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Identification of Mouse ULK1, a Novel Protein Kinase Structurally Related to *C. elegans* UNC-51

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A novel protein kinase related to the *C. elegans* serine/threonine kinase UNC-51 was cloned from mouse. The UNC-51-Like Kinase (ULK)1 is encoded by a cDNA of 1051 amino acids with calculated MW of 113 kDa. Comparison of the ULK1 and UNC-51 shows the highest conservation in the amino-terminal kinase domain, which is followed by a proline/serine-rich (PS) domain and a conserved carboxyl-terminal (C) domain. ULK1 mRNA is expressed in various tissues, and is mapped to mouse chromosome 5F and rat chromosome 12q16.3, by fluorescent *in situ* hybridization. HA-tagged ULK1 is expressed as a protein of ~150 kDa in COS7 cells and is auto-phosphorylated *in vitro* in its PS domain. We propose that ULK1, UNC-51 and a yeast protein kinase Apg1p comprise a novel subfamily of protein kinase, which is structurally conserved among eukaryotes. © 1998 Academic Press

The protein kinase family contains hundreds of diverse but related enzymes that regulate various aspects of growth, differentiation, metabolism and gene expression in the eukaryotic cells. A large number of genes encoding protein kinases have been identified from various species, revealing that they form one of the largest gene family (1). For example, completion of *S. cerevisiae* genome sequencing has unraveled 113 protein kinase genes, which correspond to about 2% of the total genes with most of the major vertebrate kinase subfamilies being represented in yeast (2).

The nematode *C. elegans* has been proved an excellent organism for understanding many developmental processes based on the ease and power of genetic, molecular and phenotypic analyses. The rapidly accumulating genome data from *C. elegans* has substantially emphasized its importance as a model organism, since many genes have turned out to be homologous between nematode and mammals. In fact, more than half of positionally cloned genes associated with human diseases show similarities to *C. elegans* genes, and occasionally the *C. elegans* gene is the only similar gene available in all the public databases (3). These similarities have in some cases suggested function of predicted genes and in other cases have been used to find candidate mammalian genes associated with certain mutants.

More than 10 genes have been reported to be required for axonal guidance in *C. elegans,* many of which have homologous genes in mammals or other vertebrates. For example, UNC-6 and netrin (4), UNC-40 and DCC (deleted in colorectal cancer) (5), UNC-33 and CRMP-62 (collapsin response mediator protein) (6), UNC-44 and human brain ankyrin (7), and UNC-5 and UNC-5H(8), RCM (rostral cerebellar malformation gene) (9) are homologues between *C. elegans* and mammalian species. However, mammalian counterpart of UNC-51, a recently cloned serine/threonine protein kinase essential for axonal elongation and guidance (10) has not been identified. Based on the conservation of molecules involved in axon guidance and elongation, it is conceivable that UNC-51 also has a mammalian counterpart, which may play an important role in this process.

Here we show the cloning, expression and gene mapping of a novel mouse protein kinase ULK1, which is structurally related to UNC-51. ULK1 was expressed among various tissues, suggesting that it may be involved in some of the basic cellular processes.

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MATERIALS AND METHODS

Cloning of ULK1 cDNA. The initial fragment of ULK1 cDNA was cloned by PCR using degenerate oligonucleotides, 5′-ca(tc)cg(gatc)-ga(ct)tt(ag)aa(ag)cc-3′, 5′-tc(gatc)gg(gatc)gccat(ag)tacat-3′ from rat brain cDNA. The cycling parameters for PCR were 94 °C for 30 sec, 37 °C for 30 sec, 2 min ramp to 72 °C then 72 °C for 1min for the first 3 cycles, followed by 94 °C for 30 sec, 54 °C for 30 sec, and 72 °C for 1 min for 35 cycles. PCR products were separated on polyacrylamide gel electrophoresis and DNA fragments of desired size were isolated, subcloned into a pT7-7 vector (Novagene, Madison, WI) and were sequenced. The insert of the PCR-derived fragment was labeled with [32P]α-dCTP (>3000 Ci/mmol) using Megaprime kit (Amersham, Buckinghamshire, UK) to screen mouse embryo and brain cDNA libraries (Clontech, Palo Alto, CA). To obtain the 5′ end of the cDNA, 5′RACE was performed using specific primers 5′-gaattagcc-atttcctggaag-3′, 5′-cttgttaatgcatttgacggccac-3′ and neonatal mouse brain mRNA as a template. Reverse transcription and PCR reaction were carried out by using 5′RACE System (GIBCO BRL, Gaithersburg, MD) according to the manufacturer's protocol. Sequencing was done using PRISM dye terminator cycle sequence kit (Perkin Elmer, Foster City, CA) and an ABI377 automated sequencer.

Construction of ULK1 expression plasmids. ULK1 cDNA was inserted into *Xho*I-*Not* I cloning sites of pME18S-HA vector, a version of SRα expression vector (11) to express fusion proteins with HA tag at the amino-terminus. A kinase defective mutant ULK1Δ and truncation mutants ULK1(507), ULK1(427), ULK1(351), and ULK1(287) were constructed by using GeneEditor (Promega, Madison, WI) with mutagenesis primers 5′-cgacctggaggtggccgttaactgcat-taacaagaagaacc-3′, 5′-cttctccccaagtgtaaaccatcccagagc-3′, 5′-gcaaca-ggtacggttaatcggtccccattcc-3′, 5′-agcagcaaagactcctaagacacagatgac-3′ and 5′-ccatcaagaaatcctaacctgtgcctgtgc-3′, respectively. Mutation sites were checked by sequencing and subcloned into the pME18S-HA expression vector.

Transfection and immunoblotting analysis of ULK1. Expression plasmids for HA-tagged ULK1 or mutants were transfected into

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1     gcgccgccccggggcccgagcccgctcgcgcccccggccccgcgcgcgct 49
50    ATGGAGCCGGGCCGCGGCGGCGTCGAGACCGTGGGCAAGTTCGAGTTCTCTCGCAAGGAC 109
1     M  E  P  G  R  G  G  V  E  T  V  G  K  F  E  F  S  R  K  D  20
110   CTGATTGGACACGGCGCCTTCGCGGTGGTCTTCAAGGGTCGACACCGCGAGAAGCACGAC 169
21    L  I  G  H  G  A  F  A  V  V  F  K  G  R  H  R  E  K  H  D  40
170   CTGGAGGTGGCCGTCAAATGCATTAACAAGAAGAACCTTGCCAAGTCCCAAACACTGCTG 229
41    L  E  V  A  V  K  C  I  N  K  N  L  A  K  S  Q  T  L  L  60
230   GGAAAGGAAATCAAAATCCTGAAGGAACTAAAGCACGAAAACATCGTGGCGCTGTATGAC 289
61    G  K  E  I  K  I  L  K  E  L  K  H  E  N  I  V  A  L  Y  D  80
290   TTCCAGGAAATGGCTAATTCTGTCTACCTGGTCATGGAGTATTGTAATGGTGGAGACCTG 349
81    F  Q  E  M  A  N  S  V  Y  L  V  M  E  Y  C  N  G  G  D  L  100
350   GCTGACTACCTGCACACTATGCGCACACTGAGTGAAGACACTGTCAGGCTTTTCCTACAG 409
101   A  D  Y  L  H  T  M  R  T  L  S  E  D  T  V  R  L  F  L  Q  120
410   CAGATCGCTGGCGCCATGCGGCTGCTGCACAGCAAGGGCATCATCCACCGGGACCTGAAG 469
121   Q  I  A  G  A  M  R  L  L  H  S  K  G  I  I  H  R  D  L  K  140
470   CCCCAGAACATCCTGCTGTCCAATCCTGGGGGCCGCCGGGCCAACCCCAGCAACATCCGA 529
141   P  Q  N  I  L  L  S  N  P  G  G  R  R  A  N  P  S  N  I  R  160
530   GTCAAGATTGCTGACTTTGGATTCGCTCGGTACCTCCAGAGCAACATGATGGCGGCCACA 589
161   V  K  I  A  D  F  G  F  A  R  Y  L  Q  S  N  M  M  A  A  T  180
590   CTCTGTGGTTCTCCTATGTACATGGCTCCTGAGGTCATTATGTCCCAGCACTACGATGGA 649
181   L  C  G  S  P  M  Y  M  A  P  E  V  I  M  S  Q  H  Y  D  G  200
650   AAGGCTGACCTGTGGAGCATTGGCACCATTGTCTACCAGTGTCTGACAGGGAAGGCCCCT 709
201   K  A  D  L  W  S  I  G  T  I  V  Y  Q  C  L  T  G  K  A  P  220
710   TTTCAGGCCAGCAGCCCTCAGGATTTGCGCCTGTTTTATGAGAAGAACAAGACACTAGTT 769
221   F  Q  A  S  S  P  Q  D  L  R  L  F  Y  E  K  N  K  T  L  V  240
770   CCTGCCATCCCCCGGGAGACATCAGCTCCCCTGCGGCAGCTGCTCCTGGCTCTGTTGCAG 829
241   P  A  I  P  R  E  T  S  A  P  L  R  Q  L  L  L  A  L  L  Q  260
830   CGGAACCACAAGGACCGCATGGACTTTGATGAATTTTTCCACCACCCTTTCTTGGATGCC 889
261   R  N  H  K  D  R  M  D  F  D  E  F  F  H  H  P  F  L  D  A  280
890   AGCACCCCCATCAAGAAATCCCCACCTGTGCCTGTGCCCTCATATCCAAGCTCAGGGTCT 949
281   S  T  P  I  K  K  S  P  P  V  P  V  P  S  Y  P  S  S  G  S  300
950   GGCAGCAGCTCCAGCAGCAGCTCTGCCTCCCACCTGGCCTCTCCACCGTCCCTGGGGGAG 1009
301   G  S  S  S  S  S  S  S  A  S  H  L  A  S  P  P  S  L  G  E  320
1010  ATGCCACAGCTACAGAAGACCCTTACCTCCCCAGCCGATGCTGCTGGCTTTCTTCAGGGC 1069
321   M  P  Q  L  Q  K  T  L  T  S  P  A  D  A  A  G  F  L  Q  G  340
1070  TCCCGGGACTCTGGTGGCAGCAGCAAAGACTCCTGTGACACAGATGACTTTGTCATGGTC 1129
341   S  R  D  S  G  G  S  S  K  D  S  C  D  T  D  D  F  V  M  V  360
1130  CCAGCCCAGTTTCCAGGTGATCTAGTTGCTGAGGCAGCCAGTGCCAAGCCCCCACCTGAT 1189
361   P  A  Q  F  P  G  D  L  V  A  E  A  A  S  A  K  P  P  P  D  380
1190  AGCCTGCTGTGTAGTGGGAGCTCATTGGTGGCCTCTGCTGGCCTAGAGAGCCACGGCCGT 1249
381   S  L  L  C  S  G  S  S  L  V  A  S  A  G  L  E  S  H  G  R  400
1250  ACCCCCTCTCCCTCTCCGACCTGCAGCAGCTCTCCCAGCCCCTCTGGCCGGCCTGGCCCC 1309
401   T  P  S  P  S  P  T  C  S  S  S  P  S  P  S  G  R  P  G  P  420
1310  TTCTCCAGCAACAGGTACGGTGCCTCGGTCCCCATTCCTGTCCCCACTCAGGTGCACAAT 1369
421   F  S  S  N  R  Y  G  A  S  V  P  I  P  V  P  T  Q  V  H  N  440
1370  TACCAGCGCATCGAGCAAAACCTGCAATCGCCCACTCAACAGCAGACAGCCCGGTCCTCT 1429
441   Y  Q  R  I  E  Q  N  L  Q  S  P  T  Q  Q  Q  T  A  R  S  S  460
1430  GCCATCCGAAGGTCAGGGAGCACCACCCCCTGGGCTTTGGCCGGGCCAGCCCATCACCC 1489
461   A  I  R  R  S  G  S  T  T  P  L  G  F  G  R  A  S  P  S  P  480
1490  CCCTCCCACACCGATGGGGCCATGCTGGCCAGGAAGCTGTCACTTGGAGGTGGCCGTCCC 1549
481   P  S  H  T  D  G  A  M  L  A  R  K  L  S  L  G  G  G  R  P  500
1550  TACACACCTTCTCCCCAAGTGGGAACCATCCCAGAGCGACCCAGCTGGAGCAGAGTGCCC 1609
501   Y  T  P  S  P  Q  V  G  T  I  P  E  R  P  S  W  S  R  V  P  520
1610  TCCCCACAAGGAGCTGATGTGCGGGTTGGCAGGTCACCACGACCCGGTTCCTCTGTGCCT 1669
521   S  P  Q  G  A  D  V  R  V  G  R  S  P  R  P  G  S  S  V  P  540
1670  GAGCACTCTCCAAGAACCACTGGGCTGGGCTGCCGCCTGCACAGTGCCCCTAACCTGTCC 1729
541   E  H  S  P  R  T  T  G  L  G  C  R  L  H  S  A  P  N  L  S  560
1730  GACTTCCATGTTGTGCGTCCCAAGCTGCCTAAGCCCCCAACAGACCCACTGGGAGCCACC 1789
561   D  F  H  V  V  R  P  K  L  P  K  P  P  T  D  P  L  G  A  T  580
1790  TTTAGCCCACCCCAGACCAGCGCACCCCAGCCATGCCCAGGGCTACAGTCTTGCCGGCCA 1849
581   F  S  P  P  Q  T  S  A  P  Q  P  C  P  G  L  Q  S  C  R  P  600

1850  CTGCGTGGCTCACCTAAGCTGCCTGACTTCCTACAGCGGAGTCCCCTACCCCCCATCCTA 1909
601   L  R  G  S  P  K  L  P  D  F  L  Q  R  S  P  L  P  P  I  L  620
1910  GGCTCTCCTACCAAGGCCGGGCCCTCCTTTGACTTCCCCAAAACCCCCAGCTCTCAGAAT 1969
621   G  S  P  T  K  A  G  P  S  F  D  F  P  K  T  P  S  S  Q  N  640
1970  TTGCTGACCCTGTTGGCTAGGCAGGGGGTAGTAATGACACCACCCTCGGAACCGTACACTG 2029
641   L  L  T  L  L  A  R  Q  G  V  V  M  T  P  P  R  N  R  T  L  660
2030  CCTGACCTCTCCGAGGCCAGTCCTTTCCATGGCCAGCAGCTGGGCTCTGGCCTTCGGCCC 2089
661   P  D  L  S  E  A  S  P  F  H  G  Q  Q  L  G  S  G  L  R  P  680
2090  GCTGAAGACACCCGGGGTCCCTTTGGACGGTCCTTCAGCACCAGCCGCATTACGGACCTG 2149
681   A  E  D  T  R  G  P  F  G  R  S  F  S  T  S  R  I  T  D  L  700
2150  CTGCTTAAGGCTGCATTTGGGACTCAGGCCTCTGACTCAGGCAGCACAGACAGCCTACAG 2209
701   L  L  K  A  A  F  G  T  Q  A  S  D  S  G  S  T  D  S  L  Q  720
2210  GAGAAACCTATGGAGATTGCTCCCTCTGCTGGCTTTGGAGGGACTCTGCATCCAGGAGCT 2269
721   E  K  P  M  E  I  A  P  S  A  G  F  G  G  T  L  H  P  G  A  740
2270  CGTGGTGGAGGGGCCAGCAGCCCAGCACCTGTGGTATTTACTGTAGGCTCCCCACCCAGT 2329
741   R  G  G  G  A  S  S  P  A  P  V  V  F  T  V  G  S  P  P  S  760
2330  GGTGCCACCCCACCCCAGAGTACCCGTACCAGAATGTTCTCAGTGGGCTCTTCCAGCTCC 2389
761   G  A  T  P  P  Q  S  T  R  T  R  M  F  S  V  G  S  S  S  S  780
2390  CTGGGCTCTACTGGCTCCTCCTCTGCCCGCCACTTAGTGCCTGGGGCCTGTGGAGAGGCC 2449
781   L  G  S  T  G  S  S  S  A  R  H  L  V  P  G  A  C  G  E  A  800
2450  CCGGAGCTTTCTGCCCCAGGCCACTGCTGTAGCCTTGCTGACCCCCTTGCTGCCAACTTG 2509
801   P  E  L  S  A  P  G  H  C  C  S  L  A  D  P  L  A  A  N  L  820
2510  GAGGGGGCTGTGACCTTCGAGGCTCCTGACCTCCCAGAGGAGACCCTCATGGAGCAAGAG 2569
821   E  G  A  V  T  F  E  A  P  D  L  P  E  E  T  L  M  E  Q  E  840
2570  CACACGGAAACCCTACACAGTCTGCGCTTCACACTAGCGTTTGCACAGCAAGTTCTGGAG 2629
841   H  T  E  T  L  H  S  L  R  F  T  L  A  F  A  Q  Q  V  L  E  860
2630  ATTGCAGCCCTGAAGGGAAGTGCCAGTGAGGCCGCCGGTGGCCCTGAGTACCAGCTCCAG 2689
861   I  A  A  L  K  G  S  A  S  E  A  A  G  G  P  E  Y  Q  L  Q  880
2690  GAAAGTGTGGTGGCTGACCAGATCAGTCAGTTGAGCCGAGAGTGGGGCTTTGCAGAGCAA 2749
881   E  S  V  V  A  D  Q  I  S  Q  L  S  R  E  W  G  F  A  E  Q  900
2750  CTGGTTCTGTACTTGAAGGTGGCTGAGCTGCTGTCCTCAGGCCTACAGACTGCCATTGAC 2809
901   L  V  L  Y  L  K  V  A  E  L  L  S  S  G  L  Q  T  A  I  D  920
2810  CAGATTCGAGCTGGCAAACTCTGCCTTTCATCTACTGTGAAGCAGGTGGTACGCAGACTA 2869
921   Q  I  R  A  G  K  L  C  L  S  S  T  V  K  Q  V  V  R  R  L  940
2870  AATGAGCTGTACAAGGCCAGCGTGGTATCCTGCCAGGGCCTCAGCTTGCGACTTCAGCGC 2929
941   N  E  L  Y  K  A  S  V  V  S  C  Q  G  L  S  L  R  L  Q  R  960
2930  TTCTTTCTGGACAAACAACGGCTGCTGGACGGGATCCATGGTGTCACTGCAGAGCGGCTC 2989
961   F  F  L  D  K  Q  R  L  L  D  G  I  H  G  V  T  A  E  R  L  980
2990  ATCCTCAGCCATGCTGTGCAAATGGTACAATCAGCTGCCCTTGATGAGATGTTCCAGCAC 3049
981   I  L  S  H  A  V  Q  M  V  Q  S  A  A  L  D  E  M  F  Q  H  1000
3050  CGAGAGGGCTGTGTACCGAGATATCACAAAGCCCTGCTATTGCTGGAGGGGTTGCAGCAC 3109
1001  R  E  G  C  V  P  R  Y  H  K  A  L  L  L  L  E  G  L  Q  H  1020
3110  ACTCTCACGGACCAGGCAGACATTGAGAACATTGCCAAATGCAAGCTGTGCATTGAGAGG 3169
1021  T  L  T  D  Q  A  D  I  E  N  I  A  K  C  K  L  C  I  E  R  1040
3170  AGACTCTCGGCCCTGCTGAGTGGTGTCTATGCCTGActacctgctgccaacctgcagggt 3229
1041  R  L  S  A  L  L  S  G  V  Y  A  *
3230  agggtctgagacctggcagactgtcctcaacactgatcagatccgatggtgctgagactg 3289
3290  ctgccagccaactccagcagggatgctccacagtggacctgtgcagactggtgcaactct 3349
3350  tgcttcctagactgccagtctctgctggcaggtagacatcagagtgccaggggtcccttg 3409
3410  cccactgggacaggagtttctgaacatattcttcctagctggctccctggcaagcaggta 3469
3470  tggtgccgagaagtggcacctgccacctgaagagcccatggcagccctgtgtcaggcaag 3529
3530  ggcctgagaccgttgctgactccaagccaaagcaagctttttcctcacagttcacttgccc 3589
3590  cattgcttgtccaagaaaaaagggtatggtctttctggccccctgttctctctaggaactc 3649
3650  cg                                                          3651
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FIG. 1. Nucleotide and deduced amino acid sequence of mouse ULK1 cDNA. The kinase domain is in bold letter. The GenBank accession number for the nucleotide sequence of mouse ULK1 cDNA is AF053756.

COS7 cells using Lipofectamine (GIBCO BRL), according to manufacturer's protocol. Cells were harvested 48 hrs after transfection and cell lysates were prepared in an extraction buffer containing 50 mM Tris-HCl pH 7.5, NaCl 150 mM, 1% NP-40, PMSF 1 mM. Samples were fractionated by 7.5% SDS PAGE and transferred to PVDF membrane (BIO-RAD, Hercules, CA). After blocking with 5% nonfat milk in T-TBS (TBS with 0.1% Tween 20), immunodetection was done by using 12CA5 monoclonal HA antibody (Boehringer Mannheim) as a first antibody and HRP-conjugated anti-mouse Ig antibody (Amersham) as a second antibody, followed by a visualization with ECL detection kit (Amersham).

In vitro kinase assay. COS7 cell lysates expressing HA-tagged ULK1 or mutants were treated with 10 μg of 12CA5 and then with 20 μl of protein G-Sepharose (Pharmacia). Immune complex was collected by centrifugation, washed twice in the extraction buffer and once in a kinase buffer (50 mM Hepes pH 7.5, 10 mM $Mg(OAc)_2$ and 1 mM DTT). The immune complex was incubated for 15 min at 30 °C in a kinase buffer containing 10 μCi of [32P]γATP (>3000 Ci/mmol) in a total volume of 25 μl. The reaction was terminated by the addition of Laemmli sample buffer, separated by 7.5% SDS-PAGE, and analyzed by BAS-2000 bio-image analyzer (Fuji Photo Film, Tokyo, Japan).

Northern blotting. A 0.6 kb *Stu*I fragment from ULK1 cDNA was labeled using Megaprime kit (Amersham) and hybridized to a multiple tissue Northern blot membrane (Clontech) which contains 2 μg of mRNA from various tissues. The blots were hybridized at 65 °C for 2 hrs in QuikHyb (Stratagene, La Jolla, CA) and washed twice with 2XSSC, 0.1% SDS for 20 min each at room temperature, followed by a final wash with 0.2XSSC, 0.1% SDS for 20 min at 65 °C. The blots were analyzed by BAS-2000 bio-image analyzer.

Chromosome preparation and in situ hybridization. The direct R-banding FISH method was used for chromosomal assignment of the ULK1 gene to mouse chromosomes. Preparation of R-banded chromosomes and FISH were performed as previously described (12, 13). Mitogen-stimulated mouse and rat splenocyte culture was synchronized by thymidine block, and the incorporation of 5-bromodeoxyuridine during the late replication stage was made for differential replication staining after the release of excessive thymidine. R-band staining was performed by exposure of chromosome slides to UV light after staining with Hoechst 33258. The chromosome slides were hardened at 65 °C for 2 hrs and then denatured at 70 °C in 70% formamide in 2XSSC and dehydrated in a 70-85-100% ethanol series at 4 °C. The mouse ULK1 fragment inserted in pBluescript KS(−) was labeled by nick translation with biotin 16-dUTP (Boehringer Mannheim) following the manufacturer's protocol. The labeled DNA fragment was ethanol precipitated with salmon sperm DNA and *E. coli* tRNA, and then denatured at 75 °C for 10 min in 100% formamide. The denatured probe was mixed with an equal volume of hybridization solution to make final concentration of 50% formamide, 2XSSC, 10% dextransulfate, and 2 μg/μl BSA (Sigma). A 20 μl mixture containing 250 ng labeled DNA was put on the denatured slide, covered with parafilm and incubated overnight at 37 °C. The slides were washed for 20 min in 50% formamide in 2XSSC at 37 °C, and in 2XSSC and 1XSSC for 20 min each at room temperature. After rinsing in 4XSSC, they were incubated under coverslip with anti-biotin antibody (Vector Laboratories) at a 1: 500 dilution for 1 hr at

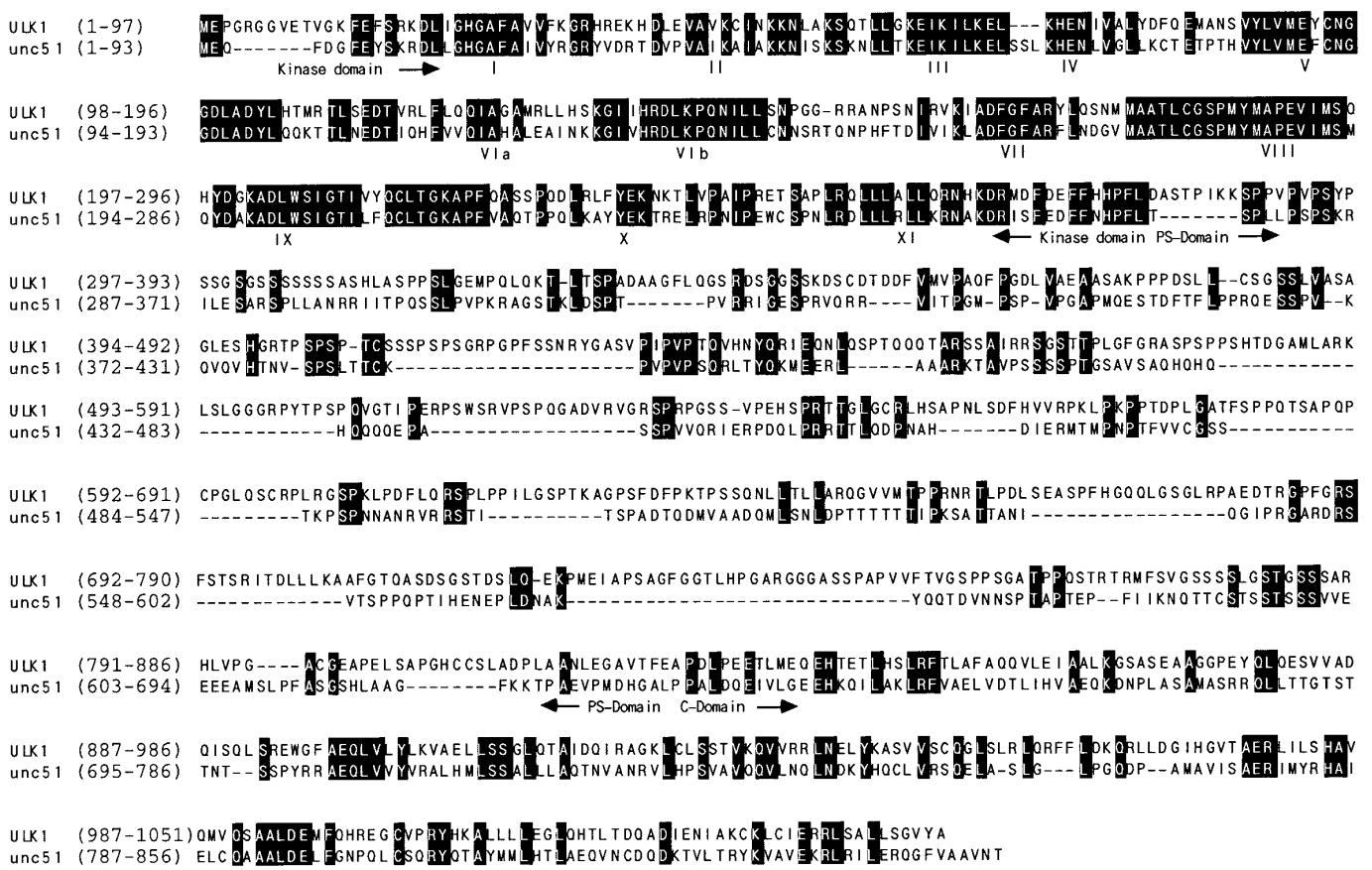

FIG. 2. Amino acid sequence alignment of ULK1 and UNC-51. The alignment was first done by using ALIGN ver.2 and then modified by eyes.

37 °C. After washing with 4XSSC, 0.1% Nonident P-40 in 4XSSC, 4XSSC for 10 min on the shaker, the slides were rinsed with 2XSSC and stained with 0.75 μg/μl propidium iodide. Excitation at wave length 450-490 nm (Nikon filter set B-2A) and near 365 nm (UV-2A) were used for observation. Kodak Ektachrome ASA100 films were used for microphotography.

RESULTS AND DISCUSSION

cDNA cloning and sequencing of mouse ULK1. Using degenerate primers based on the sense and anti-sense sequences of HRDLKP (conserved domain VIb) and RYMAPE (conserved domain VIII), partial cDNA fragments were amplified by PCR from first strand cDNA prepared from neonate rat brain. The PCR products were fractionated on a 5 % PAGE and amplified fragments of expected size were recovered, subcloned and sequenced. Homology search of the sequences revealed that three clones out of about 200 clones sequenced encoded an ORF which showed the highest homology to the domain between VIb and VIII of *C. elegans* protein kinase, UNC-51. Using this cDNA fragment as a probe, we screened about 10^6 clones from mouse embryo and brain cDNA libraries and obtained three partial overlapping clones. The 5′ end was further cloned by 5′-RACE. The resultant ULK1 cDNA was 3651 bp, having a predicted GC rich 5′UTR, an ATG start codon which matches the Kozak's consensus, and an ORF of 1051 amino acids (Fig. 1). The nucleotide sequence has been deposited in GenBank under the accession number of AF053756. From the overall structural similarity of the deduced amino acid to UNC-51 (see below), we designated this clone as mouse ULK1 (UNC-51-Like Kinase 1).

ULK1 is homologous to UNC-51. Using the deduced 1051 amino acid sequence of ULK1 to query the protein databases of National Center of Biotechnology Information (NCBI), ULK1 showed the highest overall homology to *C. elegans* UNC-51 (32% identity and 41% similarity). Alignment of ULK1 and UNC-51 delineated three domains; the amino-terminal kinase domain, the intervening proline/serine-rich (PS) domain and the carboxyl-terminal conserved (C) domain (Fig. 2).

The kinase domain of ULK1 possesses all of the subdomain motifs characteristic for protein kinases (14). ULK1 (21-278) and UNC-51(14-275) is 62% identical and 74% similar at the amino acid level, and there was no mammalian protein kinases in the Swiss-Prot databank sharing this level of identity. Typically, the kinase domain of ULK1 had lower homologies to other mammalian protein kinases such as human PKCμ (Acc. No. Q15139, 34% identity in 261 aa overlap), human NRK2 (Acc. No. P51957, 33% identity in 264 aa overlap), mouse NEK1 (Acc. No. P51954, 32% identity in 264 aa overlap), and mouse ribosomal protein S6 kinase II α 1 (Acc. No. P18653, 33% identity in 262 aa overlap), all of which apparently belong to different kinase subfamilies.

The PS domain of ULK1 (279-828) and the corresponding region of UNC-51 (276-636) show the least homology and are different in length. Although primary structure of this domain is not conserved, this region is characterized by a high percentage of proline and serine residues; ULK1 (S: 16%, P: 15%) and UNC-51 (S: 13%, P: 12%). A motif of ϕPXP(S/T) (where ϕ stands for hydrophobic amino acids) is found at 290-294, 437-441 of ULK1 and 280-284, 385-389 of UNC-51. Stretches of serine and/or threonine are also found at 297-308, 777-788 of ULK1 and 521-526, 592-599 of UNC-51. Whether these short conserved motifs in the PS domain has specific roles is not known at present.

The C domain spanning about 220 aa at the carboxyl-terminus of ULK1 and UNC-51 are homologous. The sequenecs of ULK1 (829-1051) and UNC-51 (637-856) are 29% identical and 44% similar at the amino acid level. It is worth noting that the UNC-51 mutations attributing to *unc-51* phenotype have often been found in this region (10). Also an UNC-51 interacting protein, UNC-14, binds to a region in this C domain (15). These lines of evidence and the sequence conservation of this domain point to its functional importance.

The mRNA for mouse ULK1 is widely expressed. We examined the tissue distribution of ULK1 mRNA by Northern blot analysis. As shown in Figure 3, ULK1 mRNA was detected as a single 4.7 kb transcript in

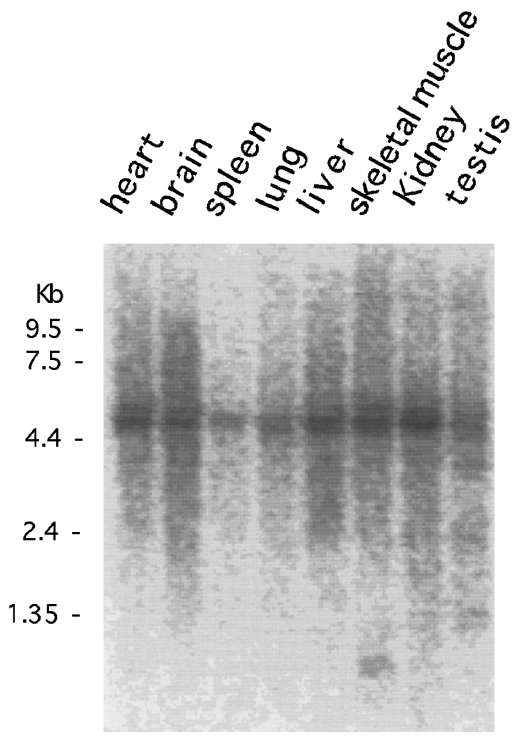

FIG. 3. Northern blot analysis of ULK1 gene in mouse tissues. The Clontech mouse multiple tissue blot carrying 2 μg of polyA RNA per lane was probed with a URK1 fragment. ULK1 mRNA is detected as an approximate 4.7 kb band in all of the tissue examined.

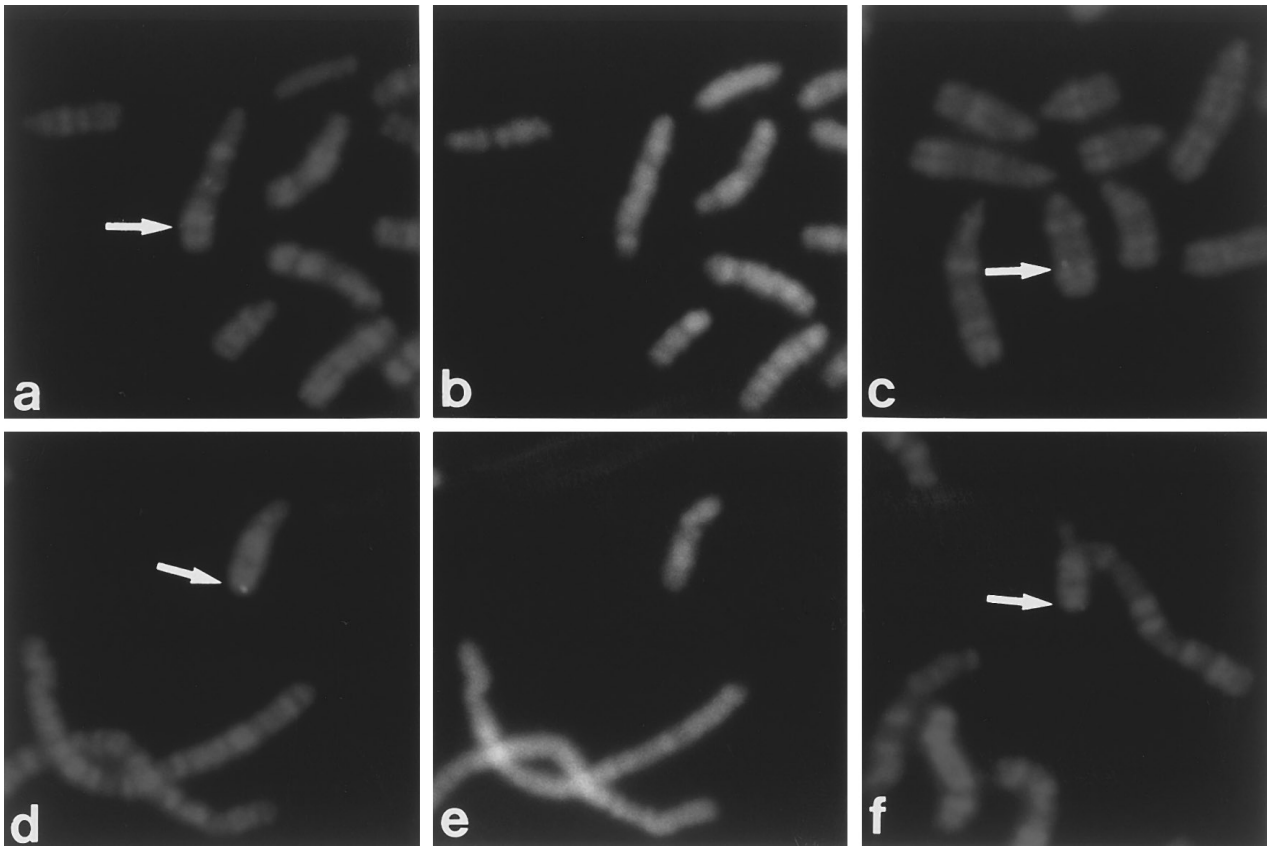

FIG. 4. Chromosomal localization of the ULK1 gene on mouse (a, b, c) and rat (d, e, f) R-banded chromosomes. The mouse ULK1 cDNA fragment was used as a biotinylated probe. The hybridization signals are indicated by arrows. The ULK1 gene was located to mouse chromosome 5F and rat chromosome 12q16.3. The metaphase spreads were photographed with Nikon B-2A (a, c, d, f), and UV-2A (b, e) filters. R-band and G-band patterns are demonstrated in (a, c, d, f) and (b, e), respectively.

all tissues examined. While UNC-51 is predominantly expressed in neurons and in some muscles in *C. elegans* (10), expression of ULK1 mRNA is ubiquitous in mouse tissues. This suggests that ULK1 may have roles in non-neural tissues as well as in neural tissues.

Chromosomal location of ULK1 gene. The chromosomal assignment of the ULK1 gene to mouse and rat chromosomes was made by direct R-banding FISH using mouse cDNA as a probe. The ULK1 gene was localized to mouse chromosome 5F and rat chromosome 12q16.3 (Fig. 4)(12, 16, 17). They were mapped in the region where the conserved linkage homology has been identified between the two species (18). The mouse chromosome region where ULK1 was mapped includes mouse mutant loci such as, *mc* (marcel), *bl* (blebbed), *gc* (gray coat), *le* (light ear), *jg* (jagged tail), *Ph* (patch), *bf* (buff), and *Gus* (=*asd:* adipose storage deficiency) (19).

Expression and autophosphorylation of ULK1. To test the kinase activity of ULK1, kinase negative mutant ULK1Δ was constructed as a control by replacing the conserved ATP-binding Lys with Asn. HA-tagged ULK1 and ULK1Δ in expression plasmids were transfected into COS7 cells, and cell lysates were subjected to immunoblotting analysis (Fig. 5A) and immunoprecipitation kinase assay (Fig. 5B). Immunoblotting using HA antibody identified a molecule of ~150 kDa which corresponds to HA-tagged ULK1 (Fig. 5A, lane 5). Kinase negative ULK1Δ migrated slightly faster than ULK1 (Fig. 5A, lane 6), suggesting that ULK1 may be phosphorylated. In accordance with this observation, ULK1 was phosphorylated in the kinase assay (Fig 1B, lane 5). The phosphorylation was primarily due to autophosphorylation since phosphorylation of ULK1Δ was drastically reduced (Fig. 5B, lanes 6). To further determine the site of phosphorylation, truncated ULK1 mutants were tested in the assay. Phosphorylations of truncated mutants, ULK1(507), ULK1(427), ULK1(351) were readily detected, while that of ULK1(287) which only contains the kinase domain was not detected (Fig. 5A & 5B, lanes 1 to 4). These results indicate that ULK1 is autophosphorylated in the PS domain, especially between 287 and 351 where the longest serine stretch resides. Since deletions of the PS and C domains up to ULK1(351) did not significantly alter the level of autophosphorylation, these domains may not be essential for regulating kinase activity.

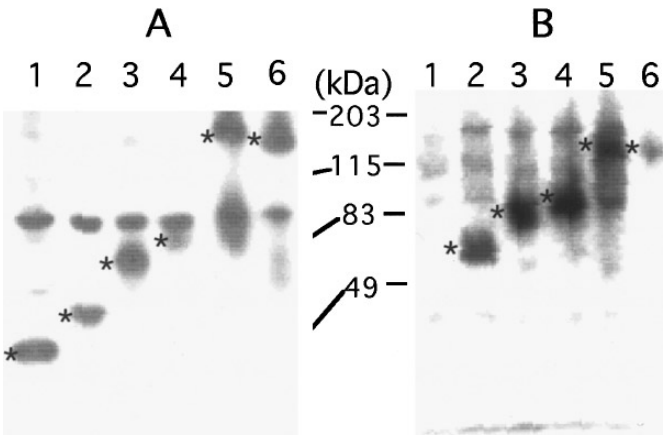

FIG. 5. Expression and *in vitro* kinase activity of HA-tagged ULK1 and its mutants. A. Immunoblotting analysis of lane 1: ULK1(287), lane 2: ULK1(351), lane 3: ULK1(427), lane 4: ULK1(507), lane 5: ULK1, and land 6: ULK1Δ. The products are shown by the asterisks. B. *In vitro* kinase assay of lane 1: ULK1(287), lane 2: ULK1(351), lane 3: ULK1(427), lane 4: ULK1(507), lane 5: ULK1, and land 6: ULK1Δ. The products are shown by the asterisks.

Although we have determined the protein kinase activity of ULK1, further study is needed to elucidate its physiological substrate and mode of regulation.

In conclusion, we have shown evidence for the presence in mammals of a novel protein kinase ULK1, which is homologous to *C. elegans* UNC-51. Recently, a yeast protein kinase Apg1p, which is homologous to UNC-51, was cloned and characterized (20). UNC-51 shows higher similarity with ULK1 than with Apg1p. Albeit the homology is less prominent, Apg1p appears to have a similar structure composed of the kinase, PS and C domains. Taken together, it is likely that ULK1, UNC-51 and Apg1p comprise a novel subclass of protein kinase, which is conserved among wide variety of species. *Apg1* mutant is defective in autophagic processes and dynamic membrane turnover (20), while *unc-51* mutants have atypical membranous vesicles and cistern-like structures in its abberent axons (21, 22). Information on these model eukaryotes may then facilitate the functional analysis of ULK1 in mammalian cells.

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