Tissue-specific and differential expression of prothymosin α gene during rat development

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We have analyzed the RNA expression of prothymosin α (ProTα) gene during rat development in several tissues and compared it to that of two proteins related to cell proliferation: proliferating cell nuclear antigen (PCNA)/cyclin and histone H3 (H3). The expression of ProTα gene was found to be regulated in a developmental and tissue-specific manner. The mRNA levels of ProTα followed a similar time-course in liver, brain, kidney, and testis, being highly increased in the early periods of postnatal development. However, in thymus ProTα mRNA showed only moderate changes throughout development. Our findings suggest that ProTα participates in developmental processes like cell proliferation and/or differentiation.

Prothymosin α; Development; Gene expression

1. INTRODUCTION

Prothymosin α is a highly acidic (pI = 3.55) polypeptide of 12.500 Da that has been traditionally implicated in various aspects of immune function [for review, see 1]. Nevertheless, several investigations have questioned such immunological role based on its wide tissue distribution [2], its broad phylogenetic presence from yeast to man [3] and the absence of a signal peptide [4]. Moreover, since we postulated a nuclear location for ProTα [5], several contradictory data on its subcellular distribution have been reported, indicating either nuclear [6,7] or cytoplasmic localization [3,8]. However, microinjection of ProTα into the cytoplasm of *Xenopus* oocytes showed that this protein indeed migrates to the nucleus [6]. On the other hand, structural and functional studies on ProTα gene expression suggested that this protein could have an intracellular role tied to cell proliferation [4,9]. In this sense, we have shown that ProTα mRNA is present in a wide spectrum of adult rat tissues and that its levels change with T-lymphocyte proliferation and maturation [10].

In the present work we analyzed the developmental expression of the ProTα gene in a normal physiological context to determine whether this gene is regulated in a tissue-specific manner and/or parallels the proliferative activity of cells. Expression of ProTα gene in normal ontogenic processes was studied in tissues of lymphoid (thymus), neural (brain), genital (testis) and general type (liver and kidney), which present different growth patterns during development. Concurrently, we analyzed the gene expression of two evolutionary conserved nuclear proteins related to cell proliferation: PCNA/cyclin, the auxiliary factor for DNA polymerase δ [11,12] and histone H3, which is a structural component of the nucleosome core [13]. Our results revealed that ProTα gene is regulated following a developmental and tissue specific program. Comparison with PCNA/cyclin and H3 showed that the three genes present the same temporal pattern of expression during development except in testis, where only PCNA/cyclin is highly activated after the onset of puberty.

2. MATERIALS AND METHODS

RNA preparation, Northern blots and hybridization analyses were carried out as previously described [10]. After hybridization, the blots were washed twice in 2× SSPE, 0.5% sodium dodecyl sulphate at room temperature for 10 min each and twice in 0.1× SSPE, 0.5% sodium dodecyl sulphate at 65°C for 30 min each. The filters were air-dried and exposed to X-ray films at −80°C in the presence of intensifying screens. After the first hybridization, the probe was removed by boiling in 0.1% sodium dodecyl sulfate, 5 mm EDTA and the blots sequentially hybridized with the PCNA/cyclin, H3 and COII probes. The probes were a 1.15-kb *Eco*RI fragment containing the human ProTα cDNA [10], a 1.12-kb *Bam*HI fragment containing the human PCNA/cyclin cDNA [14], a 2.5-kb *Eco*RI-*Hind*III fragment containing the human H3 gene [15], and a 5.2-kb plasmid containing the rat COII cDNA [16]. Exposure times of autoradiograms were 2 days for ProTα, 6 days for PCNA/cyclin and H3 and 12 h for COII, thus direct comparison in the figures between band intensities can be made only within each panel.

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3. RESULTS AND DISCUSSION

Expression of ProTα, PCNA/cyclin, and H3 genes in normal ontogenic processes was analyzed in liver, brain, thymus and testis. In addition, we used the mitochondrial cytochrome c oxidase subunit II (COII) as a control probe to exclude that differential expression of the genes were due to altered amounts of RNAs. The levels of each mRNA at the different developmental stages were determined by electrophoresis/blotting procedures for sequential hybridization to the four probes mentioned. The relative levels of every transcript were quantitatively evaluated by densitometric scanning of autoradiograms and expressed as a percentage of its level in the stage in which the expression was lowest.

We first studied the expression of these genes in rat liver during pre- and postnatal development (Fig. 1). The highest levels of ProTα mRNA were detected in fetal liver at 18 days of gestation decreasing in 20 day fetus and newborn. Densitometric analysis of autoradiograms showed more than 20-fold less ProTα mRNA in the later developmental stages. A very similar pattern was found for PCNA/cyclin and H3 mRNAs, with approximately 10-fold higher expression in the 18 day fetus compared to postnatal and adult liver. The analogy of the developmental profiles of ProTα, PCNA/cyclin and H3 indicated a relationship between cellular proliferation during liver ontogeny and the ProTα mRNA content. In this sense, we have found that ProTα is activated in adult rats during liver regeneration after partial hepatectomy (manuscript in preparation), although less than in the initial periods of development. Thus, the difference of ProTα expression between fetal and adult liver could be also attributed to the presence of differentiating hematopoietic cell populations in the fetal organ.

In developing brain, ProTα mRNA levels were 8-fold higher in newborn as compared to adult, showing a common pattern to those of PCNA/cyclin and H3 transcripts (Fig. 2). Similarly to liver, the PCNA/cyclin and H3 mRNAs were barely detectable after 2 weeks of age whereas ProTα mRNA was observable even at 48-week-old rats. During the ontogeny of rat nervous system, some neuron populations and a large number of glial precursor cells continue to proliferate after birth [17]. Thus, our hybridization results evidence this active cell proliferation in neonates brain. In kidney, we have found a common developmental pattern to that of liver and brain (data not shown). The downregulation during development of PCNA/cyclin and H3 genes in brain, kidney and liver could be explained by the replacement of active cell divisions for hypertrophy as a major means of growth since both proteins are tightly related to cell proliferation. Accordingly, the similar results found for ProTα gene reveal that its expression is also linked to proliferative activity of cells from different origins.
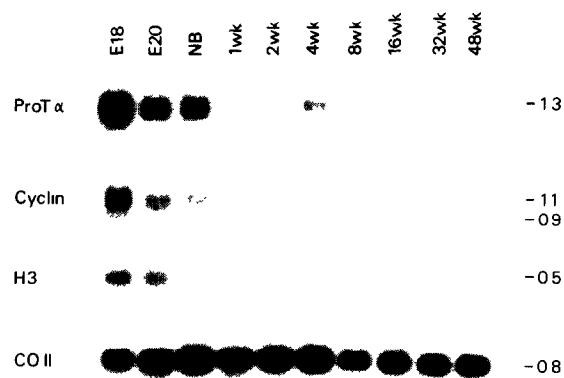

Fig. 1. Stage-specific expression of ProTα, PCNA/cyclin, H3 and COII genes in rat liver during pre- and postnatal development. Total cellular RNA was prepared from Sprague-Dawley rat liver at 18 (E18) and 20 (E20) embryonic days and the indicated postnatal weeks (WK). Ten micrograms of RNA were applied to a denaturing formaldehyde-agarose gel. The same Northern blot was sequentially hybridized to the ^{32}P-labeled ProTα, PCNA/cyclin, H3 and COII probes (sp.act. $1-2 \times 10^8$ cpm·μg^{-1}). The sizes (kb) of the detected transcripts are indicated on the right.

Contrary to the other tissues, in thymus the relative amounts of ProTα, PCNA/cyclin and H3 mRNAs did not show major changes during thymic postnatal development (Fig. 3). A moderate increase was detected, mainly in the PCNA/cyclin mRNA, at the age where thymus reaches its maximum size. The descent in the levels of H3 mRNA at the 48-week stage is striking compared to what was found with the other probes. The high expression of these genes from birth onwards is not associated with the involution of this organ after puberty, and can be related to the presence of exogenously precursor cells that colonize this tissue starting to proliferate [18] and to an intrathymic pool of self-renewing precursor cells that maintains its lymphoid component
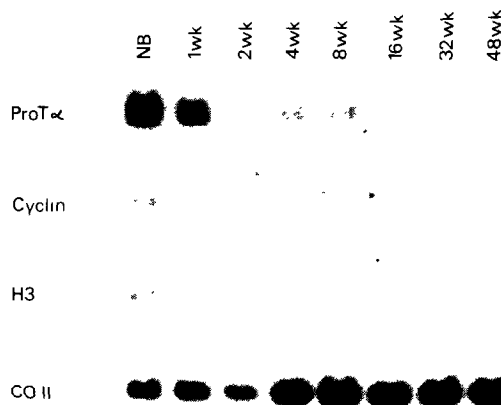

Fig. 2. Stage-specific expression of ProTα, PCNA/cyclin, H3 and COII genes in rat brain during postnatal development. Total cellular RNA (10 μg) from rat brain at the indicated postnatal weeks (WK) were hybridized to ProTα, PCNA/cyclin, H3 and COII probes. For further details see legend to Fig. 1.
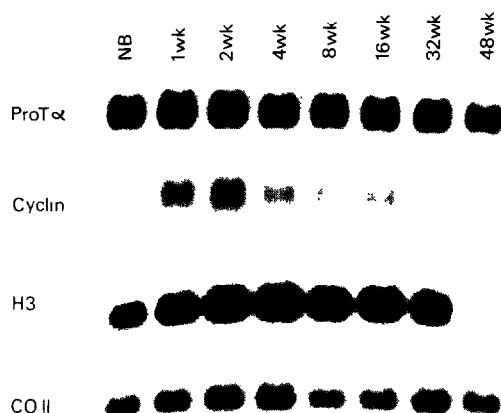
Fig. 3. Stage-specific expression of ProTα, PCNA/cyclin, H3 and COII genes in rat thymus during postnatal development. Total cellular RNA (10 μg) from rat thymus at the indicated postnatal weeks (WK) were hybridized to ProTα, PCNA/cyclin, H3 and COII probes. For further details see legend to Fig. 1.

[19]. Interestingly, other developmentally regulated genes related to cell proliferation, like the *myc* [20], *myb* and *ets* [21] protooncogenes, were also seen not to vary with thymus ageing.

In testis, the levels of ProTα and H3 mRNAs during the first 2 weeks of age were more than 17-fold greater than in adults showing an abrupt decrease coincident with the onset of puberty (Fig. 4). PCNA/cyclin mRNA also presented a similar pattern in the prepubertal stages but a dramatic rise was observed at puberty, remaining high in the postpubertal periods. The sizes of ProTα, H3 and PCNA/cyclin mRNAs were the same as in the nongerminal tissues (Fig. 1). However, the 1.1 and 0.9 kb PCNA/cyclin mRNAs, which use different polyadenylation signals [22], followed distinct regulation programs: while the 1.1 kb mRNA was the most
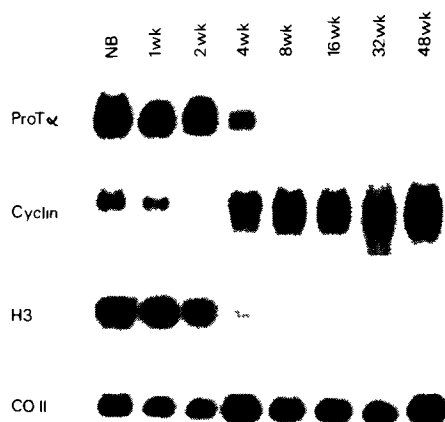

Fig. 4. Stage-specific expression of ProTα, PCNA/cyclin, H3 and COII genes in rat testis during postnatal development. Total cellular RNA (10 μg) from rat testis at the indicated postnatal weeks (WK) were hybridized to ProTα, PCNA/cyclin, H3 and COII probes. For further details see legend to Fig. 1.

abundant in all tissues and prepubertal testis, the concentration of both mRNAs was quite similar in the adult testis (Fig. 4). After birth, testis cell populations are mostly represented by self-renewing type A spermatogonia and by proliferating immature Sertoli cells [23]. Accordingly, the expression of ProTα, PCNA/cyclin and H3 genes in the first 2 weeks of age correlates with the proliferative state of testis. It is noteworthy that testis was the only organ in which ProTα and H3 displayed different developmental patterns compared to PCNA/cyclin. The remarkable increase in the expression of PCNA/cyclin gene after puberty indicates that this protein is needed in spermatogenesis. On the other hand, it is well-known that during spermatogenesis a dramatic change in chromatin structure, involving a complete replacement of nucleosomal histones by protamines, occurs in many species; concurrently, virtually all the nonhistone nuclear proteins are eliminated [24]. Thus, the low expression of ProTα and H3 genes in adult testis would reflect this exchange of nuclear proteins during spermatogenesis.

In this study we demonstrated differential transcriptional activity of ProTα gene during rat development. The mRNA levels of this protein followed a similar time-course to that of PCNA/cyclin and H3 in liver, brain, kidney, thymus and prepubertal testis accounting for the proliferative activity of cells in such tissues during postnatal development. Since ProTα gene is dramatically activated during development at stages containing many proliferating but also differentiating cells, a possible role in cellular differentiation deserves consideration. Future studies on the regulation of ProTα gene during development will help to the understanding of its biological role.

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