial hepatectomy. $H1^0$ mRNA accumulation is thus linked to DNA replication as is accumulation of H3 mRNA.

We then showed that $H1^0$ protein accumulation can be uncoupled from that of its coding mRNA. Indeed we [3], and others [10] have reported previously that the amount of $H1^0$ decreased and reached a minimum at 50–65 h after surgery. We have confirmed these data in the present study, and have shown that, nevertheless, the amount of $H1^0$ mRNA increased during this period.

A difference between the accumulation of $H1^0$ and its coding mRNA has also been observed in vitro during the induced differentiation of MEL cells [8]. These results and those presented previously in MEL cells [7,8] can account for the following model. The accumulation of $H1^0$ mRNA, similar to that of the other histone-coding mRNAs is coupled to DNA replication. Therefore $H1^0$ mRNA is mainly produced during the proliferation phase of cell differentiation and can be used to direct $H1^0$ synthesis when needed by cells, eventually when cells are arrested. If this is true, cells need a strong control for uncoupling $H1^0$ mRNA from that of $H1^0$ which should not accumulate during cell proliferation.

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