Expression and Chromosomal Assignment of a Novel Protein-Tyrosine Kinase Gene Related to the Insulin Receptor Family

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A. Introduction

There is growing evidence in recent literature for the involvement of protein-tyrosine phosphorylation in the early events of leukocyte activation. Interleukin-3 (IL-3), a growth factor essential for the growth of hematopoietic stem cells, myeloid cells, and possibly early lymphoid cells induces rapid phosphorylation of several cellular proteins in different IL-3-dependent cells (B. Isfort and J. Ihle, personal communication; [1]). One of the early events in T-cell activation is the tyrosine phosphorylation of a component of the T-cell antigen receptor [2]. Macrophage proliferation in response to M-CSF is mediated through the activation of the tyrosine kinase moiety of its receptor, the *c-fms* proto-oncogene [3].

The protein-tyrosine kinases described to date are either transmembrane proteins having an extracellular ligand binding domain [3–8] or cytoplasmic proteins related to the *v-src* oncogene [9–11]. Most of these proteins are expressed in a wide variety of cells and tissues; few are tissue specific [12]. Previous studies have suggested that lymphokines may mediate hematopoietic cell survival via their action on glucose transport [13, 14] which is regulated in some cells through the protein-tyrosine kinase activity of the insulin receptor [15]. As activation of glucose transport may be essential to hematopoietic cell growth, we investigated the possibility that insulin receptor-like genes are expressed specifically in hematopoietic cells. Using the insulin-receptor related avian sarcoma oncogene, *v-ros* as a probe [16], we isolated and characterized the complementary DNA of a novel gene denoted *ltk* (leukocyte tyrosine kinase), which is expressed mainly in leukocytes and has so far unique structural properties; *ltk* appears to encode a transmembrane protein devoid of an extracellular domain and is related to several tyrosine kinase receptor genes of the insulin receptor family [17].

B. Results and Discussion

The polypeptide predicted from the *ltk* cDNA sequence has a molecular mass of 52212 Daltons, and when plotted according to the hydrophilicity table of Hopp and Woods [18], reveals a predominant stretch of 26 hydrophobic amino acids at the N-terminus of the polypeptide (positions 9–34), followed by three polar amino acids (Fig. 1). This region, albeit having an unusual position, is compatible with the properties of a transmembrane domain and the positively charged peptide (Asn-Gln-Lys) may serve as a membrane transfer stop signal. Following the hydrophobic stretch is a region homologous to the cytoplasmic region of protein kinases having the characteristics of a protein-tyrosine kinase domain [12].

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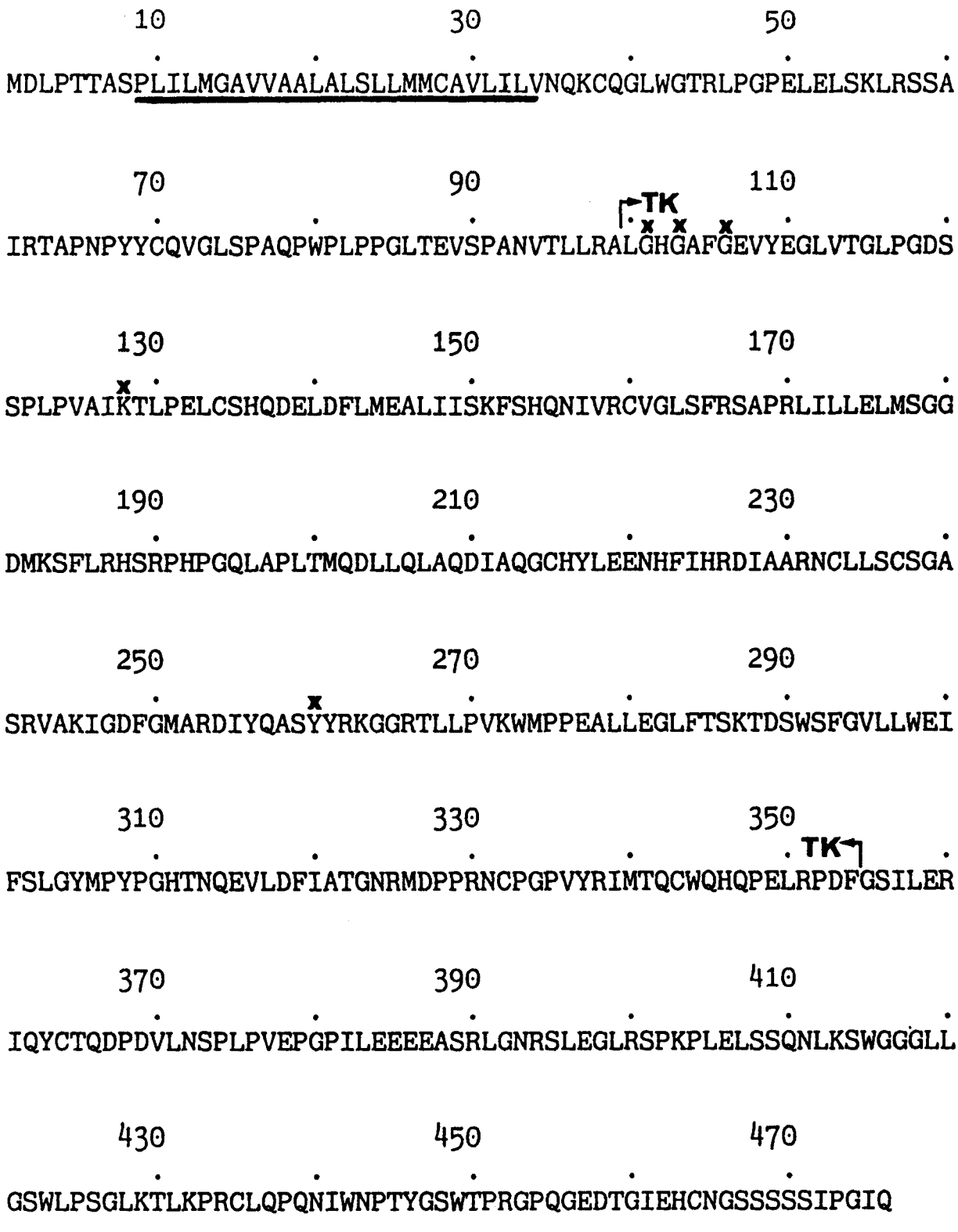

Fig. 1. Predicted protein sequence of *ltk*. The putative transmembrane region is underlined with a *solid bar*. *Asterisks:* Gly 101, Gly 103, Gly 106, and Lys 128 indicate the potential ATP binding site; and Tyr 260, the putative autophosphorylation site. The tyrosine kinase (TK) domain is indicated by *arrows*

A search through the GenBank protein data-bank reveals that *ltk* shares a high degree of similarity throughout the tyrosine kinase domain with receptor proteins. These include the human *c-ros* protein protein (53%) [19], the *Drosophila* hemeotic protein *sev* (49%) [20], the human receptors for insulin and insulin-like growth factor-1 (IGF-1) (48%) [5, 8] and the receptor portion of

the human *trk* protein (45%) [21]. Comparison of *ltk* with other kinase-receptor proteins reveals a lower degree of similarity: 30%–38%. With the former group of receptor proteins *ltk* shares a unique peptide: Tyr-Tyr-Arg-Lys-X-Gly-X-X-Leu-Leu-Pro-Val at the autophosphorylation site. Interestingly, the insulin and IGF-1 receptors are among the few kinases where autophosporylation activates the kinase activity of the protein [22–24]. It therefore seems that *ltk*, together with the former group of kinase-receptor genes, forms a subfamily within the protein-tyrosine kinase family. It remains to be seen whether the members of this subfamily are also functionally related.

In the mouse and human genome *ltk* is transcribed from a single copy gene. Using a series of mouse-human and rat-human cell hybrids we assigned the human *ltk* gene to chromosome 15 (Fig. 2, Table 1). Interestingly, the closely related IGF-1 receptor gene is also located on human chromosome 15.

The expression of *ltk* was analyzed in different murine tissues. Northern blot analysis revealed two transcripts in Rad-LV transformed T-cell lines, Tc38 and Tc23 [25] (Fig. 3a) and in the thymus (not shown) of approximately 2.6 kb and 3.0 kb. The expression of *ltk* in the various mouse tissues and cell lines was quantified by RNA slot-blot hybridization analysis using a 5′ *ltk* probe (excluding the kinase coding region; Fig. 3b), *ltk* was abundant in the thymus and observed in the spleen and kidney. Among the different cell lines, *ltk* was expressed in an interleukin-2 (IL-2) dependent *ltk* line, CTLD4; a macrophage colony stimulating factor (M-CSF) dependent macrophage cell line, Mac26; in the IL-3 dependent cell line, Ba/F3, which is thought to be an early pre-B-lymphocyte line (R. Palacios, personal communication); and in mouse lymphokine activated killer (LAK) cells. Rehybridization of the blot with a *ltk* kinase region probe shows a similar tissue distribution except for an additional faint signal in the brain (not shown). It thus appears that *ltk* is expressed mainly in cells of hematopoietic origin.

Considering the homology of *ltk* to transmembrane protein kinases, it was expected to include an extracellular recognition unit. However, the putative external portion of the protein is only nine amino acids long and unlikely to be sufficient for ligand binding. It is possible that one of the two transcripts detected by the *ltk* probe (Fig. 3a) encodes another protein with a larger extracytoplasmic domain. N-terminal variation has been observed in several other protein-tyrosine kinases as a result of alternative mRNA splicing [11, 26]. The *c-abl* protooncogene generates two mRNA transcripts of 5.5 and 6.5 kb by alternative splicing, each encoding a protein with a

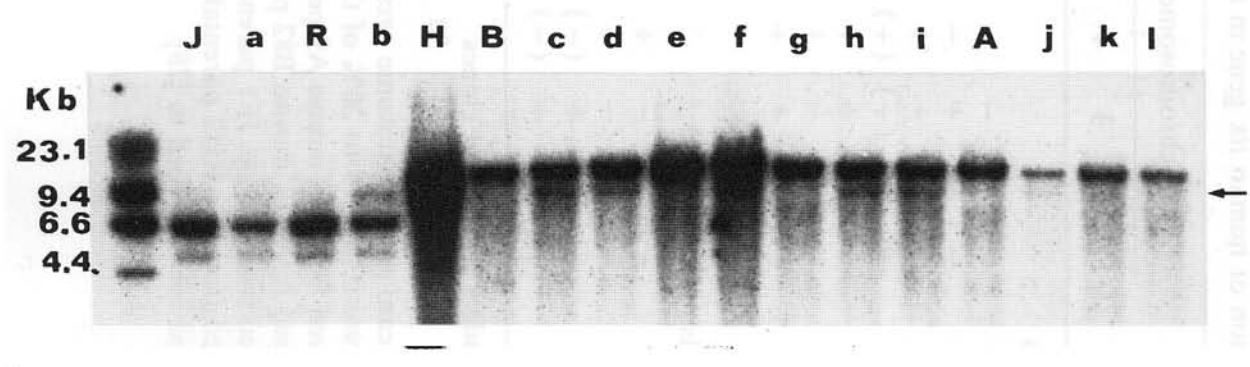

Fig. 2. Mapping of the *ltk* gene to human chromosome 15. Sourthern blot analysis of DNA (10 μg) from mouse cell lines, A9 (*A*) and B82 (*B*); rat cell lines, JF1 (*J*) and Rat 2 (*R*); human HeLa cell line (*H*); mouse-human and rat-human hybrids, whose chromosomal content is indicated in Table 1. The DNA was digested with an excess of the restriction enzyme HindIII, fractionated in 1% agarose, transferred to a nylon membrane filter (Zetabind), and hybridized to a ^{32}P radiolabeled *ltk* cDNA probe. The position of human *ltk* is indicated by an *arrow*

Table 1. Detection of human *ltk* gene in human × rat and human × mouse hybrids

Hybrids	Human chromosomes																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
Positive hybrids																								
j-HA221[a]	−	−	−	+	−	+	+	−	−	−	+	−	−	−	+	−	+	−	+	−	+	−	+	−
i-HB26[b]	−	−	+	−	−	+	+	−	−	+	(+)	(−)	+	+	+	+	+	+	(+)	+	+	+	−	−
h-HB29[b]	−	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
g-HB33[b]	−	−	(+)	(+)	−	+	−	+	−	−	(+)	−	−	+	−	−	(+)	(−)	−	−	−	+	−	−
f-HB43[b]	+	+	+	+	−	+	+	+	−	−	+	+	−	+	+	+	+	+	+	+	+	+	+	−
d-HB142.2[b]	−	−	−	+	−	−	−	−	−	−	−	−	−	+	+	−	+	−	−	−	−	−	−	−
c-HB182[b]	−	−	+	+	+	+	+	+	−	−	+	+	+	+	+	−	+	+	+	+	+	+	−	−
b-HR40C8[d]	−	−	−	−	−	−	+	−	−	+	−	+	+	+	+	(−)	+	−	+	+	+	(+)	+	−
Negative hybrids																								
1-HA11[a]	−	−	−	+	+	+	−	+	−	−	+	−	−	−	−	−	+	−	−	+	+	−	−	−
k-HA13[a]	+	−	−	+	+	+	−	+	−	−	+	+	−	−	−	+	+	−	+	+	+	−	+	−
e-HB112[b]	−	−	+	(−)	−	−	−	(−)	−	−	+	+	−	+	−	−	+	−	−	−	+	−	−	−
a-JV211[c]	+	(−)	+	(−)	+	+	+	+	−	+	+	+	−	−	−	+	−	−	−	+	−	+	−	
Percent independent discordant clones	80	60	40	30	70	40	30	60	70	60	20	60	40	20	10	60	20	30	30	60	40	30	50	70

The symbols mean: +, chromosome present in at least 60% of the metaphases; (+), chromosome present in 30% to 50% of the metaphases; (−), chromosome present in 10% to 20% of the metaphases; −, chromosome absent.

[a] Hybrids derived from the mouse A9 parental cell.

[b] Hybrids derived from the mouse B82 parental cell.

[c] Hybrid derived from the rat JF1 parental cell.

[d] Hybrid derived from the Rat2 parental cell.

The hybrids used are described in [28].

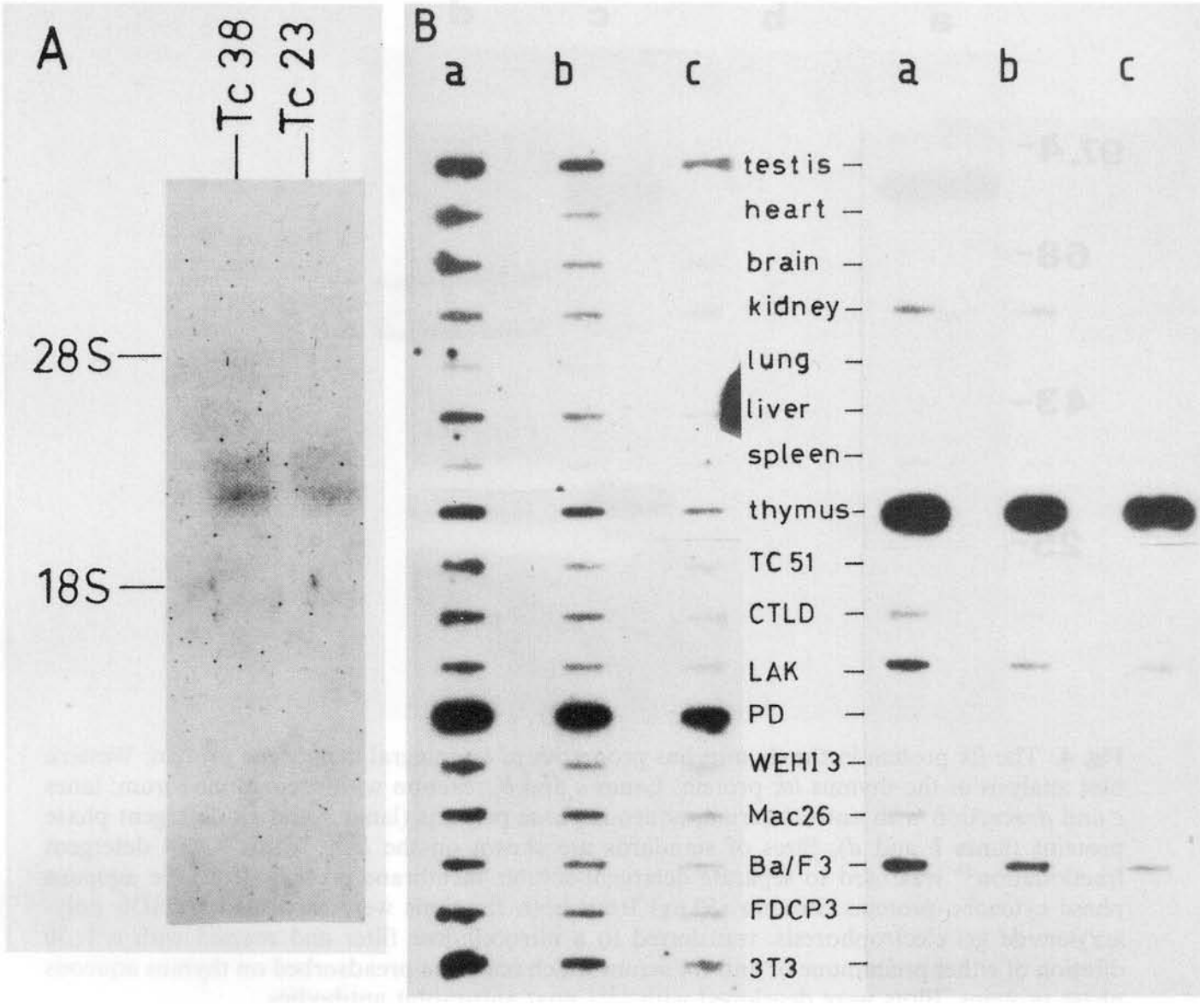

Fig. 3 A, B. Expression of *ltk* in different murine tissues and cell lines. **A** Northern blot analysis of poly(A)$^+$ RNA from Rad-LV transformed T-cell lines [22]. Poly(A)$^+$ RNA was extracted by $LiCl_2$-urea lysis and phenol/chloroform extraction, followed by Oligo(dT) selection and separated on a formaldehyde-containing 1% agarose gel (5 μg/lane). The RNA was transferred to Zetabind nylon membrane and hybridized to radiolabeled *ltk* cDNA probe. **B** Slot-blot analysis of total cellular RNA isolated from different murine tissues and cell lines. RNA, 30 μg (*a*), 10 μg (*b*), and 3 μg (*c*) was spotted onto nitrocellulose using the Minifold II slot-blotter (Schleicher and Shuell) and hybridized with a radiolabelled *c-abl* kinase region probe as a control, *left panel;* and a radiolabelled 5′ *ltk* fragment, *right panel*. Cell lines: TC51, Rad-LV transformed T-cell line [22]; CTLD, an IL-2 dependent T-cell line; LAK, lymphokine activated killer cells were prepared by incubating splenocytes with IL-2 (1000 U/ml) for 5 days; PD, Abelson-MuLV transformed pre-B cell line; WEHI 3, macrophage/monocyte line; Mac 26, a M-CSF dependent bone marrow derived macrophage line; Ba/F3, and IL-3 dependent lymphoid line; FDCP 3, a GM-CSF dependent myeloid line; 3T3, a fibroblast line from C3H mouse

different N-terminus [11]. If the origin of the two *ltk* transcripts is similar, we may have characterized one alternative splice form, while the other may encode a different N-terminus. Nevertheless, S1 nuclease analysis showed transcripts fully complementary to the *ltk* cDNA clone in the thymus.

To demonstrate that *ltk* encodes a transmembrane protein, *ltk*-specific antiserum was prepared against an *ltk*/β-galactosidase fusion protein and used to

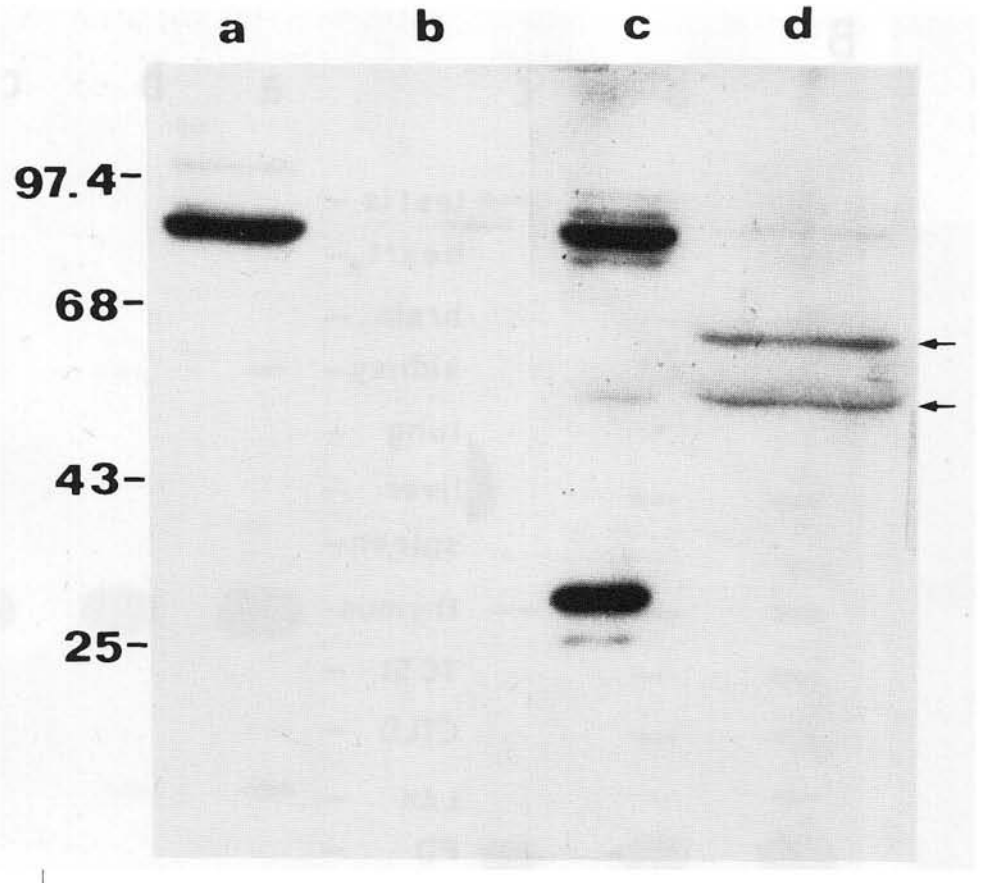

Fig. 4. The *ltk* protein in the thymus has properties of an integral membrane protein. Western blot analysis of the thymus *ltk* protein. Lanes *a* and *b*, reaction with preimmune serum; lanes *c* and *d*, reaction with anti-*ltk* serum; aqueous phase proteins (lanes *a* and *c*); detergent phase proteins (lanes *b* and *d*). Sizes of standards are shown on the *left*. Triton X-114 detergent fractionation[28] was used to separate detergent-soluble membrane proteins from the aqueous phase cytosolic proteins. Protein (50 μg) from both fractions were separated by SDS polyacrylamide gel electrophoresis. transferred to a nitrocellulose filter and reacted with a 1:50 dilution of either preimmune or anti-*ltk* serum which had been preadsorbed on thymus aqueous phase proteins. Blots were developed with ^{125}I goat anti-rabbit antibodies

demonstrate the presence of *ltk* protein in a thymus membrane protein fraction (Fig. 4). The specificity of the serum was shown by immunoprecipitation of *ltk* protein. The largest protein band synthesized in rabbit reticulocyte lysate has a molecular mass of 52 kD in agreement with the predicted size of the protein encoded by the long open reading frame of *ltk* and is immunoprecipitated by *ltk*-specific antisera (not shown).

Western blot analysis of membrane fractionated thymus proteins showed that anti-*ltk* serum reacts specifically with two protein bands of 56 and 64 kD in the thymus membrane fraction (Fig. 4). The 56 kD band is consistent with the expected size of the encoded protein, while the 64 kD band may represent either a second *ltk* gene product due to alternative splicing alternative translation-initiation or a cross-reactive protein.

These results show that authentic *ltk* transcripts exist in thymocytes encoding a protein with features of a transmembrane catalytic subunit. The unusual structure of the putative *ltk* protein implies a role in signal transduction. Thymocytes, B cells, and the various other cells which express the gene, grow and differentiate in response to various extracellular stimuli. Protein-tyrosine phosphorylation appears to be involved in the signal transduction of such stimuli. However, with the exception of the M-CSF receptor, a specific tyrosine-kinase has yet to be identified in the other signal transduction pathways. Therefore, *ltk* is

a candidate transducer for early activation events in leukocytes.

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