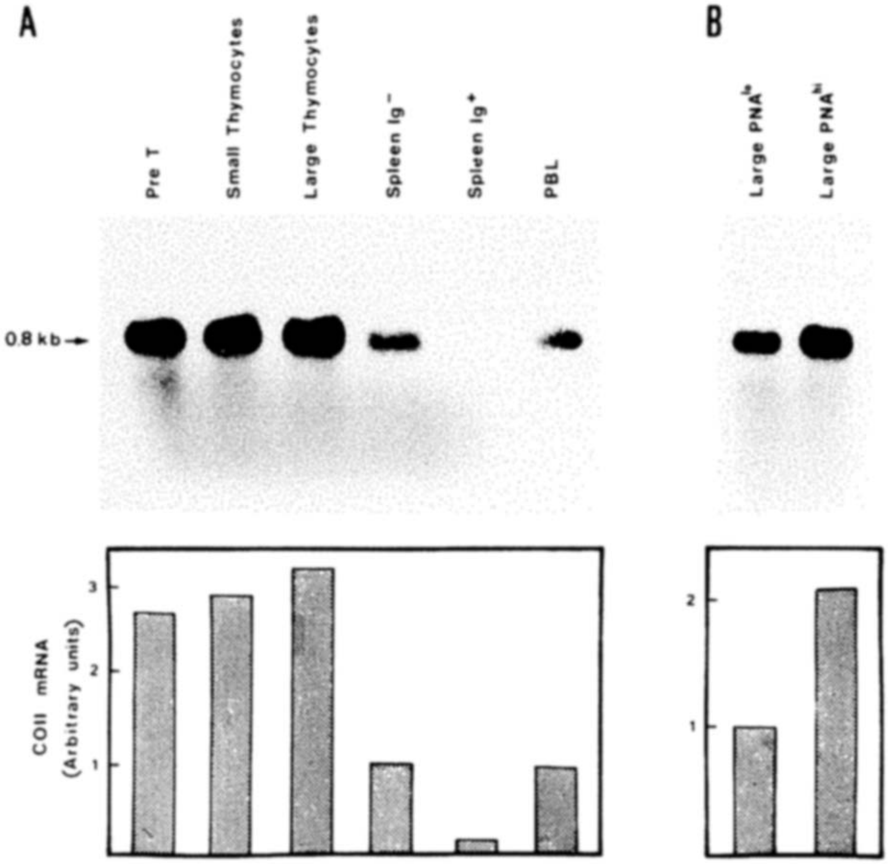

Fig. 1. (A) Analysis of RNA from T-cells at different stages of T-cell development. 15 μg of total RNA from bone-marrow pre-T cells, small thymocytes, large thymocytes, splenic Ig^- lymphocytes, splenic Ig^+ lymphocytes and peripheral blood lymphocytes were electrophoresed in a 1.5% agarose/formaldehyde gel, transferred to nitrocellulose, and probed with $5 \cdot 10^6$ $\text{cpm} \cdot \text{ml}^{-1}$ of the nick-translated PstI fragment of a rat COII cDNA. (B) Analysis of RNA from large thymocytes fractionated by PNA binding. 15 μg of total RNA from PNA^{lo} and PNA^{hi} thymocytes were fractionated electrophoretically in an agarose/formaldehyde gel and hybridized to the nick-translated COII cDNA. Hybridizations were carried out at 42° C for 20 h in 10 ml of a solution containing 50% formamide, 5 × SSPE (1 × = 0.18 M NaCl, 10 mM sodium phosphate buffer (pH 7.4), 1 mM EDTA), 2 × Denhardt's (1 × = 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin), 10% dextran sulphate, 0.1% SDS and 100 $\mu\text{g} \cdot \text{ml}^{-1}$ denatured sonicated salmon testis DNA. Filters were washed twice at room temperature in 2 × SSPE, 0.5% SDS, three times at 65 ° C in 0.1 × SSPE, 0.5% SDS, and autoradiographied 6.5 h at −70 ° C with intensifying screens (DuPont Cronex Lighting Plus). Upper panels show the Northern blot analyses of the RNAs, and lower panels the quantitation of COII mRNA levels by densitometric analysis. PBL, peripheral blood lymphocytes.

ous divisions, were found to contain about 2-fold more COII mRNA than PNA^{lo} mature thymocytes (Fig. 1B). All these results indicate that the COII mRNA levels decrease from immature cells (pre-T-cells, small thymocytes and immature thymocytes) to mature T-cells (mature thymocytes, splenic Ig^- lymphocytes and circulating lymphocytes).

Previous reports have shown that several enzymes change during T-cell development. Thus, adenosine deaminase, deoxycytidine kinase, and terminal deoxynucleotidyltransferase are maximal in immature thymocytes and decrease in the final steps of T-cell differentiation [14–17]. In contrast, purine nucleoside phosphorylase increases in the course of T-cell maturation [14,18]. In the present study we have demonstrated that the COII mRNA levels decrease throughout the maturation of T-lymphocytes. This pattern of expression could be explained on the basis of the intense proliferative activity occurring within the thymus which would require an elevated oxidative metabolism. In this sense, the expression of proliferating-cell nuclear antigen/cyclin and prothymosin α, two proteins linked to cell proliferation [19,20], was found to be similar in rat thymocytes [7] to that observed for COII mRNA. However, the presence of relative high levels of COII mRNA in small thymocytes, a non-proliferative cell population, does not agree with such a hypothesis. Furthermore, it has been found that variations in COII mRNA are independent of proliferative activity in 3T3 fibroblasts [6] and GH_4C_1 cells [5], and that cytochrome *c* oxidase activity in neurons is correlated with ion pumping rather than with cell growth [4]. On the other hand, proliferative and non-proliferative thymocytes share higher deoxyribonucleoside triphosphate levels than mature T-lymphocytes [21]. It has been suggested that the higher ratio of adenosine deaminase to purine nucleoside phosphorylase activities in thymocytes compared to mature cells may be partially responsible for maintaining the intracellular levels of deoxyribonucleoside triphosphates in thymocytes [22]. In this view, the cytochrome *c* oxidase activity could supply the energy requirements for the generation and maintenance of these pools.

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