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Immunology

Ntk: A Csk-related protein-tyrosine kinase expressed in brain and T lymphocytes

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ABSTRACT **The activity of Src-related protein-tyrosine kinases is repressed by the phosphorylation of a conserved carboxyl-terminal tyrosine by another cytoplasmic protein-tyrosine kinase termed p50^{csk}. In this study, we characterize Ntk, a protein-tyrosine kinase bearing striking similarities to p50^{csk}. Like p50^{csk}, Ntk possesses Src homology 3 and Src homology 2 domains and lacks the consensus tyrosine phosphorylation and myristoylation sites found in members of the Src family. Expression of *ntk* transcripts was maximal in brain, and was observed at significant levels in thymus and spleen. *ntk* RNA levels were dramatically reduced upon mitogenic stimulation of normal T lymphocytes and were minimal in transformed T-cell populations. Firm evidence that Ntk is a Csk-related enzyme was provided by the observation that it phosphorylated a Src-related polypeptide on the inhibitory carboxyl-terminal tyrosine. These findings indicate that Ntk is a Csk-related enzyme that may play an inhibitory role in the control of T-cell proliferation.**

Members of the Src family of protein-tyrosine kinases participate in a variety of signal transduction responses (for reviews, see refs. 1–3). The most convincing illustration of this role is perhaps provided by studies of intracellular signaling in T lymphocytes. In mature T cells, antigen receptor stimulation is associated with a rapid rise in intracellular protein-tyrosine phosphorylation, which is critical for subsequent T-cell mitogenesis. Because the T-cell antigen receptor lacks intrinsic catalytic properties, this biochemical signal must involve coupling to other protein-tyrosine kinases. From biochemical and genetic evidence, it is now clear that T-cell receptor signaling requires the action of Lck and FynT, two Src-related enzymes abundantly expressed in T lymphocytes.

The activity of Src-related enzymes is repressed by phosphorylation of a conserved carboxyl-terminal tyrosine residue (4, 5). This inhibitory phosphorylation is not a consequence of autophosphorylation but is mediated (at least in part) by a 50-kDa cytoplasmic protein-tyrosine kinase termed Csk or p50^{csk} (6–8). p50^{csk} is ubiquitous, but is most abundant in thymus and spleen, as well as in neonatal brain (9, 10).

The importance of p50^{csk} in normal cellular physiology has been documented in at least two independent systems. First, mutant mice lacking Csk have significantly altered development of the nervous system, which is associated with early embryonic lethality (11, 12). Second, in antigen-specific T-lymphocyte lines, overexpression of Csk negatively regulates T-cell responsiveness following antigen receptor stimulation (13).

The exon–intron structure of the *csk* gene differs significantly from that of other protein-tyrosine kinase genes (14). Based on this finding, as well as on the unique structural and biochemical properties of its product, the *csk* gene has been considered to be the sole member of a distinct family. This view is further supported by the fact that previous attempts to identify additional *csk*-like genes by cross hybridization under low-stringency conditions were unsuccessful (9).

In this report, we describe a Csk-related tyrosine protein kinase termed Ntk (for nervous tissue and T-lymphocyte kinase),†† with demonstrable Csk-like enzymatic activity. While *ntk* RNA is predominantly expressed in brain, significant levels are also noted within the T-cell lineage. *ntk* RNA expression is markedly diminished both in antigen-activated proliferating normal T cells and in transformed T-cell populations, indicating that the Ntk protein may play an inhibitory role in the regulation of T-cell growth.

MATERIALS AND METHODS

Construction and Screening of cDNA Library. RNA was isolated from thymuses of day 16 embryonic mice. Poly(A)$^+$ RNA was then used to generate a cDNA library in the Uni-ZAP vector (Stratagene). Total library cDNA was isolated following helper phage rescue as described (15) and used as template in PCR with degenerate oligonucleotide primers corresponding to conserved regions in the catalytic domain of protein-tyrosine kinases (16). Amplified products were cloned into the pBluescript vector (Stratagene) by standard protocols. Library screening and phagemid rescue of positive clones were then performed according to protocols provided by Stratagene. Finally, DNA sequencing was performed by the dideoxynucleotide chain-termination method (17).

RNA Isolation and Expression Studies. RNA was isolated from tissues of 6- to 8-week-old BALB/c mice (Charles River Breeding Laboratories), thymuses of BALB/c mouse fetuses (day 16), and a variety of T-cell populations (see below) by the guanidine isothiocyanate method (18). For isolation of yeast RNA, cells were lysed in guanidine isothiocyanate buffer while mixing vigorously in the presence of glass beads. Ribonuclease (RNase) protection assays were performed as described (19), using 30 μg of total cellular RNA. To generate the RNA probe, an *Msc* I–*Bst*EII fragment from the *ntk* cDNA was cloned into the *Sma* I site of pGEM-3 (Promega). This probe is within the sequences coding for the catalytic domain of Ntk. After linearization with *Hin*dIII, radiolabeled antisense RNA probes of 292 nt were synthesized with T7 RNA polymerase. The length of the predicted fragment protected by cellular *ntk* transcripts was 227 nt. End-labeled

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Abbreviations: PMA, phorbol 12-myristate 13-acetate; mAb, monoclonal antibody; PHA, phytohemagglutinin.

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††The sequence reported in this paper has been deposited in the GenBank data base (accession no. L27738).

Msp I-digested pBR322 fragments were used as size markers. The relative levels of *ntk* RNA expression were assessed with a phospho-imager (BAS 2000; Fuji).

Northern blot analyses were carried out in standard fashion, with 10 μg of total cellular RNA. While *ntk* transcripts were detected by hybridization with a full-length mouse *ntk* cDNA, *csk* transcripts were revealed by probing with a complete rat *csk* cDNA.

Cell Culture. The $CD4^+$ antigen-specific mouse T-cell clone 5.32.10 (20), was activated with concanavalin A (Con A, 1 μg/ml; Calbiochem) in the presence of irradiated syngeneic spleen cells. Purified T lymphocytes were obtained from lymph nodes of 6- to 8-week-old BALB/c mice by using a T-cell-enrichment column (Biotex Laboratories, Edmonton, Canada) and were stimulated for 24 hr with anti-CD3ε monoclonal antibody (mAb) 145-2C11 (1 μg/ml; ref. 21) plus irradiated syngeneic spleen cells. Whole-spleen cell suspensions were also obtained from these mice and were stimulated for 36 hr with Con A (5 μg/ml; Sigma) and phorbol 12-myristate 13-acetate (PMA) (0.1 μM; Sigma). In all cases, controls were without addition. Transformed mouse T-cell lines were obtained from the American Type Culture Collection (Rockville, MD) and were stimulated as detailed in the legend to Fig. 3.

Coexpression of Ntk and Lck in Yeast Cells. Stable transformants of the *Saccharomyces cerevisiae* strain S150-2B expressing a kinase-defective $Lys^{273} \rightarrow Arg$ (R273) $p56^{lck}$ mutant or a variant carrying an additional $Tyr^{505} \rightarrow Phe$ mutation (R273F505 Lck) have been reported (22). To coexpress Ntk, the full-length *ntk* cDNA clone 4A was inserted into the yeast expression vector pGAL-A4. Yeast were transformed by the lithium acetate method (23), and transformants were selected in growth medium lacking uracil. After induction in galactose-containing medium (22), Lck polypeptides were recovered by immunoprecipitation and probed by immunoblotting with either a rabbit polyclonal anti-Lck serum or a mouse anti-phosphotyrosine mAb (4G10; Upstate Biotechnology, Lake Placid, NY), according to published protocols (22). Expression of *ntk* was confirmed by RNase protection assay. Yeast cells coexpressing Lck and Csk have been described (22).

RESULTS

Cloning and Sequencing of *ntk* cDNAs. A PCR-based strategy was employed to identify protein-tyrosine kinases in normal T lymphocytes. Degenerate primers corresponding to conserved regions in the kinase domain of protein-tyrosine kinases (16) were used to amplify cDNAs derived from a day 16 murine fetal thymus library, which were then cloned and sequenced. One clone represented a kinase with greatest homology to Csk. Using this PCR-generated insert as a probe

A

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                10         20         30         40         50
NTK:  MARRSSRVSW LAFEGWESRD LPRVSPRLFG AWHPAPAAAR MPTRWAPGTQ
CSK:                                    MSA IQAS.PS..E

      SH3
          60         70         80         90        100
NTK:  CMTKCENSRP KPGELAFRKG DMVTILEACE DKSWYRAKHH GSGQEGLLAA
CSK:  .IA.YNFHGT AEQD.P.C.. .VL..VAVTK .PN..K..NK -V.R..IIP.

                SH3 SH2
         110        120        130        140        150
NTK:  AALRQREALS TDPKLSLMPW FHGKISGQEA IQQLQPPEDG LFLVRESARH
CSK:  NYVQK..GVK AGT..... .....TREQ..ERL.Y...T. .......TNY

         160        170        180        190        200
NTK:  PGDYVLCVSF GRDVIHYRVL HRDGHLTIDE AVCFCNLMDM VEHYTKDKGA
CSK:  ....T....C EGK.E...IM YHASK.S... E.Y.E...QL .....T.ADG

                     SH2  Kinase
         210        220        240        250
NTK:  ICTKLVKPRR KQGAKSAEEE LAKAGWLLDL QHLTLGAQIG EGEFGAVLQG
CSK:  L..R.I..KV ME.TVA.QD. FYRS..A.NM KE.K.LQT.. K....D.ML.

         260        270        280        290        298
NTK:  EYLGQKVAVK NIKCDVTAQA FLDETAVMTK LQHRNLVRLL GVILHH--GL
CSK:  D.R.N..... C..N.A.... ..A.AS...Q .R.S...Q.. ...VEEKG..

         308        318        328        338        348
NTK:  YIVMEHVSKG NLVNFLRTRG RALVSTSQLL QFALHVAEGM EYLESKKLVH
CSK:  ...T.YMA.. S..DY..S.. .SVLGGDC.. K.S.DVC.A. ....GNNF..

         358        368        378        388        398
NTK:  RDLAARNILV SEDLVAKVSD FGLAKAERKG LDSSRLPVKW TAPEALKNGR
CSK:  .......V.. ...N..... ...T.EASST Q.TGK..... ......REKK

         408        418        428        438        448
NTK:  FSSKSDVWSF GVLLWEVFSY GRAPYPKMSL KEVSEAVEKG YRMEPPDGCP
CSK:  ..T..... .I....IY.F ..V...RIP. .D.VPR.... .K.DA.....

                        Kinase
         458        468        488        498
NTK:  GSVHTLMGSC WEAEPARRPP FRKIVEKLGR ELRSVGVSAP AGGQEAEGSA
CSK:  PA.YDV.KN. .HLDA.T..T .LQLR.Q.EH IRTHELHL

         505
NTK:  PTRSQDP
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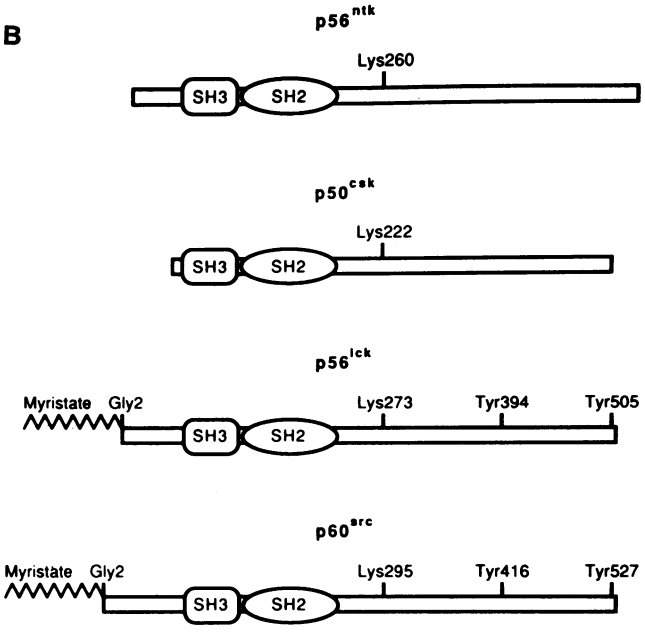
B



C

Domain	Ntk vs. Csk	Ntk vs. Src	Ntk vs. Lck	Src vs. Lck
SH3	43% (69%)	29% (59%)	30% (57%)	50% (75%)
SH2	52% (76%)	38% (57%)	38% (56%)	52% (70%)
Kinase	58% (78%)	46% (66%)	46% (64%)	67% (82%)
Overall	51% (72%)	38% (59%)	38% (57%)	54% (72%)

FIG. 1. Primary structure of Ntk. (*A*) Amino acid sequence of Ntk (upper line), compared with that of rat Csk (lower line) (7). The initiating methionine for Ntk and for Csk is in bold type and underlined. The boundaries of the Src homology 3 (SH3), Src homology 2 (SH2), and kinase domains are indicated above the sequences. Dots in the Csk sequence indicate identical amino acids with Ntk. (*B*) Schematic diagram of the structure of Ntk compared with those of Csk, Lck, and Src. The critical lysine residue which lies in the presumed phosphotransfer motif of the kinase domain (24) and the tyrosine residues which are known to be phosphorylated are indicated. The glycine residue at position 2 of Src-like enzymes is required for myristoylation and membrane association. (*C*) Amino acid identity (and homology in parentheses) between Ntk and Csk, Lck, or Src. Identity and homology between Ntk, rat Csk (7), murine Lck (25), and avian Src (26) were defined.

to screen the day 16 fetal thymus library, two independent clones, 2A and 4A (1759 and 1697 bp long, respectively), were obtained. Sequence analysis revealed that they were identical, except that clone 2A possessed a longer 5′ untranslated sequence and contained a 3-nt insertion within the presumed coding sequence (discussed below).

The 4A cDNA contained a 1515-nt open reading frame, which encoded a 505-aa polypeptide of 56,144 Da (Fig. 1*A*). The sequence of the predicted product was most closely related to that of Csk (51% identity and 72% homology; Fig. 1*C*). Because of its pattern of expression (see below), the protein encoded by 4A was termed Ntk (for nervous tissue and T-lymphocyte kinase).

By analogy with $p50^{csk}$ (7), several independent domains could be defined from the amino terminus to the carboxyl terminus of Ntk (Fig. 1 *A* and *B*): (*i*) a unique domain of 50 aa, (*ii*) a Src homology 3 motif, (*iii*) a Src homology 2 domain; (*iv*) a catalytic domain characteristic of protein-tyrosine kinases (27), and (*v*) a 29-aa carboxyl-terminal tail. Like $p50^{csk}$, Ntk lacked the amino-terminal myristoylation signal, as well as the sites of autophosphorylation and carboxyl-terminal tyrosine phosphorylation characteristic of Src-related enzymes (Fig. 1*B*). The degrees of identity and homology between Ntk and Csk were similar to those existing between two typical members of the Src family, Src and Lck (Fig. 1*C*).

In contrast to cDNA clone 4A (Fig. 1*A*), clone 2A harbored a 3-nt insertion in the *ntk* coding sequence. This modification caused the addition of a Gln after Thr^{43}. Preliminary evaluations of the structure of the *ntk* gene indicated that this insertion resulted from alternative usage of two juxtaposed splice acceptor sites (D. Davidson and A.V., unpublished data). As RNase protection assays showed that transcripts bearing these 3 nt were less abundant than the alternative RNAs (data not shown), the corresponding Ntk polypeptide is likely to accumulate at lower levels.

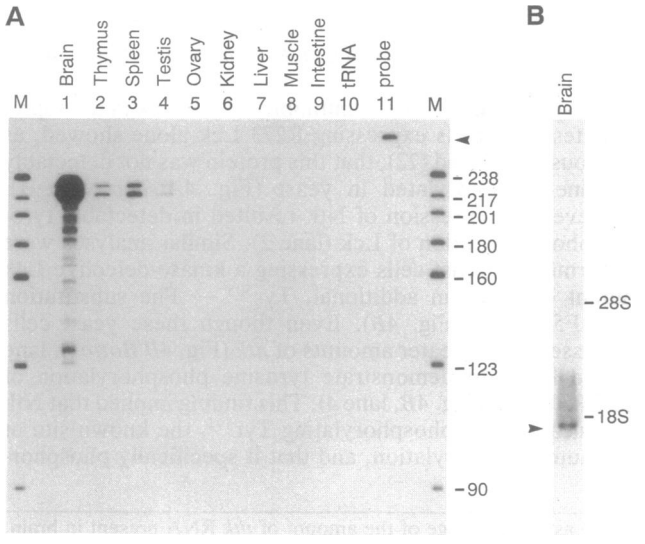


FIG. 2. Expression of *ntk* transcripts. (*A*) RNase protection assay. The arrowhead at right indicates the undigested 292-nt probe. The expected protected fragment is 227 nt in length. The doublet occurring at this position presumably arises as a result of incomplete protection due to "breathing" at the ends of the hybridized RNA duplexes. A similar pattern of protection was noted when RNA samples from yeast expressing a single *ntk* cDNA were tested (see Fig. 4 *Bottom*). The positions (in nucleotides) of DNA molecular size markers (lanes M) are shown at right. Autoradiographic exposure was for 48 hr. (*B*) Northern blot analysis. The arrowhead at left indicates the position of the major 1.8-kb transcript. The positions of 28S and 18S ribosomal RNAs are indicated at right. Exposure was for 7 days.

Expression Pattern of *ntk* Transcripts. RNA was prepared from a variety of adult mouse tissues and the pattern of expression of *ntk* was determined by RNase protection assays. Unlike *csk*, which is expressed ubiquitously (9, 10), *ntk* was expressed in a few tissue types (Fig. 2*A*). While *ntk* RNA was most abundant in brain (lane 1), significant expression was also observed in thymus and spleen (lanes 2 and 3). Additionally, much lower but detectable levels of *ntk* RNA were found in testis (lane 4), ovary (lane 5), and intestine (lane 9). No expression was noted in kidney (lane 6), liver (lane 7), or muscle (lane 8). The level of *ntk* RNA was also assessed in spleens from athymic (*nu/nu*) mice and found to be reduced by 75% as compared to spleens from euthymic control mice (data not shown). This result confirmed the predominant expression of *ntk* in T cells relative to the B cells and monocytic cells which populate the spleens of athymic mice but also suggested that *ntk* may be variably expressed in other hematopoietic cell lineages. Northern blot analysis of adult brain RNA showed a predominant transcript of 1.8 kb, consistent with the length of the longest cDNA clone (clone 2A; 1759 bp) (Fig. 2*B*). The possible existence of minor transcripts of larger size was also suggested by the weak hybridization above 1.8 kb.

***ntk* Expression in T Lymphocytes Correlates Inversely with Cellular Proliferation.** Because our *ntk* cDNAs had been isolated from fetal thymus, we wished to examine in more detail the expression of *ntk* within the T-cell lineage (Fig. 3). Expression early in thymic maturation was evident: at day 16 of fetal development, *ntk* RNA was present in thymic tissue at levels equivalent to those of adult thymus (Fig. 3*A*, lanes 5 and 6). Interestingly, activation of freshly isolated mouse splenocytes with Con A and PMA resulted in a 50-fold decrease in the abundance of *ntk* RNA (Fig. 3*A*, lanes 1 and 2). Although decreased levels of *ntk* were observed by 12 hr after cellular activation (data not shown), the most profound *ntk* down-regulation correlated with the onset of DNA synthesis (24–36 hr), as determined by [^{3}H]thymidine incorporation (data not shown). Analogous changes were also noted upon stimulation of an antigen-specific mouse T-cell line, 5.32.10, with Con A and irradiated spleen cells (lanes 3 and 4), or upon activation of *ex vivo* lymph node T cells with anti-CD3ε mAb 145-2C11 and irradiated spleen cells (Fig. 3*C*; data not shown).

Given the apparent inverse correlation of *ntk* expression with T-lymphocyte growth, we also examined the accumulation of *ntk* RNA in a variety of transformed murine T-cell tumor lines. *ntk* expression was low or undetectable in all seven lines tested (Fig. 3 *B* and *C*). Several of these lines were also exposed to the mitogenic lectin PHA. Paradoxically, in two of the tumor lines, S49.1 and EL4, treatment with PHA or with PHA plus PMA, respectively, was associated with a slight up-regulation of *ntk* RNA (Fig. 3*B*, lanes 3–6). This finding may relate to the fact that, unlike normal T lymphocytes, transformed T cells are growth inhibited by activating stimuli. In contrast to *ntk*, the levels of *csk* RNA were not significantly affected by activation of mouse splenocytes (Fig. 3*D*, lanes 1 and 2). Moreover, *csk* was expressed in transformed T-cell lines (Fig. 3*D*, lanes 3–8) in amounts comparable to those found in resting splenocytes (lane 1). Thus, the abundance of *ntk* transcripts, but not that of *csk* RNAs, appeared to correlate negatively with active T-cell proliferation.

Ntk Specifically Phosphorylates a Src-Related Enzyme at its Negative Regulatory Carboxyl-Terminal Tyrosine Residue. To determine whether the homology between Ntk and Csk extended to their enzymatic activities, we examined the ability of Ntk to phosphorylate the negative regulatory carboxyl-terminal tyrosine residue of a Src-like enzyme in a yeast coexpression system. The *ntk* cDNA (4A) was coexpressed in yeast cells carrying a kinase-defective variant of

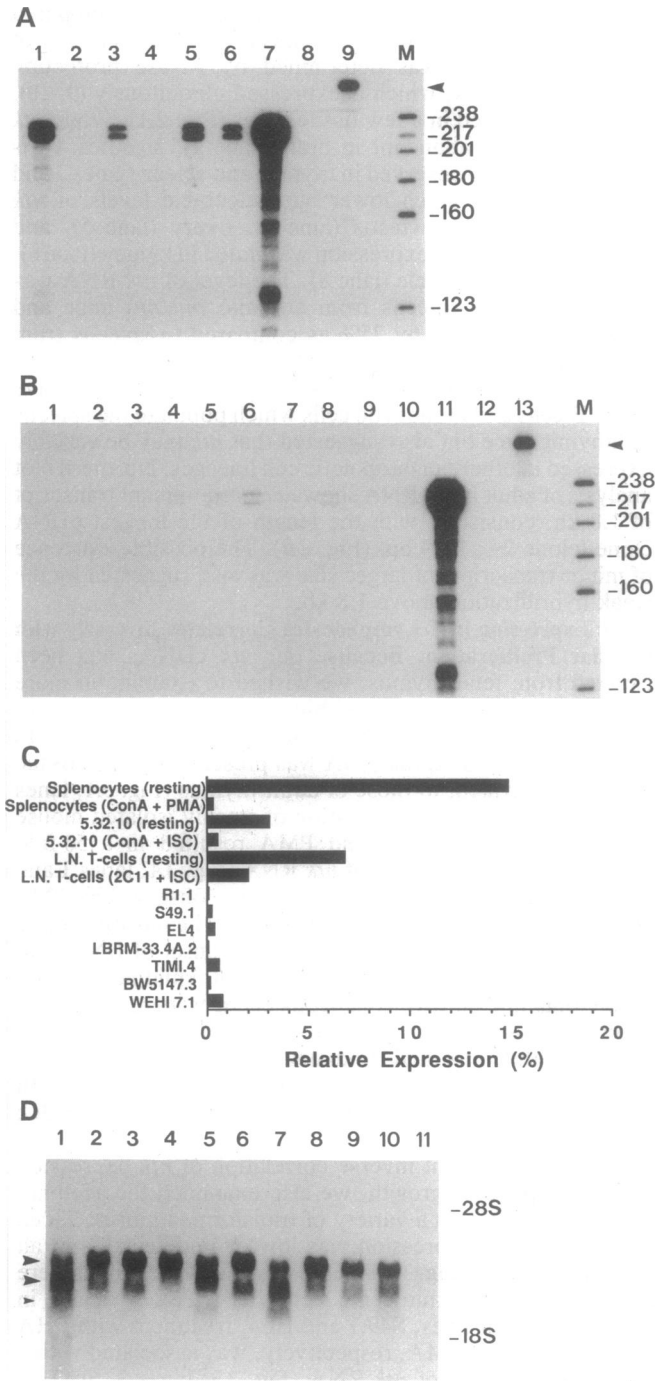


FIG. 3. Pattern of *ntk* expression in T cells. (*A*) RNase protection assay. Total cellular RNA was prepared and probed for *ntk* RNA expression as in Fig. 2*A*. Lanes: 1, resting splenocytes; 2, splenocytes stimulated with Con A (5 μg/ml) and PMA (0.1 μM); 3, unstimulated 5.32.10 T cells; 4, 5.32.10 T cells stimulated with Con A (1 μg/ml) and irradiated spleen cells; 5, fetal thymus (day 16); 6, adult thymus; 7, brain; 8, tRNA; 9, undigested probe. The arrowhead at right indicates the position of the 292-nt probe. The positions (in nucleotides) of DNA molecular size markers (lanes M) are shown on the right. Exposure was for 5 days. (*B*) RNase protection assay on RNA from T-cell tumor lines. Lanes: 1, unstimulated R1.1 cells; 2, R1.1 cells stimulated with phytohemagglutinin (PHA, 10 μg/ml); 3, unstimulated S49.1 cells; 4, S49.1 cells stimulated with PHA (5 μg/ml); 5, unstimulated EL4 cells; 6, EL4 cells stimulated with PHA (20 μg/ml) and PMA (0.1 μM); 7, unstimulated LBRM-33.4A.2 cells; 8, unstimulated TIMI.4 cells; 9, unstimulated BW5147.3 cells; 10, unstimulated WEHI-7.1 cells; 11, brain; 12, tRNA; 13, undigested probe. Exposure was for 7 days. (*C*) Quantitation of *ntk* expression. Expression was quantitated with a phospho-imager and is represented as a percentage of the amount of *ntk* RNA present in brain. ISC, irradiated spleen cells; L.N. T-cells: lymph-node T lymphocytes; 2C11, anti-CD3ε mAb 145-2C11 (used at 1 μg/ml). (*D*) Northern blot analyses of *csk* expression. Lanes: 1, resting splenocytes; 2, splenocytes stimulated with Con A (5 $\mu g/ml^{-1}$) and PMA (0.1 μM); 3, unstimulated R1.1 cells; 4, unstimulated S49.1 cells; 5, unstimulated EL4 cells; 6, unstimulated TIMI.4 cells; 7, unstimulated BW5147.3 cells; 8, unstimulated WEHI-7.1 cells; 9, fetal thymus (day 16); 10, adult thymus; 11, adult brain. The two large arrowheads at left indicate the positions of the major 3.2-kb and 2.6-kb *csk* transcripts, whereas the smaller arrowhead points to a less abundant 2.2-kb RNA. While the exact identity of these various transcripts is unclear, it has been suggested that they differ within untranslated sequences (10). The migration of the 28S and 18S ribosomal RNAs is indicated at right. Exposure was for 5 hr.

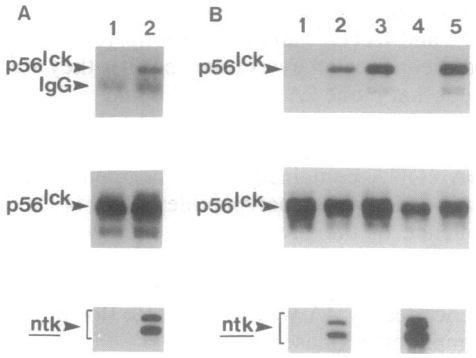


FIG. 4. Coexpression of Ntk and Lck in yeast. (*A*) Phosphorylation of Lck by Ntk. The extent of tyrosine phosphorylation of kinase-defective (R273) Lck polypeptides expressed with or without Ntk was examined in the yeast *S. cerevisiae*. Lck was immunoprecipitated from lysates of yeast cells expressing R273 Lck (lanes 1) or R273 Lck plus Ntk (lanes 2). The extent of tyrosine phosphorylation of Lck polypeptides was determined by anti-phosphotyrosine immunoblotting (*Top*), while the presence of equivalent amounts of Lck was established by an anti-Lck immunoblot (*Middle*). Expression of *ntk* in transformants was confirmed and quantitated by RNase protection assays (*Bottom*). The migration of $p56^{lck}$ and IgG heavy chain is indicated at left, as is that of the protected *ntk* transcripts. Exposures were 3 days (*Top*), 3 hr (*Middle*), and 2 hr (*Bottom*). (*B*) Ntk phosphorylates Lck at Tyr^{505}. Experimental procedures were as for *A*. Lck was immunoprecipitated from lysates of yeast cells expressing R273 Lck (lanes 1), R273 Lck plus Ntk (lanes 2 and 3), R273F505 Lck plus Ntk (lanes 4), or R273 Lck plus Csk (lanes 5). Lanes 2 and 3 represent two independent immunoprecipitations, in which different amounts of yeast lysates were used as starting material (lanes 3 represent a standard immunoprecipitation, whereas lanes 2 were based on half the amount of lysate). (*Top*) Anti-phosphotyrosine immunoblot, (*Middle*) Anti-Lck immunoblot, (*Bottom*) *ntk* RNase protection assay. Exposures were 9 hr (*Top* and *Middle*) and 2 hr (*Bottom*).

$p56^{lck}$. This Lck mutant (R273 Lck), which contains a $Lys^{273} \rightarrow Arg$ substitution, is devoid of catalytic activity (28), thus eliminating the possibility of Lck autophosphorylation in this system.

Anti-phosphotyrosine immunoblotting of Lck immunoprecipitates from cells expressing R273 Lck alone showed, as previously reported (22), that this protein was not detectably tyrosine-phosphorylated in yeast (Fig. 4*A Top*, lane 1). However, coexpression of Ntk resulted in detectable tyrosine phosphorylation of Lck (lane 2). Similar analyses were performed on yeast cells expressing a kinase-defective Lck mutant carrying an additional, $Tyr^{505} \rightarrow Phe$ substitution (R273F505 Lck; Fig. 4*B*). Even though these yeast cells expressed even greater amounts of *ntk* (Fig. 4*B Bottom*, lane 4), we failed to demonstrate tyrosine phosphorylation of R273F505 Lck (Fig. 4*B*, lane 4). This finding implied that Ntk was incapable of phosphorylating Tyr^{394}, the known site of Lck autophosphorylation, and that it specifically phosphor-

ylated Lck at Tyr^{505}. Collectively, these data showed that Ntk was capable of phosphorylating the carboxyl-terminal tyrosine residue of a Src-like enzyme in yeast cells, in a manner similar to $p50^{csk}$.

DISCUSSION

We have identified a protein-tyrosine kinase, Ntk, that appears to represent a second member of the Csk family of inhibitory protein-tyrosine kinases. This conclusion is based on several observations. First, the structure and sequence of Ntk are closely related to those of Csk. Second, as is the case for $p50^{csk}$, the Ntk protein is capable of efficiently phosphorylating the negative regulatory tyrosine of a Src-like enzyme (Tyr^{505} of $p56^{lck}$) in yeast cells. Finally, preliminary evaluation of the exon–intron structure of the *ntk* gene suggests that it is identical to that of the *csk* gene (D. Davidson and A.V., unpublished data; ref. 14).

Previous studies have demonstrated that Csk can phosphorylate the carboxyl terminus of several Src-related protein-tyrosine kinases (6, 7, 10, 29). Based on this observation, it has been postulated that Csk may be responsible for the regulation of all Src family members *in vivo* (5). However, the observation that $p60^{c\text{-}src}$ from Csk-deficient mouse embryos possessed residual carboxyl-terminal tyrosine phosphorylation *in vivo* (11, 12) suggested the existence of additional Csk-like protein-tyrosine kinases. Our characterization of Ntk is consistent with this prediction.

Our findings raise a number of possibilities. It is conceivable that Csk and Ntk have differing affinities toward the various members of the Src family. Alternatively, while these two enzymes may have identical substrates, their functions may be controlled by different regulatory mechanisms, thereby allowing for more subtle modulation of the activity of Src-related enzymes.

In addition to a presumed role in neuronal cell physiology, the pattern of Ntk expression in the T-cell lineage suggests a specific role for this protein in the regulation of T-cell proliferation. Thus, *ntk*, but not *csk*, transcripts in normal T cells were dramatically diminished upon mitogenic activation. Moreover, levels of *ntk* RNA were extremely low in transformed T-cell lines compared with normal T-cell populations. These findings imply that the Ntk protein may suppress T-cell proliferation, perhaps functioning as a tumor suppressor. Such a role could be mediated by phosphorylating the carboxyl-terminal tyrosine residue of Lck and/or FynT. This would be consistent with the fact that mice overexpressing $p56^{lck}$ or expressing a mutant form of Lck ($Tyr^{505} \rightarrow$ Phe mutant) develop thymic tumors at an early age (30).

In view of the central function of protein-tyrosine phosphorylation in antigen-induced T-cell receptor-mediated signal transduction, control of the activity of Src-related kinases most likely represents an important mechanism in the overall regulation of T-cell activation.

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