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Biochemistry

The growth factor-inducible immediate-early gene *3CH134* encodes a protein-tyrosine-phosphatase

(mitogen-activated protein kinase/signal transduction/growth control)

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ABSTRACT **Stimulation of fibroblasts with serum growth factors results in the rapid activation of a set of immediate-early genes, among them *3CH134*. We have purified a bacterially expressed form of the *3CH134*-encoded polypeptide and demonstrated that it has intrinsic protein-tyrosine-phosphatase (PTPase; protein-tyrosine-phosphate phosphohydrolase, EC 3.1.3.48) activity *in vitro*. This activity is optimal at pH 7.5, is sensitive to vanadate and cysteinyl modifying agents, and is insensitive to a panel of serine/threonine phosphatase inhibitors. Purified 3CH134 protein displays a high degree of selectivity among the tyrosine-phosphorylated polypeptide substrates tested. Under our assay conditions, the rates of dephosphorylation are in the order EDNDYINASL peptide < myelin basic protein < reduced, carboxyamidomethylated, and maleylated lysozyme (RCML) < $p42^{mapk}$. There is a 200-fold range in rates for these substrates, with $p42^{mapk}$ dephosphorylated 15-fold more rapidly than RCML. Although 3CH134 is most closely related to the tyrosine/serine dual-specificity phosphatase VH1, we failed to detect any 3CH134-directed activity on casein or RCML phosphorylated on serine/threonine residues by cAMP-dependent protein kinase. Since *3CH134* expression is controlled transcriptionally and posttranscriptionally, it may represent a class of PTPases whose activity is regulated at the level of protein synthesis and degradation.**

Serum growth factors stimulate cell proliferation through interactions with their specific cell-surface receptors, activating the intrinsic receptor protein-tyrosine kinases (PTKs; ATP: protein-tyrosine *O*-phosphotransferase, EC 2.7.1.112). This leads to signal transduction events that include reversible phosphorylation of a number of cellular substrates on tyrosyl residues. Some of these signals are transmitted to the nucleus, where the activation of a set of immediate-early genes occurs. These immediate-early genes encode a diverse array of regulatory proteins including transcription factors and cytokines (1–3). Their expression has been hypothesized to mediate the biological effects of the stimulatory growth factor.

An important feature of these phosphorylation events is their reversibility. The actions of PTKs in conjunction with the activity of a family of protein-tyrosine-phosphatases (PTPases; protein-tyrosine-phosphate phosphohydrolase, EC 3.1.3.48) control the phosphorylation state of tyrosyl residues in signaling proteins. The PTPases include transmembrane, receptor-like, and cytoplasmic enzymes defined by a unique sequence motif, (I/V)HCXAGXXR(S/T)G (4). We have noted that this motif, which contains a cysteinyl residue essential for activity, is present in the predicted sequence of the protein product encoded by the immediate-early gene *3CH134* (5).

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3CH134 is activated rapidly and transiently in quiescent fibroblasts treated with serum growth factors (6). *3CH134* is also expressed during liver regeneration soon after partial hepatectomy (7), further suggesting a link between its expression and the control of cell growth. Its transcription in fibroblasts is detected within minutes of growth factor addition, reached a peak level by 10–20 min, and is repressed after 1 h (8). The *3CH134* mRNA has a very short half-life and accumulates for only 1–2 h during the G_0/G_1 transition. Likewise, the 3CH134 protein is transiently synthesized and has a half-life of 40 min (5).

In this communication, we show directly that the *3CH134*-encoded protein dephosphorylates phosphotyrosyl protein substrates *in vitro*, including the p42 mitogen-activated protein kinase (MAP kinase) ($p42^{mapk}$). 3CH134 may thus represent a class of tyrosine phosphatases whose genes are mitogen activated and whose activities may regulate the signal transduction cascade stemming from the receptor tyrosine kinases.

MATERIALS AND METHODS

Substrate Preparation. Reduced, carboxyamidomethylated, and maleylated lysozyme (RCML) and myelin basic protein were prepared as described (9, 10) and phosphorylated on tyrosyl residues using a cytoplasmic fragment of the β subunit of the insulin receptor kinase (BIRK; ref. 11) produced in recombinant baculovirus-infected SF9 cells. The phosphorylation reaction mixture, consisting of 50 mM Tris·HCl, pH 7.5/4 mM $MnCl_2$/10 mM $MgSO_4$/2 mM 1,4-dithiothreitol (DTT)/2 mM ATP/0.1 mM Na_3VO_4/2 mg of RCML per ml/1 mCi of [γ-^{32}P]ATP per ml (1 Ci = 37 GBq), was incubated for 6 h at 30°C. The reaction was then stopped with the addition of trichloroacetic acid (TCA) to 10% and incubated on ice for 20 min. The precipitated protein was washed three times with ice-cold TCA, resuspended in 2 M Tris base on ice, and dialyzed against several changes of 50 mM imidazole (pH 7.2). A phosphotyrosyl synthetic peptide derived from the site of phosphorylation by v-Abl of PTP1B (EDNDYINASL) was phosphorylated under similar conditions and purified by reverse-phase HPLC. Autophosphorylated BIRK was prepared as described (12). Serine phosphorylated substrates were prepared with the catalytic subunit of protein kinase A in a reaction mixture consisting of 50 mM Tris·HCl, pH 7.2/10 mM $MgCl_2$/2 mM ATP/2 mM DTT/1 mCi of [γ-^{32}P]ATP per ml/2 mg of RCML per ml or 10 mg of casein per ml. Phosphorylated substrates were purified as described above and stored at 4°C.

Abbreviations: PTK, protein-tyrosine kinase; PTPase, protein-tyrosine-phosphatase; RCML, reduced, carboxyamidomethylated, and maleylated lysozyme; MAP kinase, mitogen-activated protein kinase; BIRK, β subunit of insulin receptor kinase; DTT, 1,4-dithiothreitol; BSA, bovine serum albumin.

Preparation of a Substrate Affinity Column. Thiophosphotyrosyl RCML (10) was prepared by incubation of RCML with the BIRK enzyme in the reaction mixture described above except that ATP was replaced by 1 mM adenosine 5'-[γ-thio]triphosphate (ATP[γS]) and 250 μCi of ATP[γ-^{35}S]. This reaction mixture was incubated for 12 h at 30°C and terminated by addition of TCA; then the thiophosphoprotein was washed and solubilized as described above. Thiophosphotyrosyl RCML (0.7 mol of thiophosphate per mol of protein) was dialyzed against 0.1 M $NaHCO_3$/0.5 M NaCl and coupled to CNBr-activated Sepharose-4B beads (Pharmacia); 1.5 mg of RCML protein was coupled per ml of CNBr Sepharose.

Protein Purification. A DNA fragment containing nucleotides 138–1930 of the *3CH134* cDNA (5) was cloned into the T7 RNA polymerase-based expression vector pET3b (13) and transformed into *Escherichia coli* strain BL21 DE3. This construct expresses a recombinant 3CH134 protein that is full-length with an N-terminal extension of 16 amino acids (MASMTGGQQMGRRDPA). An overnight culture of bacteria was diluted 1:50 into LB medium and grown at 30°C until A_{600} = 0.8. Synthesis of 3CH134 protein was induced with 0.8 mM isopropyl β-D-thioglucopyranoside as the cells continued to grow at room temperature for an additional 4 h. All subsequent steps were performed at 4°C. Cells were harvested by centrifugation at 3000 × *g* and resuspended in lysis buffer consisting of 20 mM Hepes, pH 7.5/10 mM NaCl/10% (vol/vol) glycerol/5 mM EDTA/5 mM DTT/5 μg of aprotinin per ml/5 μg of leupeptin per ml/1 mM benzamidine/2 μM pepstatin/0.1 mM phenylmethylsulfonyl fluoride (PMSF). Cells were lysed by two passes through a French press. Cell debris was removed by centrifugation at 10,000 × *g* for 20 min and the supernatant containing the soluble 3CH134 protein was assayed for PTPase activity.

To purify the recombinant 3CH134 protein, the lysate was adsorbed batchwise to S-Sepharose fast flow ion-exchange resin (Pharmacia), which had been equilibrated with buffer A (10 mM Hepes, pH 7.5/0.1% 2-mercaptoethanol/1 mM EDTA/1 mM benzamidine/0.2 mM PMSF). The phosphatase activity was eluted with buffer A containing 500 mM NaCl, and the eluate was dialyzed against buffer A. The dialysate was applied to a Mono S HR 5/5 column (Pharmacia), which had been equilibrated with buffer B (10 mM Hepes, pH 7.5/1 mM EDTA/2 mM DTT/1 mM benzamidine/0.2 mM PMSF). Proteins were eluted from the column at 1 ml/min with a linear gradient of 0–1 M NaCl in buffer B. Activity was recovered in the fractions containing 350–450 mM NaCl, pooled, diluted 1:10 with buffer A, and loaded onto the 1.5-ml thiophosphotyrosyl RCML affinity column. The column was washed with 15 ml of buffer A and then with 15 ml of buffer A containing 50 mM NaCl. 3CH134 protein was eluted with incremental washes of 50 mM NaCl up to 300 mM in buffer A. Active fractions in 100–150 mM NaCl were pooled, mixed with 0.2 mg of bovine serum albumin (BSA) per ml to stabilize enzyme activity, and frozen in 50% glycerol at −70°C.

Phosphatase Activity Assay. A standard assay of phosphatase activity contained 50 mM Hepes (pH 7.5), 1 mg of BSA per ml, 2 mM DTT, 1 μM tyrosine-phosphorylated RCML [Tyr(P)-RCML], and purified 3CH134 protein in a final reaction vol of 60 μl. After incubation for 10 min at 30°C, reactions were stopped by using an activated charcoal mixture as described (12).

Immunoblotting. Proteins were separated by SDS/PAGE, transferred to nitrocellulose (14), and detected by immunoblotting. 3CH134 was detected with affinity-purified anti-3CH134 antibody (5), MAP kinase was detected with polyclonal antibody R2 (UBI, Lake Placid, NY), while phosphotyrosine was detected with the monoclonal antibody 4G10 (UBI). All antibodies were diluted in TTBS (20 mM Tris/0.05% Tween 20/0.5 M NaCl) containing 5% nonfat dry milk. Blots were washed three times in TTBS and then incubated with the appropriate secondary antibodies. After washing, immunoreactive proteins were detected through the use of enhanced chemiluminescence (ECL; Amersham).

Dephosphorylation of MAP Kinase. Bacterially expressed mouse p42^{mapk}, phosphorylated primarily at tyrosine-185 using purified MAP kinase kinase (15), was generously provided by Timothy Haystead (University of Virginia). Dephosphorylation was carried out in 40 mM Hepes, pH 7.5/0.1 mg of BSA per ml/2 mM DTT/1 μM phosphorylated p42^{mapk} using either purified 3CH134 or PTP1B purified from human placenta (10). The mixtures were incubated at 30°C for various times, stopped by addition of 2× SDS sample buffer (14), resolved by SDS/10% PAGE, and immunoblotted. The reactive protein bands detected by ECL were quantitated with the computing densitometer Image Quant (Molecular Dynamics).

RESULTS

The Immediate-Early Gene *3CH134* Encodes a PTPase. To test whether the *3CH134*-encoded protein, which contains the sequence motif (I/V)HCXAGXXR(S/T)G, is indeed a tyrosine phosphatase, we produced the 3CH134 protein by using a bacterial expression vector. PTPase activity, as judged by dephosphorylation of Tyr(*P*)-RCML, was readily detected in lysates from cells transformed with the 3CH134 expression construct but not in lysates from cells transformed with the parental vector (data not shown). To demonstrate that the 3CH134 protein contains an intrinsic PTPase activity, we purified and characterized the recombinant 3CH134 polypeptide. Lysates from cells expressing 3CH134 were passed sequentially over an S-Sepharose fast flow column, a Mono S HR 5/5 ion-exchange column, and a thiophosphotyrosyl-RCML affinity column (Table 1). Each step of the purification was monitored by assay of PTPase activity and immunoblotting with affinity-purified anti-3CH134 antibodies (5).

Despite the use of a protease-deficient strain of *E. coli* and the inclusion of a broad spectrum of protease inhibitors, the bacterially expressed 3CH134 protein is highly labile. In bacterial cell lysates, the major antigenic 3CH134 species include a full-length form, migrating with the expected mobility of 40 kDa, and a truncated 35-kDa protein. After ion-exchange and affinity chromatography a more extensively truncated 3CH134 polypeptide, running as a doublet at 23 kDa, was enriched >500-fold as an active PTPase to near homogeneity (Fig. 1). Activity correlated with the appearance of the immunoreactive proteins during purification (Table 1). Dephosphorylation was linear with time and enzyme concentration. In addition to Tyr(*P*)-RCML, 3CH134

Table 1. Purification of 3CH134

	Vol, ml	Protein concentration, mg/ml	Total protein, mg	Total activity, pmol/min	Specific activity, pmol·min^{-1}·mg^{-1}	Yield, %	Purification, -fold
Crude extract	40	14.3	572	709	1.2	100	1
S-Sepharose	54	0.38	20.5	2000	97.6	282	81
Mono S	2	0.30	0.6	229	381	32	317
RCML Sepharose	2.5	0.06	0.15	104	693	15	577

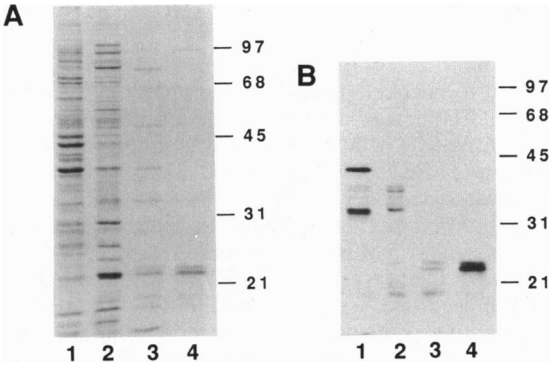

FIG. 1. Purification of recombinant 3CH134 protein expressed in bacteria. (*A*) Coomassie blue-stained SDS/12% PAGE analysis of proteins after each stage of purification. Lanes: 1, 2.5 μg of total lysate; 2, 2.5 μg of S-Sepharose eluate; 3, 0.5 μg of Mono S eluate; 4, 0.5 μg of thiophosphotyrosyl RCML Sepharose eluate. Mobilities of molecular mass markers are indicated (kDa). (*B*) Immunoblot of a gel similar to that in *A* (except lanes 3 and 4 contain ≈0.25 μg of protein) probed with affinity-purified anti-3CH134 antibodies and peroxidase-coupled donkey anti-rabbit IgG (Amersham) detected with enhanced chemiluminescence.

also dephosphorylated Tyr(*P*)-myelin basic protein and the EDNDYINASL peptide, albeit with lower efficiency (data not shown).

Characterization of 3CH134 PTPase Activity. Assay of purified 3CH134 across the pH range 6.0–9.0 showed that the enzyme is most active at pH 7.5, with little activity at pH 6.0 or 9.0 (Fig. 2*A*). The PTPase activity was sensitive to the classic PTPase inhibitor vanadate, with >90% inhibition at 1 mM (Fig. 2*B*). However, it was not sensitive to the alkaline phosphatase inhibitor tetramisole, the acid phosphatase inhibitor tartrate, or inhibitors of phosphatases 1 and 2A, okadaic acid and microcystin-LR. The PTPase activity of 3CH134 required the presence of a reducing agent and was abrogated by cysteinyl-modifying agents such as iodoacetic acid or *N*-ethylmaleimide (Fig. 2*B*).

3CH134 Does Not Dephosphorylate Phosphoseryl/Threonyl Substrates. Tyr(*P*)-RCML is readily dephosphorylated by 3CH134. Therefore, in order to examine the potential dual specificity of this enzyme, we tested whether it could dephosphorylate RCML phosphorylated by cAMP-dependent protein kinase. This has been used as a substrate for other phosphatases (16). Under assay conditions in which the phosphotyrosyl protein was dephosphorylated, phosphoserine/threonine RCML was not susceptible to 3CH134 (Fig. 3). Similarly, there was no detectable dephosphorylation of the more commonly used substrate phosphoseryl casein. In both cases, no activity was observed under conditions varying from pH 6.0 to pH 9.0 (data not shown).

Dephosphorylation of MAP Kinase by 3CH134. Since the synthesis of 3CH134 occurs soon after growth factor addition, one might expect its physiological substrates to be proteins phosphorylated on tyrosyl residues shortly after mitogenic stimulation. The MAP kinases, which are signal-transducing enzymes activated by tyrosine phosphorylation (17, 18), are candidate substrates. We thus examined the ability of 3CH134 to dephosphorylate recombinant $p42^{mapk}$, which was phosphorylated on tyrosyl residues *in vitro* using MAP kinase kinase (15). The reaction products were analyzed by parallel immunoblots with antibodies specific for MAP kinase and a monoclonal antibody specific for phosphotyrosine (Fig. 4). Dephosphorylation of $p42^{mapk}$ was observed accompanied by a shift in its electrophoretic mobility to a faster-migrating species and by the disappearance of the immunoreactive protein recognized by the anti-phophotyrosine antibody.

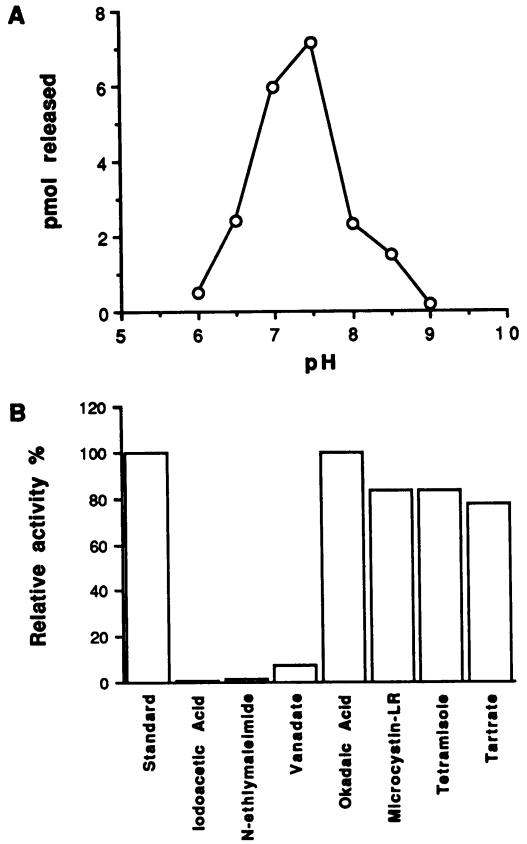

FIG. 2. Characterization of 3CH134 PTPase activity. (*A*) pH optimum. Purified 3CH134 was assayed with 2.5 μM phosphotyrosyl RCML. Reactions were assayed under standard conditions, buffered at pH 6 or 6.5 with 40 mM Mes; at pH 7.0 or 7.5 with 40 mM Hepes; and at pH 8.0, 8.5, or 9.0 with 40 mM Tris. (*B*) Effects of phosphatase inhibitors. Activity of purified 3CH134 on 2.5 μM Tyr(*P*)-RCML was measured under standard assay conditions in 40 mM Hepes (pH 7.5). Activity was measured in the presence of 10 mM iodoacetic acid, 10 mM *N*-ethylmaleimide, 1 mM sodium orthovanadate, 1 μM okadaic acid, 0.1 μM microcystin-LR, 1 mM tetramisole, or 5 mM tartrate. Dephosphorylation is presented as the percentage of that observed in the absence of inhibitors (standard).

The 3CH134 phosphatase appears to display a high degree of substrate selectivity *in vitro*. $p42^{mapk}$ was dephosphorylated with highest efficiency, some 15-fold more rapidly than RCML (Fig. 5A), which was in turn dephosphorylated 3- and 15-fold

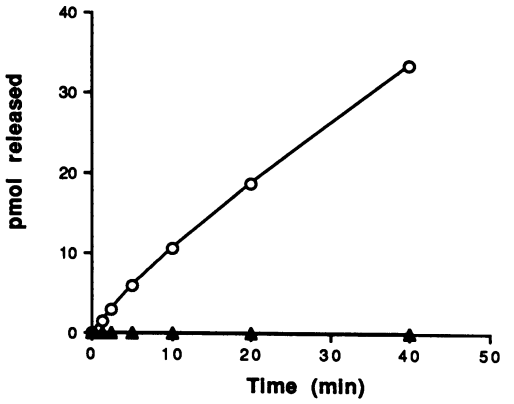

FIG. 3. Time course of dephosphorylation of RCML. Purified 3CH134 was incubated under standard assay conditions with either 2.5 μM phosphotyrosyl RCML (○) or 2.5 μM phosphoseryl/threonyl RCML (▲) for the indicated times in assay buffer containing 40 mM Hepes (pH 7.5), 1 mg of BSA per ml, and 2 mM DTT.

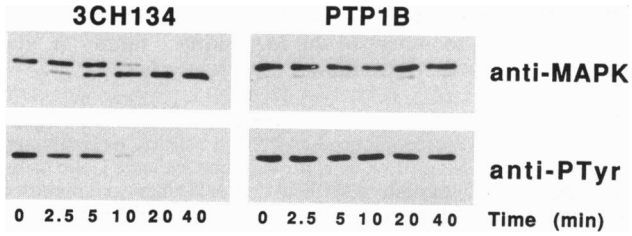

FIG. 4. Dephosphorylation of p42^{mapk} by 3CH134. 3CH134 or PTP1B was incubated with 1 μM p42^{mapk} phosphorylated primarily on Tyr-185 in 40 mM Hepes, pH 7.5/0.1 mg of BSA per ml and 2 mM DTT. Since PTP1B activity is enhanced in 1 mM EDTA, it was included in the PTP1B assay buffer. Equivalent units of activity of 3CH134 and PTP1B on Tyr(*P*)-RCML (1 μM) were used in each reaction. Incubations were allowed to proceed for the indicated times and were terminated by addition of 2× sample buffer. Reaction products were analyzed in parallel immunoblots with anti-MAP kinase (anti-MAPK) antibodies or anti-phosphotyrosine (anti-PTyr) antibody as indicated and were detected by enhanced chemiluminescence.

more rapidly than myelin basic protein and the peptide EDNDYINASL, respectively (data not shown). In contrast, PTP1B, which dephosphorylates many *in vitro* substrates efficiently (19), dephosphorylated these artificial substrates with similar efficiency but dephosphorylated p42^{mapk} at a very slow rate (Fig. 4). The possibility that 3CH134 may act on growth factor receptor PTKs was tested with BIRK, the cytoplasmic domain of the insulin receptor that was autophosphorylated on tyrosyl residues *in vitro*. Under conditions in which 3CH134 readily dephosphorylated p42^{mapk}, there was no apparent dephosphorylation of BIRK (Fig. 5*B*).

DISCUSSION

The characterization of growth factor-inducible immediate-early genes has led to the identification of regulatory molecules, including transcription factors and cytokines (1). We show here that the immediate-early gene *3CH134* (5) encodes a PTPase. 3CH134 is thus an example of a PTPase whose gene is transcriptionally activated by serum growth factors.

The PTPase family of enzymes includes a structurally diverse array of proteins consisting of transmembrane, receptor-like, and cytoplasmic forms (4). Despite this structural diversity, each member of the family is characterized by the presence of a segment of ≈240 residues that presumably represents the catalytic domain. The signature motif (I/V)HCXAGXXR(S/T)G, located near the C terminus of this domain, contains the catalytically essential cysteine residue. The 3CH134 phosphatase is most closely related to the vaccinia virus protein VH1, whose structural similarity to previously defined PTPases was restricted to ≈30 residues

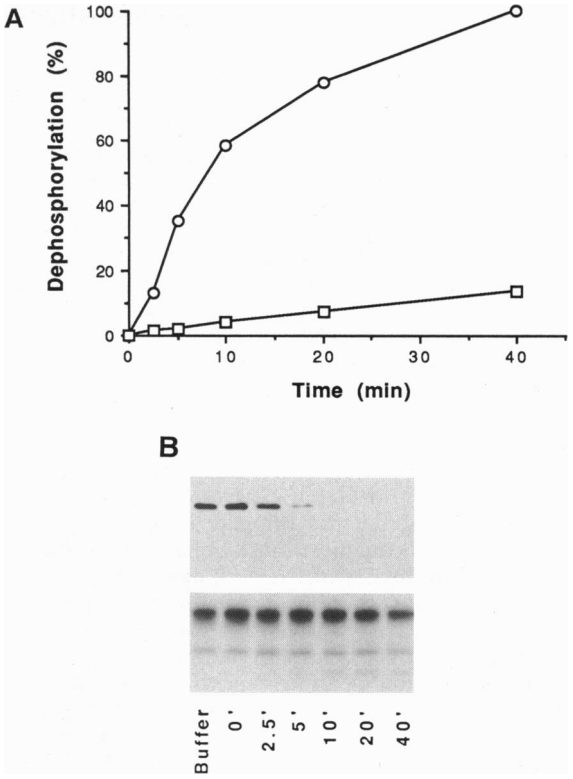

FIG. 5. Substrate specificity of purified 3CH134. (*A*) Dephosphorylation of p42^{mapk} (○) was quantitated by densitometer tracing of the appearance of the dephosphorylated p42^{mapk} species with faster electrophoretic mobility on the exposed x-ray film (see Fig. 4). Dephosphorylation of labeled Tyr(*P*)-RCML (□) was quantitated by scintillation counting of released free $^{32}P_i$. Both substrates were assayed at 1 μM Tyr(*P*) concentration for the indicated times. (*B*) Comparison of activities of 3CH134 on phosphotyrosyl p42^{mapk} and phosphotyrosyl BIRK. Each substrate was assayed at 0.5 μM phosphotyrosyl protein concentration for the indicated times (minutes) and analyzed by SDS/PAGE. Dephosphorylation of p42^{mapk} (*Upper*) was monitored by immunoblotting with anti-phosphotyrosine antibody as described in Fig. 4. Dephosphorylation of ^{32}P-labeled BIRK (*Lower*) was examined by autoradiography.

surrounding the consensus motif (20). It now appears that VH1 can be regarded as the prototype of a broad subfamily of PTPases that also includes CDC14 from *Saccharomyces cerevisiae* (21); an open reading frame from *Autographa californica* (16); cdc25, the PTPase that dephosphorylates and activates p34^{cdc2} (22, 23); YVH1 from *S. cerevisiae* (24); and VHR from human lung fibroblasts (25). An alignment of some of these protein sequences is presented in Fig. 6.

While this manuscript was being prepared, the human homolog of 3CH134, named CL100, which is activated by

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3CH134  PVEILSFLYLGSAYHASRKDMLDALGITALINVSANCPNHFEGHYQYKSIPVEDNHKADISSWFNEAIDFIDSIK
YVH1    LGGIYLGGIRPIIDHRPLGAEFNITHILSVIKFQVIPEYLIRKGYTLKNIPIDDDDVTDVLQYFDETNRFIDQ*K
VH1     MTRVTNNVYLGN-YKNAMDAPSSEVKFKYVLNLTMDKYTLPNSNINIIHIPLVDDTTTDISKYFDDVTAFLSKCD
VHR     VGNASVAQDIPKLQKLGITHVLNAAEGRSFMHVNTNANFYKDSGITYLGIKANDTQEFNLSAYFERAADFIDQA*
PTP1B   KEMIFEDTNLKLTLISEDIKSYYTVRQLELENLTTQETREILHFHYTTWPDFGVPESPASFLNFLFKVRESGSLS
cdc25M  TVDGKHQDLKYISPETMVALLTGKFSNIVEKFVIVDCRYPYEYEGGHIKNAVNLPLERDAETFLLQRPIMPCSLD

3CH134  DAGGRVFVHCQAGISRSATICLAYLMRTNRVKLDEAFEFVKQRRSIISPNFSFMGQLLQFESQVLAPHCSAE
YVH1    PQRGAVFAHCQAGLSRSVTFIVAYLMYRYGLSLSMAMHAVKRKKPSVEPNENFMEQLHLFEKMGGDFVDFDN
VH1     QRNEPVLVHCAAGVNRSGAMILAYLMSKNKESLPMLYFLYVYHSMRDLRGAFVENPSFKRQIIEKYVIDKN
VHR     QKNGRVLVHCREGYSRSPTLVIAYLMMRQKMDVKSALSIVRQNREIG-PNDGFLAQLCQLNDLAKEGKLKP
PTP1B   PEHGPVVVHCSAGIGRSGTFCLADTCLLLMDKRKDPSSVDIKKVLLEMRKFRMGLIQTADQLRFSYLAVI EG
cdc25M  KRHIILIFHCEFSSERGPRMCRFIRERDRAANDYPSLYYPEMYILKGGYKEFFPQHPNFCEPQDYRPMNHEA
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FIG. 6. Comparison of 3CH134 to other PTPase protein sequences. Shown are the amino acid sequences from 3CH134 (aa 174–320; ref. 5), YVH1 (aa 17–179; ref. 24), VH1 (aa 27 to end; ref. 20), VHR (aa 39 to end; ref. 25), human PTP1B (aa 131–279; ref. 26), and mouse cdc25 (aa 400–545; ref. 22). Identities with 3CH134 are shaded. aa 89–106 in YVH1 and aa 113 and 114 in VHR were omitted to maximize alignment and are represented by asterisks in the aligned sequences.

oxidative stress and heat shock, was demonstrated to hydrolyze the phosphate ester pNPP *in vitro* (27). However, under various assay conditions, several enzymes other than phosphatases can hydrolyze pNPP (28). Here we demonstrate directly that 3CH134 possesses intrinsic PTPase activity toward several phosphotyrosyl proteins *in vitro*. Moreover, it displays a high degree of substrate selectivity, with a preference for $p42^{mapk}$ among the substrates tested.

The specific activity of the 3CH134 PTPase is low when standard artificial substrates are used *in vitro*. A number of factors may contribute to this. (*i*) The 23-kDa form of 3CH134 may exhibit altered activity. However, the activity of the full-length form of 3CH134 in bacterial lysates toward RCML, myelin basic protein, and EDNDYINASL peptide was similar to the purified truncated form. Nonetheless, the possibility that the truncated 3CH134 may show differences in specific activity and/or substrate selectivity from the full-length form cannot be ruled out. Furthermore, as is a common problem in bacterial expression systems, a significant proportion of the protein may be denatured. (*ii*) A catalytically significant posttranslational modification may be absent due to the use of bacterial expression systems to produce recombinant protein. In this regard, it is interesting to note that the activity of cdc25 is increased in M phase, concomitant with hyperphosphorylation of the protein (29). (*iii*) The contribution of an associated protein may also be important for activity, as has been proposed for cyclin B in the activation of cdc25 (30). Thus, although several of the previously characterized PTPases, including PTP1B, display broad substrate specificity *in vitro*, many of the standard model substrates may be inappropriate for 3CH134.

3CH134 is most closely related to YVH1 (24), VHR (25), and VH1 (ref. 20; Fig. 6). Levels of both 3CH134 and YVH1 are controlled transcriptionally, the latter being induced by nitrogen starvation (24). Although VH1 and VHR possess dual specificity for dephosphorylation of phosphoseryl and phosphotyrosyl residues in proteins, neither 3CH134 nor YVH1 dephosphorylates the selection of phosphoseryl/threonyl substrates tested under conditions in which they exhibit PTPase activity. Nevertheless, given the similarity to the dual specificity PTPases VH1 and VHR, it is possible that 3CH134 may demonstrate phosphatase activity against phosphoseryl or phosphothreonyl residues in addition to phosphotyrosyl residues under physiological conditions with an appropriate substrate.

The identity of physiologically relevant substrates for 3CH134 remains to be established. In light of the rapid and transient appearance of 3CH134 mRNA and protein after stimulation of quiescent cells with serum or growth factors (5, 6, 8), a role in the regulation of early steps in signal transduction from growth factor receptor PTKs is possible. At least two sites of action would be consistent with such a role: the receptor PTKs or the downstream serine/threonine kinases that are themselves regulated by reversible tyrosine phosphorylation. As an example of the former category, we assayed Tyr(*P*)-BIRK, the recombinant catalytic domain of the insulin receptor, which has been used as a substrate for PTPases *in vitro* (12). No significant dephosphorylation by purified 3CH134 was detected. Candidate substrates in the latter category include the MAP kinases (18). A diverse array of hormones and growth factors elicit a rapid and transient activation of MAP kinases as an essential step in their signaling pathways. Maximal activation of MAP kinases requires phosphorylation of both a tyrosyl and a threonyl residue in the protein; dephosphorylation of either severely attenuates activity (31). Here we demonstrate that 3CH134 dephosphorylates Tyr(*P*)-$p42^{mapk}$ *in vitro* 15-fold more rapidly than Tyr(*P*)-RCML and 200-fold more rapidly than the phosphorylated synthetic peptide EDNDYINASL. The possibility that, among other potential physiological substrates, 3CH134 acts on members of the MAP kinase family *in vivo* clearly merits further attention.

We are very grateful to Tim Haystead of the University of Virginia for providing us with recombinant $p42^{mapk}$ substrate. Recombinant baculovirus expressing BIRK was provided by Melanie Cobb (University of Texas Southwestern Medical Center, Dallas) and prepared by Andrew Flint. We also thank Bob DelVecchio and Andrew Flint for helpful discussions and Carol Marcincuk for typing the manuscript. This work was supported by National Institutes of Health Grants CA53840 (N.K.T.) and CA46565 (L.F.L.). N.K.T. acknowledges support from the Hansen Memorial Foundation. C.H.C. was a predoctoral fellow of the National Science Foundation, H.S. is a recipient of a Damon Runyan–Walter Winchell Cancer Research Fund fellowship, N.K.T. is a Pew Scholar in the Biomedical Sciences, and L.F.L. is an Established Investigator of the American Heart Association.

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