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The human prothymosin α gene is polymorphic and induced upon growth stimulation: Evidence using a cloned cDNA

(thymosin α_1/signal peptide/mitogen-stimulated lymphocyte)

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ABSTRACT **Clones for human prothymosin α have been identified in cDNA libraries from staphylococcal enterotoxin A-stimulated normal human lymphocytes and from simian virus 40-transformed fibroblasts. The 1198-base-pair fibroblast clone has been sequenced. The encoded protein is highly acidic (54 residues out of 111) and shares >90% sequence homology with rat prothymosin α. The peptide "hormone" thymosin α_1 appears at positions 2–29 of the prothymosin α amino acid sequence. There is no N-terminal signal peptide. Examination of mouse and human tissues revealed the presence of prothymosin α mRNA in kidney, liver, spleen, normal lymphocytes (predominantly T cells), human T-cell leukemia virus-infected T cells, and myeloma cells (B-cell lineage). Prothymosin α mRNA is inducible; upon mitogen stimulation it increased >15-fold above the level found in resting lymphocytes. Similarly, serum-deprived NIH 3T3 cells responded to serum restitution with an increase in prothymosin α mRNA. Characterization of human genomic DNA by Southern blot analysis disclosed a complicated pattern consistent with genetic polymorphism. These data suggest that prothymosin α plays an intracellular role tied to cell proliferation. There is no evidence that it serves as a precursor for secreted thymic peptides. However, given the complexity at the genomic level, multiple functions, including a putative secretory capability, cannot be excluded.**

The thymus gland is the source of three related, highly acidic peptides: thymosin α_1, thymosin α_{11}, and des-(25–28)-thymosin α_1 (1, 2). These hormones or hormone-like materials are derived in the rat from a polypeptide precursor containing 113 amino acids known as prothymosin α (3). The mature peptides from several sources, either together with other members of a crude, heat-stable, thymic extract identified as thymosin fraction 5 (4–6) or, in some cases, singly, have been reported to restore, maintain, or enhance immune function, to protect against opportunistic infections, and to play a regulatory role in the differentiation of T lymphocytes (reviewed in refs. 6–10; also refs. 11–13). Thymosin α_1 and thymosin fraction 5 have also been used in the treatment of cancer (14, 15). Because thymosin α_1, a peptide with 28 amino acids, and the gag protein of human T-cell leukemia virus, type III (HTLV-III) [lymphadenopathy-associated virus (LAV) or human immunodeficiency virus (HIV)], demonstrate sequence homology, some investigators feel that this material may be useful in developing a vaccine for acquired immunodeficiency syndrome. In support of this view, the homologous region has been shown to be critical for HIV virus replication *in vitro* (16).

Here we report the sequence of a nearly full-length cDNA clone of human prothymosin α. We have also investigated the tissue distribution of prothymosin α mRNA and the regulation of expression of the gene, using hybridization techniques. As a result of these data we propose that elevated levels of prothymosin α mRNA, and, by inference, prothymosin α, play a role in cell proliferation.

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MATERIALS AND METHODS

Preparation of a cDNA Library from Human Lymphocytes. Cytoplasmic mRNA was isolated from human lymphocytes stimulated for 48 hr with staphylococcal enterotoxin A (17). Template RNA (10 μg) was mixed with primer DNA [100 ng of the unique 0.6-kilobase (kb) double-stranded (ds) *Dde* I fragment of γ-interferon] (18), denatured in methylmercuric hydroxide, and reverse transcribed (19). The second strand was synthesized using mRNA fragments as primers (20) and cleaved with 10 units of S1 nuclease per ml at 37°C for 30 min in the presence of 100 μg of tRNA per ml to eliminate hairpins. Phenol-extracted ds cDNA was purified by Sepharose CL-4B column chromatography (0.9 × 17 cm) in 50 mM NH_4HCO_3. Material larger than 0.6 kb was pooled, lyophilized, redissolved, ethanol precipitated twice with 0.2 M potassium acetate in the presence of carrier poly(A), and tailed at 37°C for 30 min by the addition of 2000 units of terminal transferase (Boehringer Mannheim) per ml and 5 μM dGTP (21). Tailed cDNA was hybridized with *Pst* I-cut dC-tailed pUC9 (21) and used to transform *Escherichia coli* HB101 (Bethesda Research Laboratories) according to the manufacturer's instructions. The library was replicated onto HAWP nitrocellulose filters (Millipore) and screened with nick-translated probes (22) by hybridization overnight at 37°C in 50% formamide, 0.6 M NaCl/60 mM sodium citrate, 50 μM Tris·HCl at pH 7.5, 10 mM EDTA, 0.1% $NaDodSO_4$, 250 μg of tRNA per ml, and 5× concentrated Denhardt's reagent (0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone).

A cDNA library prepared from GM637 simian virus 40 (SV40)-transformed human fibroblasts (23) (a gift of Edward Ginns) was screened by colony hybridization using the 310-base-pair (bp) *Hin*fI fragment isolated from the lymphocyte clone. A second fragment, hereafter called the 300-bp lymphocyte fragment, is a 340-bp *Hin*dIII/*Dde* I fragment isolated from the original library in pUC9 (see Fig. 1).

DNA Sequencing. Fragments for sequencing were cloned into pGEM3 (Promega Biotec) as follows: a 460-bp *Dra* I/*Hin*dIII fragment (see Fig. 1) from the lymphocyte clone, containing 81 bp of the γ-interferon primer, was inserted into a *Hin*dIII/*Sma* I-digested plasmid; a 1.4-kb *Bam*HI fragment from the fibroblast clone was inserted into the *Bam*HI site of the plasmid. Subsequently, six overlapping fragments, labeled A–F (see Fig. 1), were subcloned into pGEM3. DNA

Abbreviations: bp, base pair(s); kb, kilobase(s); ds, double-stranded; HTLV, human T-cell leukemia virus; SV40, simian virus 40; MHC, major histocompatibility complex.

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sequencing was performed on both strands with the GemSeq ds DNA sequencing system (Promega Biotec).

Cell Culture and Preparation of RNA. NIH 3T3 cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum and antibiotics. Resting cultures were obtained by reducing the serum to 0.5% for 40–48 hr (24) and stimulated by the reintroduction of 10% serum. Human myeloma cells were a gift from David Nelson. Total cytoplasmic RNA was prepared as described for lymphocytes (17). Mouse tissue RNAs, isolated in the presence of guanidine hydrochloride (25), were generously provided by Margaret Chamberlin and mRNA from HTLV-infected T-cell growth factor-dependent MI cells was a gift of Suresh Arya (26).

RNA Transfer and Southern Blots. RNA was fractionated electrophoretically in 1.5% agarose/formaldehyde gels (27) and blotted onto nitrocellulose (28). As controls and standards for normalization, RNA transfer blots of mouse and human material were hybridized, respectively, with chicken β actin (29) and a human class I major histocompatibility complex (MHC) (30) gene. The genes were donated by Margaret Chamberlin and Dinah Singer. Blots of human RNA were also hybridized to a γ-interferon cDNA probe (18). Other markers were 18S and 28S rRNAs and the RNA ladder (Bethesda Research Laboratories). Human lymphocyte DNA, a gift of Ann Dean, was cleaved overnight with excess restriction enzyme (Bethesda Research Laboratories), fractionated electrophoretically in 0.8% nondenaturing agarose gels, and blotted onto nitrocellulose (31). All probes were nick-translated (22). Blots were stripped of radioactivity by boiling 15 min in two changes of 0.1% $NaDodSO_4$/5 mM EDTA and subjected to lengthy autoradiography for confirmation.

RESULTS

Isolation of a Clone for Prothymosin α from a cDNA Library Derived from Mitogen-Stimulated Normal Human Lymphocytes. A cDNA library consisting of ≈8000 clones was prepared as described in *Materials and Methods* and screened with the same 600-bp *Dde* I fragment of γ-interferon used as a primer for cDNA synthesis. The purpose was to identify interferon-like mRNAs with sequence heterogenity at their 5′ ends. The library contained 2000 clones that hybridized. Of these, 2 clones, each about 900 bp in size, were chosen for further study because (*i*) when cleaved with *Dde* I they were found to contain a fragment apparently identical to the primer, a fact suggesting that the partial *Dde* I site at the 3′ end of the antisense strand of the primer had been regenerated, and (*ii*) when separated from the primer by treatment with *Dde* I and purified electrophoretically, the unique sequences generated by primer extension hybridized to an mRNA composed of ≈1300 nucleotides, the correct size for γ-interferon mRNA. These characteristics were consistent with homology to γ-interferon. Restriction mapping indicated that the clones of interest were related to each other but not to the known γ-interferon mRNA sequence immediately adjacent to the primer (18). A partial map of the "*new*" portion of the lymphocyte cDNA is shown at the top of Fig. 1*A*.

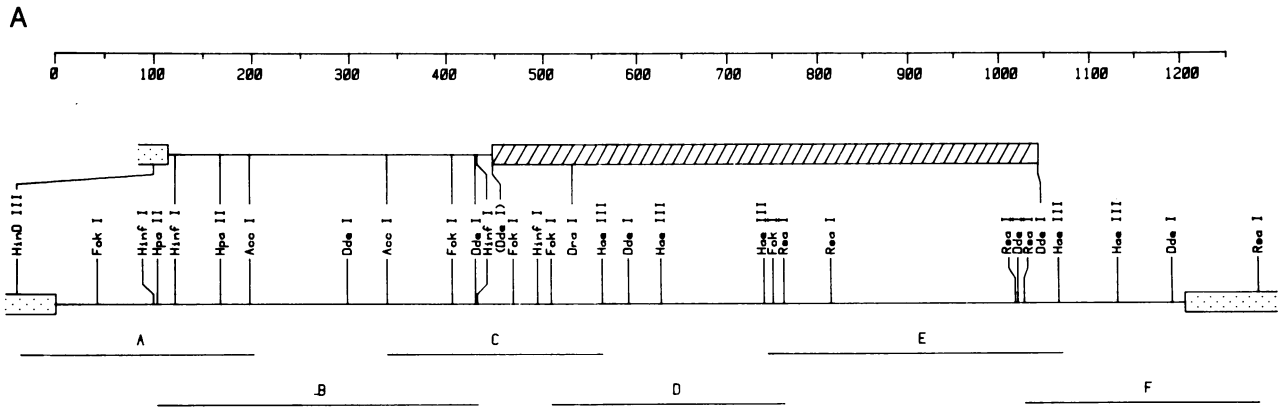
Preliminary Identification of the 300-bp Fragment. To determine whether the lymphocyte fragment coded for a T-cell-specific product, a series of RNA preparations from human T cells and normal mouse tissues was separated electrophoretically, blotted onto nitrocellulose, and screened. As shown in Fig. 2, a human RNA of about 1300 nucleotides in normal T cells and HTLV-infected T cells hybridized. Although both of these samples hybridized to a γ-interferon probe with roughly equal intensity (data not shown), the signals in response to the 300-bp fragment were clearly different. Similarly, a mouse mRNA of the same length from liver, kidney, or spleen was found to hybridize to the 300-bp human lymphocyte sequence. RNA from heart and brain contained little or no homologous RNA. Later, when human myeloma cells were probed with a longer fragment (see below), these, too, were found to be positive. The broad tissue specificity that emerged suggested that a purely immunological function was unlikely.

Isolation and Sequencing of a Nearly Full-Length cDNA Clone for Prothymosin α from SV40-Transformed Fibroblasts. Based on the widespread tissue distribution of the unique 300-bp fragment, an Okayama–Berg cDNA library from SV40-transformed human fibroblasts was obtained (23). The library was amplified, transferred to nitrocellulose filters, and screened with the 300-bp lymphocyte probe. Two positive colonies out of 20,000 were isolated; plasmid DNA from the clone containing the larger insert was purified, subcloned using a Gemini vector (pGEM3), and sequenced in its entirety. A detailed restriction map and the sequencing strategy, together with the nucleic acid and encoded amino acid sequences, are displayed in Fig. 1. An open reading frame beginning at the first AUG codon encountered, that at position 178 in the nucleotide sequence, was identified. Upon translation of the sequence into amino acids and comparison with the protein sequences in the National Biomedical Research Foundation's protein database,† homology with bovine thymosin α_1 (1) was established. The amino acids at positions 2–29 of the human sequence were identical with the bovine thymic peptide. Homology with thymosin α_{11} (2) (positions 2–36 of the human amino acid sequence) was 100%. Furthermore, when the published amino acid sequence of rat prothymosin α (3) was compared with the nucleic acid-derived human amino acid sequence, >90% sequence homology was found. These results strongly suggest that the cloned material is human prothymosin α. Further examination of the amino acid sequence, however, revealed properties inconsistent with those of a secreted protein. The amino acid sequence encoding mature prothymosin α was preceded immediately by the first AUG codon in the nucleotide sequence. No intervening N-terminal hydrophobic signal sequence could be discerned. The only hydrophobic region in the protein encompassed the 7 amino acids in positions 33–39. (The published rat protein sequence substitutes a glutamine for a glutamic acid residue, resulting in nine consecutive uncharged amino acids.) Since nine amino acids are considered to be a minimum signal peptide (33) and since the seven (human) or nine (rat) "hydrophobic" amino acids do not include leucine, isoleucine, valine, or the aromatic amino acids, but are composed exclusively of glycine, alanine, asparagine, proline, and perhaps glutamine, a signal sequence appears to be absent regardless of location.

The cloned cDNA was not full length. The presence of an AATAA poly(A) signal at position 1164 and a homopolymeric region of adenine residues following position 1198 signified that the 3′ end was intact. However, comparison of the 1.2-kb insert with the size of the mRNA determined from RNA transfer blot analysis suggested that ≈100 bp were missing. Since a poly(A) tail must be included, the data indicated that about 50 bp from the 5′ end were absent. An in-frame termination codon at position 124, upstream from the first AUG, makes it doubtful that the missing segment performs a coding function.

The complete nucleotide sequence explained why a clone for prothymosin α was serendipitously found among clones generated by priming with an interferon-specific polynucleotide using bulk lymphocyte mRNA as a template. The 3′ end of the antisense strand of the *Dde* I fragment, that which hybridizes to genuine γ-interferon mRNA, shares sequence

†National Biomedical Research Foundation (1986) Protein Sequence Database, Protein Identification Resource (Natl. Biomed. Res. Found., Washington, DC 20007), Release No. 8.



B

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ACTGGCTGCTCTGAAAAGCCATCTTTGCATTGTTCCTCATCCGCCTCCTTGCTCGCCGCAGCCGCCTCCGCCGCGCGCCTCCTCCGCCGCCGCGGACTCCGGCAGCTTTATCGCCAGA     118

GTCCCTGAACTCTCGCTTTCTTTTTAATCCCCTTCATCGGATCACCGGCGTGCCCCACC ATG TCA GAC GCA GCC GTA GAC ACC AGC TCC GAA ATC ACC ACC AAG     222
                                                             MET Ser Asp Ala Ala Val Asp Thr Ser Ser Glu Ile Thr Thr Lys      15

GAC TTA AAG GAG AAG AAG GAA GTT GTG GAA GAG GCA GAA AAT GGA AGA GAC GCC CCT GCT AAC GGG AAT GCT GAG AAT GAG GAA AAT GGG     312
Asp Leu Lys Glu Lys Lys Glu Val Val Glu Glu Ala Glu Asn Gly Arg Asp Ala Pro Ala Asn Gly Asn Ala Glu Asn Glu Glu Asn Gly      45

GAG CAG GAG GCT GAC AAT GAG GTA GAC GAA GAA GAG GAA GAA GGT GGG GAG GAA GAG GAG GAG GAA GAA GAA GGT GAT GGT GAG GAA GAG     402
Glu Gln Glu Ala Asp Asn Glu Val Asp Glu Glu Glu Glu Glu Gly Gly Glu Glu Glu Glu Glu Glu Glu Glu Gly Asp Gly Glu Glu Glu      75

GAT GGA GAT GAA GAT GAG GAA GCT GAG TCA GCT ACG GGC AAG CGG GCA GCT GAA GAT GAT GAG GAT GAC GAT GTC GAT ACC AAG AAG CAG     492
Asp Gly Asp Glu Asp Glu Glu Ala Glu Ser Ala Thr Gly Lys Arg Ala Ala Glu Asp Asp Glu Asp Asp Asp Val Asp Thr Lys Lys Gln     105

AAG ACC GAC GAG GAT GAC TAG ACAGCAAAAAAGGAAAAGTTAAACTAAAAAAAAAAAAGGCCGCCGTGACCTATTCACCCTCCACTTCCCGTCTCAGAATCTAAACGTTGGT     604
Lys Thr Asp Glu Asp Asp  *                                                                                                 111

CACCTTCGAGTAGAGAGGCCCGCCCGCCCACCGTGGGCAGTGCCACCCGCAGATGACACGCGCTCTCCACCACCCAACCCAAACCATGAGAATTTGCAACAGGGGAGGAAAAAAGAACC     723

AAAACTTCCAAGGCCCTCCTTTTTTTCTTAAAAGTACTTTAAAAAGGAAATTTGTTTGTATTTTTTATTTACATTTTATATTTTTGTACATATTGTTAGGGTCAGCCATTTTTAATGAT     842

CTCGGATGACCAAACCAGCCTTCGGAGCGTTCTCTGTCCTACTTCTGACTTTACTTGTGGTGTGACATGTTCATTATAATCTCAAAGGAGAAAAAAAACCTGTAAAAAAAGCAAAAATG     961

ACAACAGAAAAACAATCTTATTCCGAGCATTCCAGTAACTTTTTTGTGTATGTACTTAGCTGTACTATAAGTAGTTGGTTTGTATGAGATGGTTAAAAAGGCCAAAGATAAAAGGTTTC    1080

TTTTTTTTTCCTTTTTTGTCTATGAAGTTGCTGTTTATTTTTTTTGGCCTGTTTGATGTATGTGTGAAACAATGTTGTCCAACAATAAACAGGAATTTTATTTTGCTGAGTTGTTCTA    1198
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FIG. 1. (*A*) Restriction maps of the cDNA clone from staphylococcal enterotoxin-A-stimulated lymphocytes (upper) and the nearly full-length cDNA clone from SV40-transformed fibroblasts (lower). The hatched area in the lymphocyte map (upper) represents the 0.6-kb *Dde* I fragment from γ-interferon used as a primer for the generation of the lymphocyte library. Note that the *Dde* I site in parentheses was destroyed upon primer extension. Note also that the *Dde* I site at position 293 of the nucleotide sequence was found only in the fibroblast clone. The stippled regions in both maps represent plasmid sequences. Fragments A–F of the fibroblast clone were subcloned for sequencing. (*B*) Nucleotide sequence and encoded amino acid sequence of the fibroblast cDNA clone. The consensus sequence for poly(A) addition is underlined.

homology with prothymosin α mRNA; 10 of the final 13 nucleotides of the primer, including the 4 nucleotides at the 3′ end, could form perfect Watson–Crick base pairs with

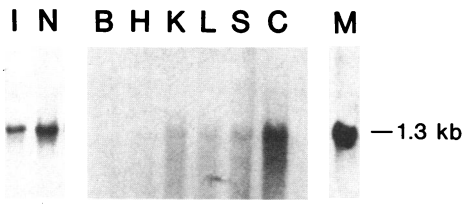

FIG. 2. Analysis of RNA from human and mouse tissues. Human poly(A) RNAs were fractionated electrophoretically in 1.5% agarose/methylmercuric hydroxide gels (32). Lane I, HTLV-infected MI cells that are T-cell growth factor-dependent (2 μg); lane N, normal human lymphocytes stimulated with staphylococcal enterotoxin A for 72 hr (0.5 μg). Mouse total RNA (30 μg per sample) was fractionated as described in *Materials and Methods*. B, brain; H, heart; K, kidney; L, liver; and S, spleen. C represents 0.7 μg of the same RNA as in lane N used as a control. Cytoplasmic RNA (14 μg) from human myeloma cells (lane M) was fractionated similarly. RNA was transferred to nitrocellulose and hybridized with a nick-translated ≈300-bp (*Dde* I/*Hin*dIII) lymphocyte fragment containing predominantly prothymosin α sequences (lanes I–C) or the full-length prothymosin α cDNA from SV40-transformed fibroblasts (see Fig. 1) (lane M). Blots were washed overnight under hybridization conditions.

prothymosin α mRNA (positions 447–458). However, the *Dde* I site thought to be located at the junction of the primer and its extension was in fact 19 bases upstream.

Function of Prothymosin α. The apparent absence of a signal peptide and the broad tissue distribution of prothymosin α mRNA suggested that this protein might participate in functions other than those already attributed to the group of peptide hormones encoded at the N terminus. Toward this end, the effect of mitogen stimulation on the levels of prothymosin α mRNA was investigated. Cytoplasmic poly(A) RNA was isolated from resting lymphocytes and from lymphocytes stimulated for 72 hr with staphylococcal enterotoxin A. The RNA was fractionated electrophoretically in denaturing formaldehyde gels, blotted, and probed with the 300-bp fragment obtained from the lymphocyte library. As shown in Fig. 3, the mRNA was present in resting lymphocytes and at dramatically higher levels after growth stimulation. Densitometric scanning of this and other autoradiographs indicated that prothymosin α mRNA increased >15-fold after 72 hr of mitogen treatment, with marked differences visible within 2 hr (data not shown). To standardize the response, the same blot was stripped of radioactivity and reprobed with a cloned human class I MHC gene (Fig. 3*A*). The data indicated that, relative to MHC expression, the induction of prothymosin α mRNA was highly significant.

In Fig. 4*A*, transfer blots of mRNA isolated from normal human lymphocytes stimulated with a variety of T-cell

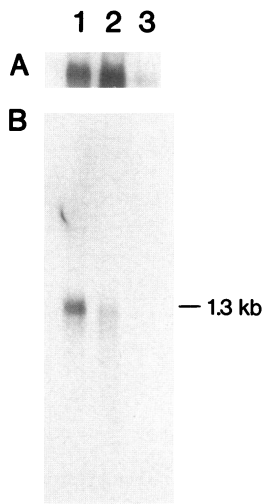

FIG. 3. Analysis of cytoplasmic poly(A) RNA from human peripheral blood lymphocytes. Lane 1, 1 μg of cytoplasmic poly(A) RNA from normal lymphocytes stimulated for 72 hr with staphylococcal enterotoxin A; lane 2, 10 μg of mRNA from normal resting lymphocytes; and lane 3, 1 μg of mRNA from normal resting lymphocytes. (*A*) RNA transfer blot screened with pSW DNA, a plasmid containing a human class I MHC gene as an insert. (*B*) The identical blot screened with the 300-bp lymphocyte prothymosin α fragment. Blots were hybridized and washed as described for Fig. 2.

mitogens for periods from 24 to 96 hr are presented. The results obtained with the 300-bp lymphocyte probe showed that prothymosin α mRNA was elevated throughout the period during which DNA synthesis occurs in mitogen-induced cells. Similar blots probed with the complete cDNA clone from SV40-transformed fibroblasts also revealed a single hybridizing species.

Since growth stimulation was accompanied by an increase

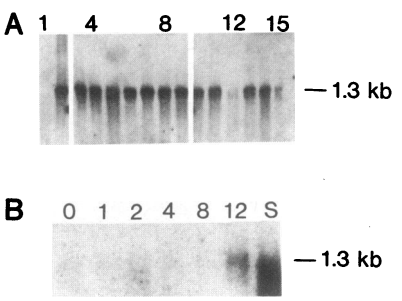

FIG. 4. Effect of growth stimulation on the level of prothymosin α mRNA. (*A*) Transfer blot of cytoplasmic poly(A) RNA (1 μg per sample) from normal human lymphocytes stimulated with the following mitogens for the stated periods: resting (lane 1), staphylococcal enterotoxin A (0.2 μg/ml) for 72 hr (lane 2), staphylococcal enterotoxin A (0.2 μg/ml) for 72 hr (lane 3), staphylococcal enterotoxin B (0.2 μg/ml) for 24, 48, 72, and 96 hr (lanes 4, 5, 6, and 7, respectively), phytohemagglutinin (2.5 μg/ml) for 24, 72, and 96 hr (lanes 8, 9, and 10, respectively), concanavalin A (25 μg/ml) for 24, 48, 72, and 96 hr (lanes 11, 12, 13, and 14, respectively), exfoliatin (0.2 μg/ml) for 96 hr (lane 15). Each sample represents mRNA from a different donor. Lane 12 contained less material based on the hybridization of the MHC gene used as a control. The blot was hybridized to the 300-bp lymphocyte fragment and washed as detailed in Fig. 2. (*B*) Transfer blot of 15 μg of cytoplasmic RNA from serum-deprived NIH 3T3 cells after serum replacement. The numbers above the lanes refer to the time (in hours) after refeeding. The blot was hybridized to a nick-translated Gemini plasmid containing 1.2 kb of prothymosin α sequences (see Fig. 1) as described in Fig. 2. The blot was washed at 70°C for 1.5 hr in 0.15 M NaCl/0.015 M sodium citrate at pH 7. Mouse spleen (15 μg of total RNA) was used as a positive control.

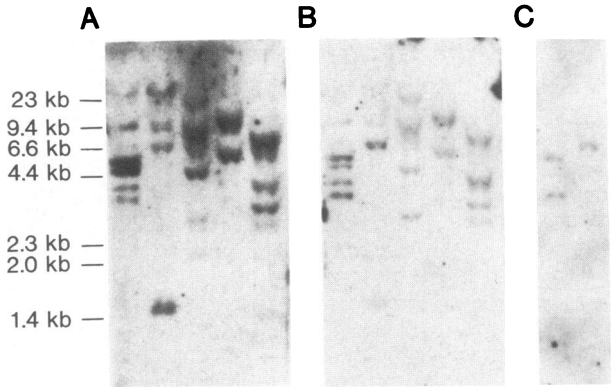

FIG. 5. Southern blot of human genomic DNA. Approximately 50 μg of DNA from the lymphocytes of a single donor were cleaved overnight (*A* and *B*) with *Bam*HI, *Bgl* I, *Eco*RI, *Hin*dIII, and *Pst* I from left to right, respectively. Only *Bam*HI and *Bgl* I were used in *C*. DNA was fractionated electrophoretically, blotted, and hybridized to nick-translated probes as follows: the 300-bp prothymosin α fragment from the lymphocyte (*A*), the Gemini plasmid containing a 1.2-kb insert of prothymosin α from the fibroblast (*B*), the fibroblast E fragment in a Gemini vector (*C*). The E fragment contains only 3′ noncoding sequences. Blots were hybridized as described in Fig. 2 and washed at 70°C in 30 mM NaCl/3 mM sodium citrate.

in prothymosin α mRNA in lymphocytes, the effect of serum deprivation, to induce quiescence, and subsequent release from starvation, on NIH 3T3 cells, was examined. The time course in Fig. 4*B* revealed that, like the lymphocyte system, growth of NIH 3T3 cells was accompanied by increased levels of prothymosin α mRNA.

Preliminary Characterization of the Prothymosin α Gene. Aliquots of human genomic DNA were cleaved with one of five different restriction enzymes that recognize sites not found in prothymosin α cDNA. The DNA was fractionated electrophoretically, denatured, blotted onto nitrocellulose, and probed with the 300-bp fragment from lymphocytes (Fig. 5*A*), the 1.2-kb clone from fibroblasts (Fig. 5*B*), or a 300-bp fragment from the 3′ noncoding region, labeled "E" in Fig. 1 (Fig. 5*C*). The lymphocyte probe revealed a complex pattern of hybridizing bands consistent with multiple genes and/or introns. When compared with the signals obtained using the nearly full-length cDNA probe, a nearly identical series of bands was disclosed. The 300-bp probe from the 3′ end hybridized to a subset of the same group of bands. These data suggest that the gene for prothymosin α is polymorphic.

DISCUSSION

Human prothymosin α is a highly acidic protein containing 111 amino acids, of which 19 are aspartic acid residues and 35 are glutamic acid residues. Comparison of the sequence of the human protein, derived from the cDNA, with that of the rat, obtained by analyzing the protein directly, revealed that the structure is highly conserved. The homology is >90%, including a perfect match of 38 consecutive amino acids at the N terminus, if position 1 of the human protein is excluded.

The nucleotide and protein sequences of prothymosin α include several critical features. There is no signal peptide. Assuming the methionine in position 1 is cleaved to expose the underlying serine residue, the mature protein contains 110 amino acids. In the rat, the N-terminal serine is acetylated. There are also no N-glycosylation sites. Thus, translocation into the endoplasmic reticulum is somewhat problematic.

Our sequence and the rat sequence contain paired basic amino acids. These residues, located at positions 20–21 and 103–104 (lysine, lysine) and at position 89–90 (lysine, arginine) of the human, are utilized in other systems as processing signals for the conversion of precursor polypeptides to mature products (34). However, cleavage at these sites would

yield none of the biologically active thymic peptides for which prothymosin α is the proposed precursor. It is interesting to note that the Horecker group failed to find peptides able to crossreact with antibody to thymosin α_1 in all tissues examined, including the thymus, when denaturing conditions were maintained during protein isolation. Significant levels of prothymosin α were found in rat thymus, brain, liver, lung, and kidney under these conditions (35). They and others found peptides only when a crude thymic extract known as thymosin fraction 5 or isotonic conditions (36) were employed as part of the protocol. It therefore seems reasonable to question the putative natural origin of prothymosin α-derived peptides.

Prothymosin α mRNA is found in many tissues and cells, including liver, spleen, kidney, normal peripheral blood lymphocytes (of which 85% are T cells), myeloma cells (100% B-cell lineage), and normal and transformed fibroblasts. Upon stimulation of normal lymphocyte populations with a series of T-cell mitogens, including staphylococcal enterotoxins A and B, concanavalin A, phytohemagglutinin, and exfoliatin (another product of *Staphylococcus aureus*), the level of prothymosin α mRNA increased above that seen in resting lymphocytes and remained elevated for at least 4 days. Similarly, serum-deprived NIH 3T3 cells also exhibited an increase in prothymosin α mRNA within the first 12 hr of serum-stimulated cell proliferation. Taken together, the combined data suggest that prothymosin α is not necessarily a precursor for a group of related peptides secreted into the serum, that prothymosin α itself, without processing, performs a vital role within the cell, and that its function is closely tied to cell growth. The major limitation to this interpretation is the presence of thymosin α_1 or thymosin-like substances in serum, coupled with the alteration in serum "hormone" levels in disease states (37). Furthermore, the functions attributed to thymosin α_1 imply an extracellular mode of action.

Genomic blots of human DNA probed with fibroblast cDNA or with fragments from the coding or 3′ noncoding regions of lymphocyte or fibroblast cDNA, respectively, produced a pattern of hybridizing bands consistent with gene polymorphism. Although some of these genes may contain introns, introns alone cannot explain the complexity of bands and signal intensities. The existence of polymorphism is also confirmed by the differences found between lymphocyte and fibroblast sequences. Deletion of three bases in frame from the lymphocyte, an event that removes the glutamic acid residue at position 40 from the protein and a *Dde* I site from the nucleic acid (Fig. 1), is consistent with, but not proof of, expression of a nonallelic gene. The possibility of a multigene family, pseudogenes, or gene rearrangements in T cells could explain the apparent discrepancy between our data and the prevailing view of prothymosin α as a precursor for secreted peptide hormones. If one or more as yet unidentified prothymosin α mRNAs had a legitimate signal peptide, the difficulties could be resolved. It has not escaped our notice that the codons in positions -17 to -4 of the fully sequenced fibroblast cDNA clone code for hydrophobic amino acids and that substitution of the termination codon at position -18 with a sense codon, together with the generation of an AUG codon upstream, could create a putative signal peptide. Although neither of the two cDNAs sequenced has such codons, minor heterogeneity among the prothymosin α genes might have given rise to the required change. Clearly, characterization of the prothymosin α genes, their mRNAs, and their encoded products is needed to understand the role played by this protein either inside or outside the cell.

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1. Goldstein, A. L., Low, T. L. K., McAdoo, M., McClure, J., Thurman, G. B., Rossio, J., Lai, C.-Y., Chang, D., Wang, S.-S., Harvey, C., Ramel, A. H. & Meienhofer, J. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 725–729.
2. Caldarella, J., Goodall, G. J., Felix, A. M., Heimer, E. P., Salvin, S. B. & Horecker, B. L. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 7424–7427.
3. Haritos, A. A., Blacher, R., Stein, S., Caldarella, J. & Horecker, B. L. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 343–346.
4. Goldstein, A. L., Guha, A., Zatz, M. M., Hardy, M. A. & White, A. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1800–1803.
5. Hooper, J. A., McDaniel, M. C., Thurman, G. B., Cohen, G. H., Schulof, R. S. & Goldstein, A. L. (1975) *Ann. N.Y. Acad. Sci.* **249,** 125–144.
6. Low, T. L. K., Thurman, G. B., Chincarini, C., McClure, J. E., Marshall, G. C., Hu, S.-K. & Goldstein, A. L. (1979) *Ann. N.Y. Acad. Sci.* **332,** 33–48.
7. White, A. (1980) in *Biochemical Action of Hormones*, ed. Litwack, G. (Academic, New York), pp. 1–46.
8. Goldstein, A. L., Low, T. L. K., Thurman, G. B., Zatz, M. M., Hall, N., Chenk, J., Hu, S.-K., Naylor, P. H. & McClure, J. E. (1981) *Recent Prog. Horm. Res.* **37,** 369–415.
9. Schulof, R. S., Low, T. L. K., Thurman, G. B. & Goldstein, A. L. (1981) in *The Lymphocytes*, eds. Sell, K. W. & Miller, W. V. (Liss, New York), pp. 191–215.
10. Wara, D. W., Barret, D. J., Ammann, A. J. & Cowan, M. J. (1979) *Ann. N.Y. Acad. Sci.* **332,** 128–134.
11. Bistoni, F., Marconi, P., Frati, L., Bonmassar, E. & Garaci, E. (1982) *Infect. Immun.* **36,** 609–614.
12. Ishitsuka, H., Umeda, Y., Nakamura, J. & Yagi, Y. (1983) *Cancer Immunol. Immunother.* **14,** 145–150.
13. Salvin, S. B. & Neta, R. (1983) *Cell. Immunol.* **75,** 160–172.
14. Schulof, R. S. (1985) *CRC Crit. Rev. Oncol. Hematol.* **3,** 309–376.
15. Lipson, S. D., Chretien, P. B., Makuck, R., Kenady, D. E. & Cohen, M. H. (1979) *Cancer* **43,** 863–870.
16. Sarin, P. S., Sun, D. K., Thornton, A. H., Naylor, P. H. & Goldstein, A. L. (1986) *Science* **232,** 1135–1137.
17. Berger, S. L., Wallace, D. M., Siegal, G. P., Hitchcock, M. J. M., Birkenmeier, C. S. & Reber, S. B. (1981) *Methods Enzymol.* **79,** 59–68.
18. Gray, P. W., Leung, D. W., Pennica, D., Yelverton, E., Najarian, R., Simonsen, C. C., Derynck, R., Sherwood, P. J., Wallace, D. M., Berger, S. L., Levinson, A. D. & Goeddel, D. V. (1982) *Nature (London)* **295,** 503–508.
19. Payvar, F. & Schimke, R. T. (1979) *J. Biol. Chem.* **254,** 7636–7642.
20. Gubler, U. & Hoffman, B. J. (1983) *Gene* **25,** 263–269.
21. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
22. Meinkoth, J. & Wahl, G. (1984) *Anal. Biochem.* **138,** 267–284.
23. Okayama, H. & Berg, P. (1983) *Mol. Cell. Biol.* **3,** 280–289.
24. Campisi, J., Gray, H. E., Pardee, A. B., Dean, M. & Sonenshein, G. E. (1984) *Cell* **36,** 241–247.
25. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18,** 5294–5299.
26. Arya, S. K., Wong-Staal, F. & Gallo, R. C. (1984) *Science* **223,** 1086–1087.
27. Lehrach, H., Diamond, D., Wozney, J. M. & Boedtker, H. (1977) *Biochemistry* **16,** 4743–4751.
28. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5201–5205.
29. Cleveland, D. W., Lopata, M. A., MacDonald, R. J., Cowan, N. J., Rutter, W. J. & Kirschner, M. W. (1980) *Cell* **20,** 95–105.
30. Sood, A., Pereira, D. & Weissman, S. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 616–620.
31. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
32. Bailey, J. M. & Davidson, N. (1976) *Anal. Biochem.* **70,** 75–85.
33. Watson, M. E. E. (1984) *Nucleic Acids Res.* **12,** 5145–5164.
34. Douglass, J., Civelli, O. & Herbert, E. (1984) *Annu. Rev. Biochem.* **53,** 665–715.
35. Haritos, A. A., Tsolas, O. & Horecker, B. L. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1391–1393.
36. Freire, M., Rey-Mendez, M., Gomez-Marquez, J. & Arias, P. (1985) *Arch. Biochem. Biophys.* **239,** 480–485.
37. Naylor, P. H., Schulof, R. S., Sztein, M., Spira, T. J., McCurdy, P. R., Darr, F., Kessler, C. M., Simon, G. L. & Goldstein, A. L. (1985) *Ann. N.Y. Acad. Sci.* **437,** 88–99.