# Prothymosin α mRNA levels vary with c-myc expression during tissue proliferation, viral infection and heat shock

Katerina Vareli, Maria Frangou-Lazaridis*, Orestes Tsolas

*Laboratory of Biological Chemistry, University of Ioannina Medical School, GR-451 10 Ioannina, Greece*

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**Abstract Expression of prothymosin α, an acidic nuclear protein implicated in cellular proliferation, has been reported to be regulated by c-myc in vitro. We have studied the correlation of expression levels between prothymosin α and c-myc, using three different in vivo systems, viz. normal ontogenic process of placental development, lytic viral infection and heat shock treatment. The two genes have been found to share a similar expression pattern, although prothymosin α mRNA remains always detectable, indicating the existence of yet another mechanism, in addition to c-myc, which regulates its expression in vivo.**

*Key words:* Prothymosin α; c-myc; Cellular proliferation; Human placenta; Adenovirus; Heat shock

## 1. Introduction

Prothymosin α (ProTα) is a small, 12.5-kDa, highly acidic (pI 3.5) protein [1] localized in the nucleus [2–4]. The isolation of ProTα from a number of vertebrate species [1,5–7], its wide tissue distribution [6–9] and its high evolutionary conservation [10–11] indicate an important role for the protein in the cell. Although this role remains to be elucidated, accumulated evidence supports a function related to proliferation events. For example, ProTα gene expression was found to be elevated in proliferating relative to quiescent cells [12–14] and ProTα antisense oligomers were able to inhibit cell division in myeloma cells [15]. Moreover, ProTα levels were reported to be elevated in malignant cells and tissues compared with healthy ones, indicating its possible participation in the tumorigenic process [16–18].

The view that ProTα is involved in proliferation is strongly supported by the finding that activation of c-myc directly induces transcription of the ProTα gene in vitro [19]. This induction was reported to be due to the consensus binding site for c-myc (E box) that exists in the first intron of the ProTα gene [20]. It is well-established that c-myc is the most prominent nuclear oncogene and its implication in the control of cellular proliferation possibly involves the induction of ProTα [19–21].

To investigate the correlation between ProTα and c-myc we studied the expression of the two genes during normal ontogenic process, viral infection and heat shock treatment. In all systems, ProTα gene expression variations coincided in time with those of c-myc, but ProTα mRNA could be detected even after c-myc had ceased to be expressed. This pattern of expression under physiological conditions, as well as under stress conditions, indicates that the oncogene could influence ProTα gene expression in vivo but also suggests multiple levels of regulation for ProTα.

## 2. Materials and methods

### *2.1 Cell culture, viral infection and thermal treatment*

F9, CV1 and HeLa cells were grown as monolayer in Dulbecco's modified Eagle's medium, DMEM, supplemented with 10% fetal calf serum (FCS). Daudi cells were cultured in suspension in Roswell Park Memorial Institute medium, RPMI 1640, supplemented with 20% FCS. F9 cells were induced to differentiate with retinoic acid ($10^{-7}$) and dibutyryl c-AMP ($10^{-3}$) as described [22]. Viral infections were carried out with adenovirus type 5 at a multiplicity of 50 pfu/cell in DMEM without serum. After 90 min of virus absorption, the cells were fed with complete medium and allowed to grow. Thermal treatment was performed by placing the cells in a water bath set at 42.5°C for the indicated time points, while the control cells remained at 37°C

### *2.2 RNA isolation, Northern analysis and dot-blot analysis*

Total RNA was extracted by the acid guanidinium/phenol/chloroform method as described by Chomczynski and Sacchi [23] For Northern analysis, aliquots of 15 μg RNA were fractionated on 0.8% agarose-2.2 M formaldehyde gels and transferred overnight to nylon membrane (Hybond N, Amersham). For dot-blot analysis, 15 or 30 μg RNA was immobilized on nylon membrane [9]. Prehybridization, hybridization and washes were carried out as described [9]. The c-DNA probes employed for detection were a 1200-bp *Eco*RI-*Eco*RI fragment for ProTα [10], a 400-bp *Pst*I-*Pst*I fragment for c-myc second exon, a 1460-bp *Eco*RI-*Sac*I fragment for E1A, and a 2460-bp *Eco*RI-*Eco*RI fragment for heat-shock protein 70 (Hsp 70), labeled with [$^{32}$P]dCTP by random priming [24].

## 3. Results and discussion

Since c-myc is known to influence cellular proliferation processes [19,21], the possibility exists that the involvement of ProTα in proliferation may be related to the induction of the ProTα gene by c-myc [19]. To test this hypothesis, we examined ProTα expression under conditions that are known to influence c-myc expression. ProTα and c-myc gene expression were initially analysed during placental development The levels of each mRNA at different developmental stages were determined by dot-blot analysis. In developing placenta, the highest levels of ProTα mRNA were detected at 5 weeks, while high levels were also detectable at 6 weeks of gestation (Fig. 1, upper panel). Considerable decrease was observed in 8 weeks, while in full-term placenta ProTα mRNA levels were very low, but still detectable (Fig. 1, upper panel). Expression of c-myc mRNA was also high at 6 weeks but undetectable in full-term placenta (Fig. 1, lower panel), in agreement with reported data that relate the appearance of c-myc to the proliferation activity of the tissue [25]. Our findings thus establish a parallel between the expression of the two genes in normal tissue development and strengthen reports supporting a similar correlation in the case of colon cancers [18].

The fact that proliferative events are often inversely corre-

*Corresponding author. Fax: (30) (651) 33442/32602.


*SSDI* 0014-5793(95)00938-8

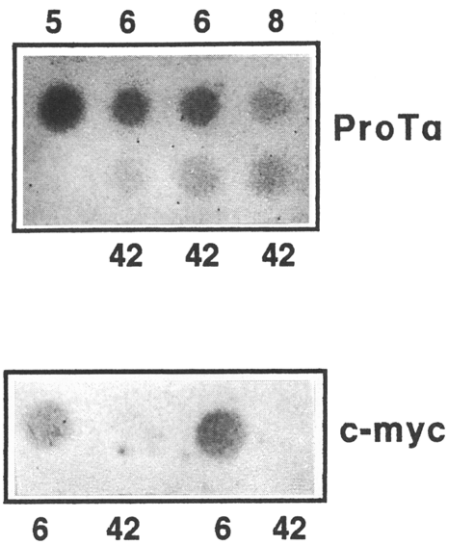


Fig. 1. Upper panel: dot-blot analysis of 15 μg total RNA, prepared from developing and full-term placenta at indicated weeks of pregnancy, using ProTα cDNA probe Lower panel: dot-blot analysis of 15 and 30 μg total RNA prepared from placenta at 6 weeks and full-term using c-myc cDNA probe for detection.

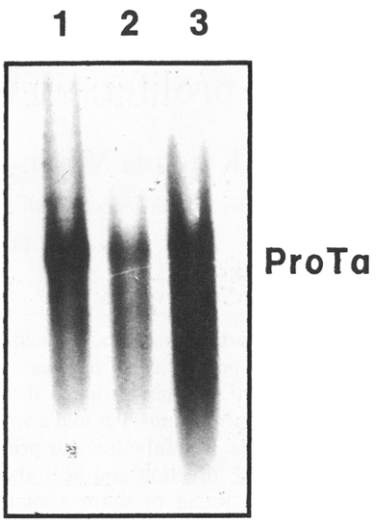


Fig. 2 Equal amounts of total RNA (15 μg) prepared from F9 cells (lane 1), differentiated F9 cells (lane 2) and differentiated F9 cells infected with adenovirus type 5 (lane 3), were separed on an agarose-formaldehyde gel, blotted to nylon membrane and hybridized with ProTα cDNA probe

lated with differentiation led us to examine ProTα mRNA levels in rat embryonal carcinoma, F9, cell differentiation. In this system, c-myc expression is known to be repressed [26]. Using Northern analysis we observed a significant decrease in ProTα mRNA levels in terminally differentiated F9 cells compared to the undifferentiated control cells (Fig. 2). It should be noted that lower levels of ProTα and c-myc mRNA have also been detected in human myeloid leukemic HL-60 differentiated cells [27–28]. Therefore, it seems likely that the link between

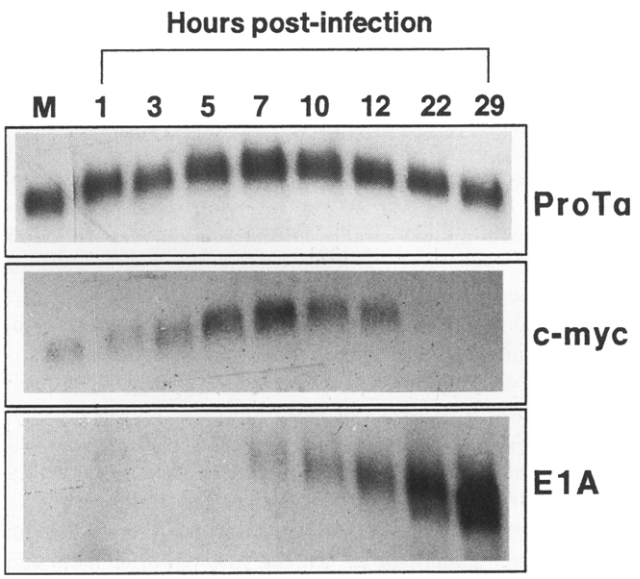


Fig. 3. HeLa cells were infected with adenovirus type 5 and total RNA was prepared at the indicated times post-infection. Total RNA was also prepared from mock infected cells (lane M). Equal amounts of RNA (15 μg) were analysed by Northern blotting utilizing the ProTα and c-myc cDNAs as probes. The same samples were hybridized to an *Eco*RI-*Sac*I fragment for the E1A region of adenovirus to monitor the progression of viral infection.

ProTα expression and differentiation is a consequence of the role of the protein in cell proliferation. To test this hypothesis we investigated ProTα mRNA expression after viral infection of differentiated F9 cells with a DNA tumor virus, since it is known that viral infections transmit proliferation signals to the otherwise terminally differentiated cells [22]. Using Northern analysis we observed that adenoviral infection indeed restores ProTα mRNA to the original undifferentiated levels (Fig. 2). This result indicates once again that restoration of proliferation leads to increased ProTα mRNA expression.

In an effort to achieve a more detailed study along these lines, we examined ProTα and c-myc mRNAs during the course of adenoviral infection of HeLa cells. RNA samples were collected at various time points post-infection and analysed by Northern blotting (Fig. 3). The detection of the early region 1A (E1A) of the virus was used to monitor the progression of viral infection. E1A mRNA was detectable at 7 h and its levels were further induced as expected during the course of the lytic infection (Fig. 3). ProTα mRNA was detected in both infected and control cells with a transient increase at 7 h in infected cells, and retained elevated levels even after 29 h of infection (Fig. 3). On the contrary, c-myc mRNA, which was strongly induced at the time point corresponding to the maximum levels of ProTα mRNA, i.e. 7 h post-infection, was found to be reduced to undetectable levels after 22 h of infection (Fig. 3). In conclusion, our results show that maximum levels of ProTα and c-myc mRNA occur at the same time point of the lytic cycle, but ProTα mRNA continues to be expressed late in infection, with a modest overall decrease.

Since viral infection can be viewed as a kind of stress, we studied the response of ProTα and c-myc genes to heat shock, utilizing two cell lines of different origin, viz. Daudi human B cells and CV1 monkey kidney cells. The above cell lines were incubated at 37°C (control) or at 42.5°C for various time periods and the mRNAs for ProTα and c-myc were analysed by Northern blotting (Fig. 4). In Daudi cells, the ProTα mRNA

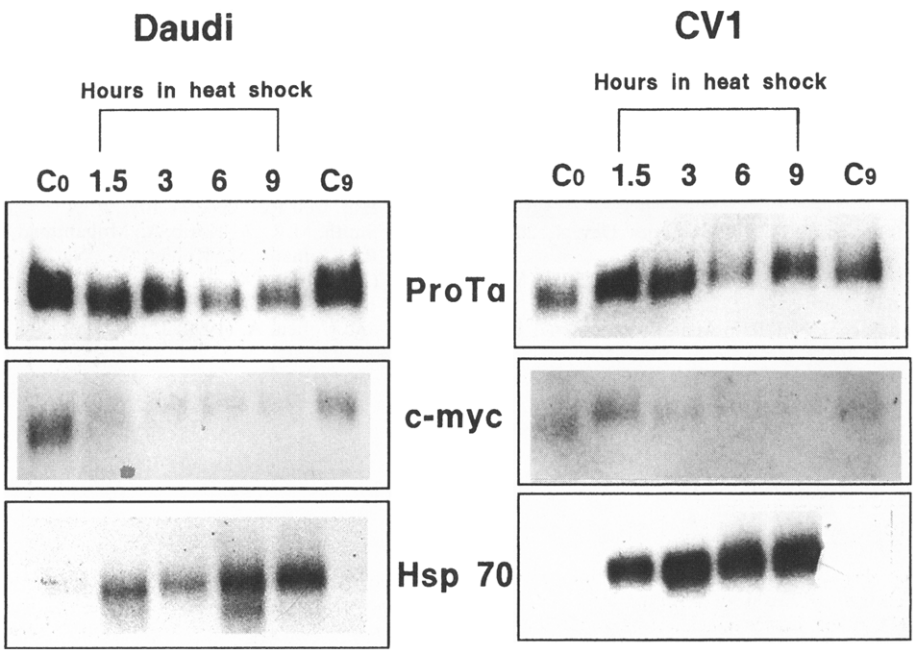


Fig. 4. Daudi and CV1 cells were heat shock treated at 42.5°C for the indicated time periods and total RNA was prepared. In both cases, total RNA was also prepared from control cells at zero time (lane C) and 9 h (lane C) of incubation at 37°C. Equal amounts of RNA (15 μg) were analysed by Northern blotting utilizing ProTα, c-myc and Hsp 70 cDNAs as probes.

accumulation was found to drop gradually after heat shock (Fig. 4). A significant decrease was observed after 6 h reaching a plateau at 9 h (Fig. 4). A different pattern of expression was observed in CV1 cells. ProTα mRNA was induced within the first 1.5 h, but the reduction observed at 6 h eventually declined to control levels (Fig. 4). Although the ProTα expression pattern was different in the two cell lines examined, its correlation to c-myc expression was retained. We found that thermal treatment of Daudi cells reduces c-myc mRNA below detection (Fig. 4), in agreement with Bukh et al. [29]. Similarly, the initial increase of c-myc mRNA observed in CV1 cells, within the first 1.5 h of heat treatment, was followed by a sharp reduction resulting in undetectable levels as early as 3 h of heat shock (Fig. 4). The Hsp 70, examined as a control, was found to be induced in both cases within the first 1.5 h as expected (Fig. 4).

In conclusion, ProTα mRNA is found to be present at various levels in all times. Nevertheless, the amount of ProTα increases under conditions that induce transcription of c-myc. Our results thus establish an apparent correlation between ProTα and c-myc mRNA expression during normal tissue development, cell differentiation, viral infection and heat shock. Our data support the hypothesis that transcription of ProTα gene is associated with that of c-myc and could be under its control. However, our findings utilizing in vivo systems show that ProTα is also expressed even in the absence of c-myc expression. Therefore, we suggest that, in addition to the c-myc transcriptional induction, ProTα expression is maintained in vivo by additional, although not yet elucidated, regulatory mechanisms.

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