Dyrk, a Dual Specificity Protein Kinase with Unique Structural Features Whose Activity Is Dependent on Tyrosine Residues between Subdomains VII and VIII*

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The cDNA of a novel, ubiquitously expressed protein kinase (Dyrk) was cloned from a rat brain cDNA library. The deduced amino acid sequence (763 amino acids) contains a catalytic domain that is only distantly related to that of other mammalian protein kinases. Its closest relative is the protein kinase Mnb of Drosophila, which is presumably involved in postembryonic neurogenesis (85% identical amino acids within the catalytic domain).

Outside the catalytic domain, the sequence comprises several striking structural features: a bipartite nuclear translocation signal, a tyrosine-rich hydrophilic motif flanking the nuclear localization signal, a PEST region, a repeat of 13 histidines, a repeat of 17 serine/threonine residues, and an alternatively spliced insertion of nine codons. A recombinant glutathione S-transferase-Dyrk fusion protein catalyzed autophosphorylation and histone phosphorylation on tyrosine and serine/threonine residues with an apparent Km of approximately 3.4 µM. Exchange of two tyrosine residues in the “activation loop” between subdomains VII and VIII for phenylalanine almost completely suppressed the activity and tyrosine autophosphorylation of Dyrk. Tyrosine autophosphorylation was also reduced by exchange of the tyrosine (Tyr-219) in a tyrosine phosphorylation consensus motif. The data suggest that Dyrk is a dual specificity protein kinase that is regulated by tyrosine phosphorylation in the activation loop and might be a component of a signaling pathway regulating nuclear functions.

Reversible phosphorylation of proteins represents the main mechanism of signal transduction in cells (Edelman et al., 1987; Cohen, 1992; Hunter, 1991). It is catalyzed by a large family of protein kinases that share structural similarities (11 subdomains) within a catalytic domain of about 300 amino acids (Hanks et al., 1988). Protein kinases appear to represent the largest family of enzymes (Hunter, 1987), and to date more than 100 mammalian protein kinases have been identified by molecular cloning and/or functional characterization. This large number of homologous proteins provides the basis of a complex signaling network that transmits and coordinates the response to extracellular stimuli.

Two major subgroups of protein kinases, the protein tyrosine kinases and the protein serine/threonine kinases, have been distinguished on the basis of functional but also of structural parameters (Hanks et al., 1988; Hunter, 1991). Initially it was assumed that the members of these subfamilies are specific for phosphorylation of either tyrosine or serine/threonine residues. More recently, however, several kinases have been identified that catalyze their autophosphorylation on both tyrosine and serine/threonine residues when isolated as recombinant proteins from Escherichia coli (Lindberg et al., 1992). In addition, two other kinases have dual specificity toward a specific substrate in vivo, i.e. in the intact cell. The dual specificity kinase MEK1 phosphorylates MAP kinase ERK2 on both threonine 183 and tyrosine 185 (Payne et al., 1991), thereby activating the kinase and presumably inducing its translocation to the nucleus. Similarly, the dual specificity kinase Wee1 of Schizosaccharomyces pombe appears to phosphorylate and inhibit the serine/threonine kinase Cdc2 by phosphorylation on tyrosine 15 (Lundgren et al., 1991).

In this paper, we report the cloning and characterization of a novel dual specificity protein kinase with unique structural features. The activity of this kinase appears to be regulated by tyrosine phosphorylation in the presumed activation loop between subdomains VII and VIII. Its sequence comprises a nuclear targeting motif, a PEST region, and two striking repeats of unknown function. It is suggested that the enzyme is part of a signaling pathway that controls nuclear functions.

MATERIAL AND METHODS

RNA Preparation and cDNA Synthesis—3T3-L1 cells (Green and Kehinde, 1974) were differentiated as described previously (Welland et al., 1993), washed twice with phosphate-buffered saline, frozen in liquid nitrogen, and lysed in a solution of 4 M guanidine thiocyanate and 7% mercaptoethanol. The lysates were layered on a cesium chloride cushion (5.88 M) and centrifuged at 28,000 rpm (rotor SW 40) for 29 h at 20 °C. Pelleted RNA was dissolved with 300 µl of 0.1 M sodium acetate/Tris buffer (pH 9.0) and was neutralized by the addition of 50 µl of 2 M potassium acetate (pH 5.5). First strand cDNA was synthesized with reverse transcriptase (First-strand cDNA synthesis kit, Pharmacia Biotech Inc.) by oligo(dT) priming.

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The abbreviations used are: MAP, mitogen-activated protein; PCR, polymerase chain reaction; bp, base pair(s); PAGE, polyacrylamide gel electrophoresis; PVDF, polyvinylene difluoride; GST, glutathione S-transferase.

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Cloning of Protein Serine/Threonine Kinases—Highly degenerate oligonucleotide primers were designed from the conserved regions V1b and IX (according to Hanks and Quinl (1991)) of the catalytic domain of protein serine/threonine kinases. Forward primer, 5′-A(C/T)/G(C/A)/T(A/G)/C/TG(A/BG)/C(T/G)/A/BG/AA-3′; reverse primer, 5′-A/G(T/A)/C(A/G)/C(T/G)/G(A/BG)/C(T/G)/A/BG/AA-3′. PCR amplification, separation of the products, and cloning was carried out as described previously (Becker et al., 1994). Plasmid DNA was isolated and characterized by sequencing or Southern blotting.

Library Screening and DNA Sequencing—Cloned PCR products were isolated with restriction enzymes and used as probes to screen a rat fetal rat brain cDNA library (Stratagene, La Jolla, CA, catalog no. 101164). Deletions were generated by exonuclease digestion and by sonication (Branson sonifier 450, cup horn, 1 min at maximum setting) and were sequenced by the method of Sanger (T7-sequencing kit, Pharmacia). Sequences were determined with overlapping fragments from both directions.

Northern Blot Analysis—Samples of total RNA (20 μg) dissolved in a denaturing solution containing formaldehyde (6.5%) and formamide (50%) and incubated at 65°C for 15 min were separated by electrophoresis through 1% agarose gels containing 6.5% formaldehyde. Gels were stained with ethidium bromide before transfer onto nylon membranes (Hybond, Amersham-Buchner, Braunschweig, Germany) in order to ensure that equal amounts of total RNA had been loaded. Membranes were hybridized at 42°C with partial cDNA probes labeled with [32P]dCTP by random oligonucleotide priming (Feinberg and Vogelstein, 1983). The blots were washed twice at 55°C in 0.1× sodium citrate, 0.1% SDS and once with 0.015× sodium citrate, 0.1% SDS.

Preparation of Recombinant GST-Dyrk—A PCR fragment (bp 112-745) of the Dyrk cDNA (clone B) comprising an EcoRI site before the initiation codon was prepared and used for construction of an expression vector pGEX-2T (Pharmacia) harboring the cDNA of Dyrk (bp 119-2414) fused with that of GST in a single open reading frame. Due to the cloning strategy, the two amino acids before the stop codon were expected to linker-derived segments. PCR products encoding full length were verified by sequencing. Site-directed mutagenesis with degenerate oligonucleotide primers (less than 20% of the template was replaced as described previously (Kunkel et al., 1987). Mutants were confirmed by sequencing and subcloned into pGEX. Transformants in E. coli DH5α were isolated, induced with isopropyl-1-thio-β-D-galactopyranoside for 2 h at 37°C, and lysed by sonication. The recombinant fusion protein was purified by affinity chromatography to glutathione-Sepharose and eluted with glutathione as described by the manufacturer (Pharmacia). The concentration of active recombinant kinase was determined by laser densitometry of the Coomassie-stained 90-kDa product in comparison with bovine serum albumin standards.

Expression of Dyrk in COS-7 Cells—The cDNA of Dyrk was subcloned in the multiple cloning site of the mammalian expression vector pSVL, which contains a translation start and a short tag sequence for immunochemical detection (HA protein of influenza virus). In addition, the C terminus was extended by a short linker-derived segment by the cloning strategy. Transfection of COS-7 cells was performed as described previously (Wandel et al., 1994). For immunochemical detection, cells were lysed by boiling in 1% SDS in 10 mM Tris buffer (pH 7.4). For immunoprecipitation, cells were lysed in buffer containing 50 mM Hepes, 150 mM NaCl, 2 mM EDTA, 20 mM NaF, 2 mM sodium pyrophosphate, 1 mM phenylmethylsulfonyl fluoride, 1 mM sodium vanadate, 2 μg/ml aprotenin, 2 μg/ml leupeptin, 1 μg/ml pepstatin, and 1% Nonidet P-40. Antiserum against the HA epitope was from BabCo, Richmond, CA. As controls, samples were isolated with protein A-Sepharose and subjected to a kinase assay (see below) that was carried with the adsorbed material in the presence of histone. The products were separated by SDS-PAGE and detected by autoradiography.

Autophosphorylation of Recombinant GST-Dyrk Transferred to PVDF Membranes—Samples of the fusion proteins were separated by SDS-PAGE, transferred onto PVDF membranes (Immobilon-P, Millipore, United Kingdom). Proteins were denatured and renatured as described previously (Ferrill and Martin, 1991), and the membranes were incubated in 2 ml of phosphorylation buffer consisting of 33 mM Hepes, 6.6 mM manganese chloride, 6.6 mM magnesium chloride, 0.7 mM dithiothreitol, and [32P]ATP (40 μCi). Membranes were washed as described by Ferrill and Martin (1991) and autoradiographed for 16 h.

Assay of Protein Kinase Activity—Samples of the fusion proteins were incubated in phosphorylation buffer containing [32P]ATP (2 μCi, tracer only or final concentration of 10–100 μM as indicated) in a total volume of 12 μl for 30 min at room temperature. Histone (type I1S, catalog no. H6005, Sigma), α-casein (catalog no. C8032), or poly-Glu/Tyr 4.1 (catalog no. P0275) were added to a final concentration of 0.66 μg/μl or as indicated. Thereafter, the samples were boiled with Laemmli’s sample buffer (Laemmli, 1970) and separated on 10 or 14% polyacrylamide gels. The gels were dried and autoradiographed for 1–4 h.

Assay of Tyrosine Phosphorylation of GST-Dyrk—Samples of the fusion protein GST-Dyrk were separated on 10% polyacrylamide gels, transferred onto nitrocellulose membranes, and probed with anti-phosphotyrosine antibodies (Transduction laboratories, Nottingham, UK).

Phosphoamino Acid Analysis—Autophosphorylation of recombinant GST-Dyrk was carried out as described above. Reaction products were precipitated with trichloroacetic acid, and hydrolyzed in 5.8 M hydrochloric acid for 2 h at 110°C. The lysates were dried under nitrogen, washed with water, and dissolved in 10 μl of water supplemented with carrier phosphoamino acids. The phosphoamino acids were separated by thin-layer chromatography (silica gel, 25% ammonium hydroxide, 6% ethanol, 1.635). In a separate experimental series, phosphorylation was carried out in the presence of histone. The reaction products were separated by SDS-PAGE and transferred onto PVDF membranes. The phosphorylated histone was identified by autoradiography, cut, and hydrolyzed as described above.

RESULTS

Cloning of Dyrk—Degenerate oligonucleotide primers matching the regions V1b and IX according to the nomenclature of Hanks and Quinl (1991) were designed to amplify a domain of about 150 bp, including the activation loop of protein serine/threonine kinases. PCR products were cloned, and 70 clones encoding 13 different sequences were identified as protein kinases by a data base search or on the basis of structural criteria. One of them (PSK47, later designated Dyrk) was chosen for further characterization.

Three hybridizing cDNA clones (A, B, and D) of different lengths were isolated from a rat brain cDNA library and characterized by restriction mapping and/or sequencing. One of these clones (clone B, 5 kilobase pairs) contained a full reading frame and a poly(A) tail. Clone A contained a poly(A) tail but lacked 2.3 kilobase pairs in the 3′-untranslated region; clone D had a deletion of 27 bp within the open reading frame (see below).

Structural Characteristics of Dyrk—The amino acid sequence as depicted in Fig. 1A was deduced from an open reading frame (clone B) in which the translation start was assigned to the first AUG codon after a stop codon. The sequence of clone D differed from that of clone B by a deletion of 27 bp within the open reading frame (codons 69–78, shaded box). In order to confirm that two different RNA species were present in rat brain, the corresponding domain was amplified by PCR with cDNA from rat brain as the template. Indeed, two bands of the expected size were identified (data not shown). Thus, it appears reasonable to conclude that the mRNA of Dyrk is alternatively spliced, generating two products that differ by 27 nucleotides.

The deduced amino acid sequence (763 amino acids) contains all conserved regions of the catalytic domain (amino acids 159–479) according to the classification of Hanks and Quinl (1991) (subdomains I–XI, Fig. 1B). In addition, the sequence exhibits several striking characteristics (Fig. 1B). First, the sequence harbors a bipartite nuclear targeting sequence (Robbins et al., 1991; Dingwall and Laskey, 1991) flanking the N-terminal side of the catalytic domain. Second, the amino acid sequence of Dyrk comprises several tyrosine residues that may represent phosphorylation sites. Two tyrosines located between the subdomains VII and VIII are potential phosphorylation sites regulating the activity of the catalytic domain, in analogy to other protein kinases, e.g. ERK/MPAK and GSK3β (see Fig. 7) (Rossomando et al., 1992; Hughes et al., 1993). Dyrk then contains two other putative consensus motifs of tyrosine phosphorylation (Tyr-112 and Tyr-219), which are preceded by lysine and glutamic acid, 7 or 3 residues to the N-terminal side,
respectively (Cooper et al., 1984). In addition, a tyrosine-rich, highly charged motif that contains 4 tyrosines and 5 aspartic acid residues is located between the nuclear targeting domain and the N terminus of the catalytic domain. As a third unique structural feature of Dyrk, the large domain flanking the C terminus of the catalytic domain contains several striking motifs. Immediately following the catalytic domain, a serine/threonine-rich region fulfills the requirement of a PEST region. These motifs represent domains abundant in proline, glutamic acid, serine, and threonine and are believed to initiate a rapid degradation of the protein (Rogers et al., 1986). Furthermore, the C terminus of Dyrk harbors a stretch of 13 consecutive histidine residues (amino acids 607–619), and a domain containing an unusually high portion of serine and threonine residues (46 serines out of a total of 284 amino acids). Within the serine/threonine-rich segment is a stretch (amino acids 659–672) of 17 subsequent serine/threonine residues.

Data base searches turned up several partial human and mouse cDNA sequences (expressed sequence tags) with high similarity to the nucleotide sequence of Dyrk. Two of these sequence tags (mouse, accession no. Z31282; human, accession no. L25452) comprise the histidine repeat, indicating that the amino acid sequence of this repeat is fully conserved. The chromosomal localization of the latter sequence tag has been determined (Cheng et al., 1994) by hybridization to a set of mapped YAC's derived from chromosome 21. Based on the high similarity of this sequence tag with Dyrk (95% identical nucleotides), it appears safe to conclude that the chromosomal localization of the human homologue of Dyrk is 21q22.2.

Relationship of Dyrk with Other Kinases—Data bases were searched (SWISS-PROT, the Protein Identification Resource, EMBL data base) in order to find relatives of Dyrk by a comparison of its catalytic domain with that of known protein serine/threonine kinases, and a dendrogram of a selected number of protein kinases was constructed (Fig. 2A). The dendrogram demonstrates that the structure of the catalytic domain of Dyrk differs considerably from that of most other kinases. Its closest relative (85% identical amino acids within the catalytic domain) is encoded by a gene from Drosophila (mnb), which has recently been found disrupted in a mutant with abnormal neurogenesis (minibrain; Tejedor et al., 1995).
FIG. 2. Comparison of the catalytic kinase domain of Dyrk with that of other protein kinases. A, dendrogram of an alignment of the catalytic domains of Dyrk and other protein serine/threonine kinases. The dendrogram was constructed with the PILEUP program. Data base accession numbers (Protein Identification Resource) are as follows: Yak1, A32582; CDC2, A29539; CDK4 (cyclin-dependent kinase 4), J N0460; CDK5, A46365; ERK2/MAP-kinase, S16444; GSK3b (glycogen synthase kinase 3b), S14708; MEK1 (MAPK/ERK-kinase), S29863; PKA (cAMP-dependent protein kinase), S21640; PKC, A26037; TIK, A40813; ESK, A44439. GenBank accession numbers were as follows: Mnb, X70794; Clk1, L29219; Clk2, L29218; Clk3, L29217; P38, L25253; JNK1 (jun-kinase 1), L26318. Asterisks indicate known dual specificity kinases. B, sequence alignment of the catalytic domains of Dyrk, Mnb, PSK-H2, and Yak1. The deduced amino acid sequences of the catalytic domains were aligned with the aid of the CLUSTAL program (gap penalty 5, open gap cost 10, unit gap cost 10). Hyphens represent gaps introduced for optimal alignment. [ ] indicates a portion of the sequence of PSK-H2 that was not shown in the source (Hanks and Quinn, 1991). The kinase subdomains according to the nomenclature of Hanks (Hanks and Quinn, 1991) are depicted on the top of the alignment by Roman numerals. Residues identical with Dyrk are boxed. Asterisks indicate amino acids identical in all compared sequences; periods designate conservative substitutions. References for the kinases are as follows: Mnb (Tejedor et al., 1995); PSK-H2 (Hanks and Quinn, 1991); Yak1 (Garrett and Broach, 1989).
The closest relative (46% identical amino acids) is a partial human serine/threonine kinase sequence (PSK-H2) that was published without further information. The dendrogram depicts an alignment of Mnb, PSK-H2, and Yak1 with Dyrk, demonstrating that the identities within this subfamily are mainly restricted to the subdomains I, II, III, V, VIb, VII, VIII, IX, and XI of the catalytic domain. There is no similarity outside the catalytic domains (comparison not shown) except for 70 amino acids flanking the N terminus of the catalytic domain of Mnb. Thus, Dyrk is a novel member of a small subfamily of protein kinases with a unique structure of both the catalytic domain and the flanking N- and C-terminal regions.

Expression of Dyrk mRNA in Various Rat Tissues—Northern blot analysis of total RNA from a series of different rat tissues detected two transcripts (2.8 and 5.4 kb) in all tissues examined (brain, heart, skeletal muscle, lung, intestine, fat cells, adrenal gland, testis, thymus, kidney, liver, and spleen; data not shown). These sizes correspond reasonably well with those derived from the isolated clones. Although mRNA levels appeared somewhat different in the various tissues, the data indicate a ubiquitous expression of Dyrk mRNA.

Protein Kinase Activity of Dyrk—In order to demonstrate the protein kinase activity of Dyrk, a recombinant GST-Dyrk fusion protein was expressed in E. coli DH5α and was purified by affinity adsorption on glutathione-Sepharose. Analysis of the partially purified fusion protein by SDS-PAGE showed a major product of an apparent molecular mass of 90 kDa and several other bands of higher mobility (60, 41, and 30 kDa; Fig. 3A). All bands appear to be derived from the recombinant GST-Dyrk fusion protein because they were not present in control preparations of GST alone. As judged from the calculated molecular weight of GST-Dyrk (112 kDa), only fragments of the protein kinase were isolated. Variation of the conditions of expression and isolation (temperature, duration of incubation, addition of protease inhibitors) failed to prevent the partial degradation of the fusion protein or allow the isolation of a 112-kDa protein. However, the truncated 90-kDa GST-Dyrk exhibited a marked protein kinase activity that was detected after renaturing of the protein on PVDF membranes (Fig. 3B). As is also illustrated in Fig. 3B, the smaller fragments lacked a detectable protein kinase activity. Preliminary experiments were carried out in order to find out which domain(s) were removed from the 90-kDa fragment. Thrombin cleavage of the 90-kDa GST-Dyrk, presumably at the cleavage site engineered in the linker between GST and Dyrk, generated an active kinase of approximately 60 kDa (data not shown). Furthermore, the 90-kDa fragment appeared to cross-react with an antibody against GST.

Fig. 4, C illustrates a series of experiments designed to further characterize the protein kinase activity of Dyrk. In vitro protein kinase assays with recombinant Dyrk in the presence of magnesium, manganese, and [32P]ATP indicated that the kinase, in addition to autophosphorylating the 90-kDa fragment, stimulated a marked 32P incorporation into its smaller fragments (Fig. 3C, first lane). Furthermore, the fusion protein catalyzed the 32P incorporation into histone and casein but failed to phosphorylate the tyrosine kinase substrate poly-Glu/Tyr. A quantitative assessment of the stoichiometry of autophosphorylation of the 90 kDa band (Fig. 3C, first lane) indicated that 1 mol of phosphate was incorporated per 8 mol of recombinant protein kinase, suggesting that the protein was already phosphorylated in E. coli (see Fig. 5A).
**Dual Specificity Protein Kinase Dyrk**

In vitro autophosphorylation in the presence of [32P]ATP. The reaction products were hydrolyzed and separated by thin-layer chromatography as described under "Materials and Methods." The positions of carrier phosphotyrosine (PY) and phosphoserine/phosphothreonine (PS/PT) as determined by ninhydrine staining are marked. C and D, tyrosine phosphorylation of histone by Dyrk. Histone was phosphorylated by partially purified Dyrk in the presence of indicated bivalent ions or EDTA. The reaction products were separated by SDS-PAGE and subjected to autoradiography (C). D, additional samples prepared by the same procedure were transferred onto PVDF membranes (14% gel, right panel). The phosphorylated 13 kDa band was cut out of the membrane and hydrolyzed. Aliquots adjusted for their content of radioactivity were separated by thin-layer chromatography and subjected to autoradiography.

**Fig. 5. Dual specificity protein kinase activity of Dyrk: autophosphorylation and histone phosphorylation on tyrosine and serine/threonine residues.** A, tyrosine autophosphorylation of the 90-kDa fragment of Dyrk as detected with anti-phosphotyrosine antibody. Partially purified GST-Dyrk was separated by SDS-PAGE (10% gel) and transferred onto a nitrocellulose membrane, and phosphotyrosine was detected immunologically with specific antisera. B, detection of phosphotyrosine in hydrolysates of phosphorylated GST-Dyrk. Partially purified GST-Dyrk was subjected to an in vitro autophosphorylation in the presence of [32P]ATP. The reaction products were hydrolyzed and separated by thin-layer chromatography as described under "Materials and Methods." The positions of carrier phosphotyrosine (PY) and phosphoserine/phosphothreonine (PS/PT) as determined by ninhydrine staining are marked. C and D, tyrosine phosphorylation of histone by Dyrk. Histone was phosphorylated by partially purified Dyrk in the presence of the indicated bivalent ions or EDTA. The reaction products were separated by SDS-PAGE and subjected to autoradiography (C). D, additional samples prepared by the same procedure were transferred onto PVDF membranes (14% gel, right panel). The phosphorylated 13 kDa band was cut out of the membrane and hydrolyzed. Aliquots adjusted for their content of radioactivity were separated by thin-layer chromatography and subjected to autoradiography.

Five B). As anticipated, phosphotyrosine as well as phosphoserine/phosphothreonine were detected. In addition, hydrolysis and amino acid analysis of histone (11–15 kDa), which was phosphorylated by Dyrk revealed that the kinase catalyzed a marked tyrosine phosphorylation of the exogenous substrate (Fig. 5, C and D).

It was reported previously that the serine kinase phosphotyrosylase kinase exhibited a tyrosine kinase activity toward angiotensin II in the presence of manganese (Yuan et al., 1993). Thus, we studied the dependence of both total and tyrosine phosphorylating activity of Dyrk on bivalent ions (Fig. 5C). Magnesium alone was sufficient for the total protein kinase activity but was much less effective than manganese; EDTA fully suppressed the kinase activity of Dyrk. The tyrosine kinase activity of Dyrk does not appear to require the presence of manganese, since the ratio of phosphotyrosine to phosphoserine/threonine was identical with both bivalent ions (Fig. 5D).

In order to further characterize the protein kinase activity of Dyrk, a preliminary kinetic analysis of the histone phosphorylation was performed (Fig. 6). The Km value of the total phosphorylation reaction obtained in the presence of 40 µM ATP was 0.044 µg/µl or 3.4 µM (slope of the Lineweaver-Burke plot as determined by linear regression: 2.01 ± 0.14 (min × µg/(pmol × µl)) and an ordinate intercept of 45.9 ± 8.8 pmol from which K_m = 0.044 µg/µl and V_max = 2.2 nmol/(min × µg) were calculated, respectively. Alliquots of the phosphorylation were subjected to phosphoamino acid analysis (inset).
\textbf{FIG. 7. Sequence comparison of protein kinases that are regulated by phosphorylation in the presumed activation loop between domains VII and VIII.} Amino acids are given in single-letter code; residues identical with the corresponding amino acids in Dyrk are designated by an asterisk. Residues above tyrosines or threonines indicate residues that have been chemically phosphorylated, and residues below tyrosines or serines indicate residues that have been immunologically phosphorylated.

\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
Dyrk & 307 & DPG & SSCPQGR & ------ & YQYQOSRFY & RAPE \\
GSK-3\beta & 264 & \ldots & AKVRRR & VRCMKH & VSTC & \ldots \\
ERK2 & 164 & \ldots & LARVAPDDHHGCPL & FTT & VAT & \ldots \\
JNK1 & 165 & \ldots & LARTA & TGF & VMTFY & VTY & \ldots \\
p38 & 168 & \ldots & LARHIDDE & ------ & VAT & \ldots \\
PKA & 184 & \ldots & FAEVKQGK & TW & ------ & TLGCTPE & \ldots \\
\hline
\end{tabular}

The subdomains VII and VIII are somewhat different from those in the other tyrosine-regulated protein kinases. They contain a single tyrosine residue or a threonine residue, and their activation loop is more conserved among the various kinases.

\textbf{DISCUSSION}

The present data indicate that Dyrk is a dual specificity protein kinase that catalyzes its autophosphorylation on both serine/threonine and tyrosine residues. Moreover, the data give a first insight into the regulation of Dyrk. Its kinase activity depends on the presence of tyrosine residues between subdomains VII and VIII (activation loop, Fig. 7). Thus, in analog to the serine/threonine kinases ERK2 (Payne et al., 1991; Rosomando et al., 1992), GSK3\beta (Hughes et al., 1993), JNK1 (Derijard et al., 1994), and p38 (Han et al., 1994), the conclusion appears to be justified in that the activation of Dyrk depends on the phosphorylation of one or both of these tyrosines. It should be noted that the activation motif in Dyrk (YQY, see Fig. 7) is somewhat different from that in the other tyrosine-regulated serine kinases, which contain a single tyrosine residue or a tyrosine and a threonine. C-terminal to the YQY motif are three residues (RFY), which are conserved in all other tyrosine phosphorylation-regulated kinases (Fig. 7) and might therefore belong to the activation motif. The YQY motif resembles that present in Mn\(b\) (YHY), Yak1 and PSK-H2 (YTY), and we anticipate on the basis of this structural similarity that these kinases are also activated by tyrosine phosphorylation in the activation loop. It remains to be elucidated whether the activation of Dyrk is regulated by a protein kinase or by an activator of autophosphorylation.
(ERK2, Wu et al. (1991); GSK3β, Wang et al. (1994)), others can stimulate both autophosphorylation and phosphorylation of exogenous substrates on tyrosine (e.g., MEK, Zheng and Guan (1993)). Dyrrk appears unique in that it catalyzed both autophosphorylation and histone phosphorylation on tyrosine, but it failed to phosphorylate poly-Glu/Tyr. Thus, the substrates of Dyrrk appear to require specific consensus motifs, which may not be identical with those of other kinases.

Dyrrk exhibits several striking structural characteristics of potential functional relevance. The sequence harbors a bipartite nuclear targeting motif at amino acids 117–134 that consists of 2 basic amino acids, a spacer of 10 amino acids, and 4 additional basic amino acids. Similar motifs have previously been found in a number of nuclear proteins, e.g., steroid hormone receptors, transcription factors, and enzymes and proteins involved in transcription or mitosis (Dingwall and Laskey, 1991). This motif appears to be a reliable indicator of nuclear localization since it is found in about 50% of nuclear proteins but in less than 5% of nonnuclear proteins.

The region of Dyrrk flanking the C terminus of its catalytic domain contains an unusual portion of uncharged hydrophilic amino acids (31 threonines and 46 serines in a total of 284 amino acids). A computerized search revealed that a portion of this region immediately following the catalytic domain fulfilled the requirement of a PEST region (Rogers et al., 1986). Because PEST regions are typical of rapidly metabolized proteins, they are believed to signal their degradation (Rogers et al., 1986). The PEST region in Dyrrk showed a score that would rank second among those listed (Rogers et al., 1986). In addition to the PEST region, the C-terminal portion of Dyrrk harbors a repeat of 13 histidines (amino acids 607–619) and a stretch of 13 subsequent serine/threonine residues (amino acids 659–672). A data base search revealed that similar histidine repeats have previously been found in other proteins, e.g., a protein kinase from yeast (SNF1, Celenza and Carlson, 1986) and several transcription factors (e.g., accession no. P39020, P15463, and P25490), but their exact function is as yet unclear.

At present, we can only speculate on the possible function of Dyrrk on the basis of its structural features and its autophosphorylation on tyrosine residues. The closest relative of Dyrrk is the mnb gene from Drosophila with 85% identity (Kunkel, T. A., Roberts, J. D., and Zakour, R. A. (1987) Methods Enzymol. 154, 367–382). The mnb gene encodes a protein that transduces receptor-initiated signals to a nuclear phosphotyrosine kinase and is a component of a similar signaling pathway, possibly mediating the specific phosphorylation of transcription factors within the nucleus.

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