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cDNA Cloning, Expression, Subcellular Localization, and Chromosomal Assignment of Mammalian Aurora Homologues, Aurora-Related Kinase (ARK) 1 and 2

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Chromosomal segregation during mitosis as well as meiosis is considered to be regulated by multiple kinases, but the precise mechanism remains largely unknown. A mutation in *Drosophila,* designated *aurora,* was identified as a responsible gene for a chromosomal segregation defect and encodes a putative serine-threonine kinase. Here we have identified mammalian aurora homologues, designated aurora-related kinase (ARK) 1 and ARK2. Kinase domains of murine ARK1 and ARK2 showed 61 and 62% identity, respectively, to that of aurora at the amino acid levels, respectively. Cell cycle analysis revealed that the expression of ARK1 was correlated with G2/M phase, while ARK2 was expressed during S and G2/M phases. Immunofluorescence analysis demonstrated that ARK2 was mainly localized to the midbody, while ARK1 has been reported to be localized to the spindle pole during mitosis. Collectively, these results suggest that these two kinases may have distinct roles with different expression timing and subcellular localization during the cell cycle progression. Interspecific backcross mapping revealed that *Ark1* is located in a distal region of mouse chromosome 2, while *Ark2* is located in a central region of mouse chromosome 11.

Accurate chromosomal segregation during mitosis as well as meiosis is an important event in the cell cycle. The segregation of sister chromatids during mitosis, or homologous chromosomes during meiosis, requires the assembly and proper function of a microtubule spindle (1). It has been shown that the protein phosphorylation plays an important role in controlling the assembly and dynamics of the mitotic spindle, and chromosome segregation (2-6). Previous studies demonstrated that structurally related protein kinases, polo of *Drosophila melanogaster* (7, 8), plo1+ of *Schizosaccharomyces pombe* (9) and cdc5p of *Saccharomyces cerevisiae* (10), are involved in the control of spindle function and chromosome disjunction. Recent identification of human polo and cdc5p homologue, designated Plk1, indicated that these machinery are evolutionary conserved through yeast to mammals (11-16). *Aurora* was identified as the responsible gene for a spontaneous chromosomal segregation defect mutant of *Drosophila* and encodes a putative serine-threonine kinase (17). *IPL1* and *IPL2* genes were identified as increase-in-ploidy mutants in yeast *Saccharomyces cerevisiae* (18). Later, *IPL1* gene encodes a putative serine-threonine kinase showing a high similarity to aurora and considered to be a yeast aurora homologue (19). On the other hand, protein phosphatase 1 has been shown to play an important role in mitosis (20) and counteract the function of Ipl1 (19). Collectively, these results have implied that the balance between protein phosphorylation and dephosphorylation is critically involved in these processes.

During the screening of a novel serine-threonine kinase, here we have identified two novel and closely related kinases, referred to aurora-related kinase (ARK) 1 and ARK2. ARK1 and ARK2 are highly homologous to aurora of *Drosophila* and Ipl1 of yeast, suggesting that these kinases constitute a subfamily of

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Abbreviations used: ARK, aurora-related kinase; EF-1α, elongation factor 1α; IPTG, isopropyl β-D-thiogalactopyranoside.

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protein kinases. ARK1 and ARK2 mRNA levels are highest in G2/M and S to G2/M phase, respectively. We also demonstrated the subcellular localization of ARK2 and chromosomal assignment of murine *Ark1* and *Ark2*. Our results demonstrated that ARK1 and ARK2 may play distinct functional roles in the cell cycle progression with the different expression timing and subcellular localization.

MATERIALS AND METHODS

Reagents and cell lines. NIH3T3 and 293 cells were maintained in DMEM supplemented with 10% FCS. Anti-Flag and biotin-conjugated anti-Flag monoclonal antibodies (mAb) were purchased from Kodak. Fluorescence isothiocyanate (FITC) conjugated-rabbit anti-mouse Ig was purchased from DAKO.

Cloning of ARK1 and ARK2 cDNA. Total RNA from murine B cell lymphoma, A20.2J, was used to prepare cDNA by using the reverse transcriptase of Molony murine leukemia virus (Superscript, Gibco BRL) according to the manufacturer's protocol. The cDNA was amplified by PCR with degenerative oligonucleotide primers corresponding to highly conserved amino acids in the domains of serine-threonine kinases. The sense and antisense primers corresponded to amino acid sequences GFG(C/D)VY (domain I) and IHGDIK (domain VIb), respectively. The PCR was performed under the following condition: 1 min at 94 °C; 2 min at 45 °C and 1 min at 72 °C for 40 cycles. Amplified DNA was subcloned, sequenced, and two novel and related sequences were identified. These sequences showed a significant similarity to a putative serine-threonine kinase, named aurora of *Drosophila* and were hence referred to as aurora-related kinase (ARK) 1 and 2. A Uni-ZAP cDNA library from the murine monocytic cell line J774A.1 and a λgt11 cDNA library from HTLV-1-transformed human T cells (a gift of M. Yoshida, Research Institute of Medical Science, University of Tokyo) were screened with a ^{32}P-labeled PCR fragments of muirne ARK1 (mARK1) or murine ARK2 (mARK2) as probes. Several overlapping clones were isolated and DNA sequence was determined on both strands using a series of oligonucleotide primers. The nucleotide sequences have been submitted to DDBJ/Gen Bank/EMBL Data Bank with accession numbers, U69106 for mARK1; AF008551 for human ARK1 (hARK1), U69107 for mARK2 and AF008552 for human ARK2 (hARK2).

Cell cycle synchronization. For G1 phase synchronization was performed as previously described (21). Briefly, NIH3T3 cells were cultured in DMEM containing 0.5% FCS for 36 h. After addition of FCS to 10%, the cells were collected periodically and used for extraction of total RNA and cell cycle analysis.

Cell cycle analysis. Cell cycle analysis was performed as described previously (22). Briefly, 1×10^6 cells were harvested at the indicated time points and resuspended in 0.5 ml of 4 mM sodium citrate containing 0.05% NP-40, 0.45 mg/ml RNase, and 50 μg/ml propium iodide, and incubated for 10 min on ice. Then 50 μl of 1.5 M NaCl was added to the suspension. The proportions of cells in G1, S, and G2/M phases of the cell cycle were analyzed by flow cytometry on a FACScan^R with Modfit LT program (Beckton Dickinson).

Northern blot analysis. Total RNA from NIH3T3 cells was extracted using Trizol^R (Gibco BRL) according to the manufacturer's instruction. Ten μg each of total RNA was subjected to electrophoresis on denaturing agarose gel, followed by transfer onto a nylon membrane (Pall). The blot was hybridized with mARK1 or mARK2 cDNA fragment. The amount of loaded RNA was evaluated by subsequent hybridization of the same membrane with a β-actin cDNA. Hybridization and washing were performed as described previously (22).

Expression vectors and generation of stable transfectants. mARK2 cDNA was fused with two copies of Flag epitope tag sequence at its N-terminal portion and subcloned into pEF-LAC vector (a gift of Hiroshi Itoh, Tokyo Institute of Technology) (23), designated pEFL-ARK2. pEF-LAC vector contains the promoter of the gene for human polypeptide chain elongation factor 1α (EF-1α) and three lactose operators. Then, the promoter activity is usually repressed by coexpressed lactose repressor by p3′SS, a modified lactose repressor plasmid (a gift of Hiroshi Itoh)(23), and induced by the addition of isopropyl β-D-thiogalactopyranoside (IPTG). To obtain stable transfectants, pEFL-ARK2 and p3′SS, were introduced into 293 cells using Lipofectamine according to a manufacture's protocol (Gibco BRL). Two days after transfection, 2 mg/ml G418 and 0.125 mg/ml hygromycin B were added to the culture medium for selection of the stable transfectants. After resistant clones were isolated, the expression of Flag-tagged ARK2 was determined by Western blotting.

Western blotting. The pEFL-ARK2 transfected 293 cells (293-ARK2.1 and -ARK2.2) (1×10^7) were treated with or without 5 mM IPTG for 12 hr. The cells were lysed with 500 μl of a lysis buffer containing 1% NP40, 50 mM HEPES (pH 7.3), 150 mM NaCl, 2 mM EDTA, 1 μg/ml aprotinin, 1 μg/ml leupeptin, and 1 μg/ml pepstatin. Nuclei were removed by centrifugation and the supernatant was precleared with protein G-Sepharose (Pharmacia) for 1 to 2 hr. The cleared lysates were incubated with anti-Flag mAb for 1 hr at 4 °C. After the addition of 30 μl of protein G-Sepharose, the lysates were incubated further for 1 hr. The immunoprecipitates were washed three times with the lysis buffer, subjected to 12% SDS-PAGE, and then transferred onto a PVDF membrane (Millipore). The membrane was probed by biotin-conjugated anti-Flag mAb, followed by avidin-biotin complex (Vectastain). Protein bands were visualized with an enhanced chemiluminescent detection system (Amersham). The intensity of the bands was evaluated by densitometry (Scan Analysis^{TM}, Biosoft).

Immunofluorescence analysis. 293-ARK2.1 cells were cultured on slide glasses and treated with 5 mM IPTG for 12 hrs. Then the cells were fixed by 4 % paraformaldehyde in PBS. After washing with PBS, the cells were permealized by 5% acetic acid/90% ethanol for 5 sec. The cells were then incubated with anti-Flag mAb for 1 hr at RT, washed in PBS, followed by incubation with FITC-conjugated rabbit anti-mouse Ig (DAKO) for 1 h. The immunostained specimens were mounted in PBS/glycerol (1:9) containing *p*-phenylenediamine and analyzed on TCS NT microscope (Leica, Tokyo). The specimens were viewed with 100 × objective lens (PLApo 100 ×, Leica, Tokyo) and obtained scanning images was analyzed by data analysis software contained in TCS NT system.

Interspecific mouse bachcross mapping. Interspecific backcross progenies were generated by mating (C57BL/6J × *M. spretus*) F_1 females and C57BL/6J males as described (24). A total of 205 N_2 mice were used to map the *Ark1* and *Ark2* loci (see text for details). DNA isolation, restriction enzyme digestion, agarose gel electrophoresis, Southern blot transfer, and hybridization were performed essentially as described (25). All blots were prepared with Hybond-N+nylon membrane (Amersham). The *Ark1* probe, a 0.3 kb *Spe*I/*Xho*I fragment of mouse cDNA from the 3′ UTR, was labeled with a [^{32}P] dCTP using a random primed labeling kit (Stratagene); washing was done to a final stringency of 0.5 × SSCP, 0.1% SDS, 65°C. A major fragment of 5.6 kb was detected in *Sac*I digested C57BL/6J DNA and a major fragment of 7.0 kb was detected in *Sac*I digested *M. spretus* DNA. The *Ark2* probe, a 1.7 kb *Eco*RI/*Xho*I fragment of mouse cDNA, detected a 4.3 kb *Bam*HI fragment in C57BL/6J DNA and a 5.4 kb *Bam*HI fragment in *M. spretus* DNA. The presence or absence of the *M. spretus*-specific fragments was followed in backcross mice.

A description of the probes and RFLPs for the loci linked to *Ark1* including *Cd40, Nfatc2, Mc3r* and *Pck1* has been reported previously (26–29); those linked to *Ark2* include *Csfgm, Myhsf1, Glut4,* and *Myo1c* (30–32). Recombination distances were calculated using Map Manager, Version 2.6.5. Gene order was determined by minimizing

the number of recombination events required to explain the allele distribution patterns.

RESULTS AND DISCUSSION

cDNA cloning of ARK1 and ARK2. To identify a novel serine-threonine kinase, we applied a degenerative oligonucleotide-based PCR method and obtained two PCR fragments containing novel sequences. These cDNA fragments were subsequently used to screen a murine monocytic cell line J774.1 cDNA library, resulting in identification of two novel serine-threonine kinases. Database analysis revealed that these two kinases showed 61 and 62% identity, respectively, to the kinase domain of *Drosophila* aurora (17) at the amino acid levels. Thus, these kinases are considered to be murine homologues of aurora, and referred to as aurora-related kinase (ARK) 1 and ARK2, respectively. *Aurora* was identified as a responsible gene for a chromosomal segregation defect in *Drosophila* (17). We subsequently screened a human HAT109 cDNA library and obtained human ARK1 and ARK2 cDNAs. Murine and human ARK1 cDNAs contained 1920 and 2077 nucleotides encoding 395 and 403 amino acids, respectively. Murine and human ARK2 cDNAs contained 1914 and 2001 nucleotides encoding 345 and 344 amino acids, respectively (Fig. 1). Within the kinase domains, hARK1 displays 74.5, 83.1, 83.1, 62.3 and 49.2% amino acid identities to the catalytic domains of hARK2, *Xenopus* XLP46APK, XLP46BPK, *Drosophila* aurora, and *S. cerevisiae* Ipl1 (17–19), respectively (Fig. 2). During the preparation of this manuscript, several groups have identified Aurora/Ipl1-related kinases. mARK1 is identical to IAK1 (33) and Ayk (34), while hARK1 is identical to Aik (35) and BTAK (36). mARK2 is identical to STK-1 (37).

Cell cycle dependent expression of ARK1 and ARK2 mRNA. It has been shown that Aik, IAK1, or Ayk (ARK1) was highly expressed in testis, and the expression peaked in G2/M phase (33–34). We then compared the expression pattern of ARK1 and ARK2 during the cell cycle. NIH3T3 cells were synchronized at G1 phase by serum deprivation as described previously (21). After the addition of FCS, the cells progressed the cell cycle. The cells were harvested at the indicated times

A

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hARK1 MDRSKENCISGPVKATAPVGGPKRVLVTQQFPCQNPLPVNSGQAQRVLCPSNSSQRIPLQ 60
mARK1 ---C----V-R---T-V-F --------E-I-S--LGSAS---------- ---V-S- 58

hARK1 AQKLVSSHKPVQNQKQKQLQATSVPHPVSRPLNNTQKSKQPLPSAPENNPEEELASKQKN 120
mARK1 ----GAGQ--    A-P---P-A---R---- ---P--NE-  -A-SG-DS-K-Q--L--T 111

hARK1 EESKKRQWALEDFEIGRPLGKGKFGNVYLAREKQSKFILALKVLFKAQLEKAGVEHQLRR 180
mARK1 -DT-----T----D-----------------R-------------T-----N------- 171

hARK1 EVEIQSHLRHPNILRLYGYFHDATRVYLILEYAPLGTVYRELQKLSKFDEQRTATYITEL 240
mARK1 ------------------------------------------------------------ 231

hARK1 ANALSYCHSKRVIHRDIKPENLLLGSAGELKIADFGWSVHAPSSRRTTLCGTLDYLPPEM 300
mARK1 -------------------------N--------------------M----------- 291

hARK1 IEGRMHDEKVDLWSLGVLCYEFLVGKPPFEANTYQETYKRISRVEFTFPDFVTEGARDLI 360
mARK1 ------------------------M-----H------R--------------------- 351

hARK1 SRLLKHNPSQRPMLREVLEHPWITANSSKPSNCQ-NKESASKQS                  403
mARK1 -------A---LT-A--------K------PTGHTS--PT--S-                  395
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B

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hARK2 MAQKENSYPWPYGRQTAPSGLSTLPQRVLRKEPVTPSALVLMSRSNVQPTAAPGQKVMEN 60
mARK2 ------A------SK-SQ---N--S--------A-T---A-VN---S-S-------LA-- 60

hARK2 SS G T PD ILTRH FTIDDFEIGRPLGKGKFGNVYLAREKKSHFIVALKVLFKSQIEK 115
mARK2 K-Q-S-ASQGSQNKQP----N----------------------R------I-------- 120

hARK2 EGVEHQLRREIEIQAHLHHPNILRLYNYFYDRRRIYLILEYAPRGELYKELQKSCTFDEQ 175
mARK2 ----------------K-----Q-------QQ---------------------R----- 180

hARK2 RTATIMEELADALMYCHGKKVIHRDIKPENLLLGLKGELKIADFGWSVHAPSLRRKTMCG 235
mARK2 ---------S---T---K-----------------Q-------------------------- 240

hARK2 TLDYLPPEMIEGRMHNEKVDLWCIGVLCYELLVGNPPFESASHNETYRRIVKVDLKFPAS 295
mARK2 ----------------M-------------M---------P--S--------------S- 300

hARK2 VPTGAQDLISKLLRHNPSERLPLAQVSAHPWVRANSRRVLPPSALQSVA             344
mARK2 --S----------K---WQ-----E-A-------------------              345
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FIG. 1. Amino acid alignment of human and murine ARK1 (A) and ARK2 (B). Identical amino acids in murine sequences to humans are indicated by dashes.

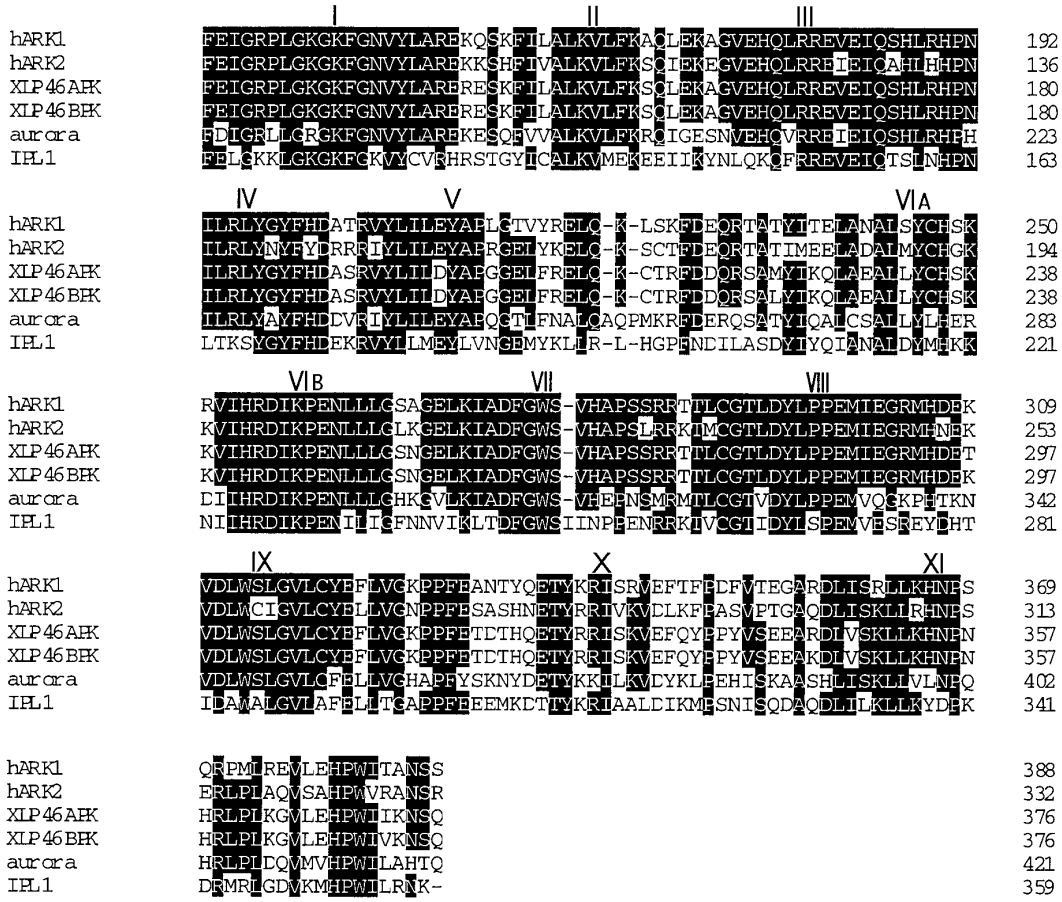

FIG. 2. Comparison of the catalytic domains of human ARK1, ARK2, *Xenopus* XLP46APK, XLP46BPK, *Drosophila* aurora, and *S. cerevisiae* Ipl1. Amino acid residue numbers are shown at the right of each line. The 11 conserved subdomains in the catalytic domains of protein kinases are numbered with roman numericals. The identical amino acids with ARK1 are shaded.

used for the cell cycle analysis and the extraction of total RNA. As shown in Fig. 3A, cell cycle analysis demonstrated that most cells arrested in G1 phase at 0 hr, and subsequently entered into S phase by 18 hr, followed by progression into G2/M phase after 24 hr. Either ARK1 or ARK2 mRNA was not detectable at 0 hr. The expression of ARK1 mRNA gradually increased by 18 hr and peaked in 24 hr, and then decreased (Fig. 3B, upper panel), suggesting that the expression of ARK1 mRNA is correlated with the population of G2/M phase as reported previously (33, 35). On the other hand, ARK2 mRNA appeared by 18 hr and persistently expressed thereafter, suggesting that ARK2 mRNA is expressed during S and G2/M phases (Fig. 3B, lower panel). Collectively, these results suggest the expression of ARK1 and ARK2 are differentially regulated during the cell cycle.

Subcellular localization of ARK2 in stable transfectants. It has been shown that Aik or IAK1 (ARK1) is mainly localized to the spindle pole during mitosis (33, 35), however, the subcellular localization of ARK2 remains to be shown. We then determined the subcellular location of ARK2 during the cell cycle. Assuming that ARK2 is implicated in the cell cycle progression, constitutive expression of ARK2 may be deleterious for cell growth and survival. To overcome this problem, we constructed an inducible expression vector for ARK2. We also added a Flag epitope tag to ARK2 to confirm the expression of exogenously expressed ARK2. We transfected pEFL-ARK2 along with p3′SS into 293 cells and subsequently obtained stable transfectants designated 293-ARK2. Two representative clones (293-ARK2.1 and -ARK2.2) are described here. We checked expression of Flag-ARK2 in these transfectants before and after treatment with 5 mM IPTG. The expressed Flag-ARK2 was detected by Western blotting using anti-Flag mAb. There was a low level expression of Flag-ARK2 in these transfectants without IPTG treatment (Fig. 4A). After the addition of IPTG, the expression levels of Flag-ARK2 increased by 2–3 fold as compared to the basal level. Then, the 293-ARK2.1 cells were treated with IPTG and stained with anti-Flag mAb. As shown in Fig. 4B, ARK2 specifically accumulated in the midbodies during cytokinesis. During the cleavage of

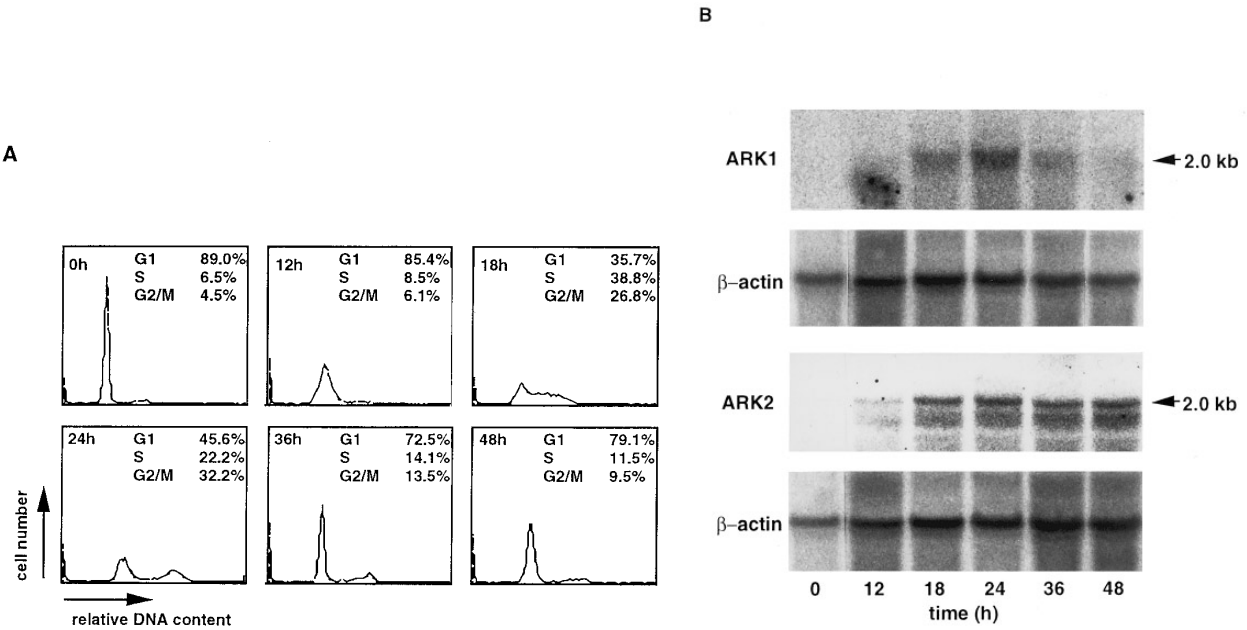

FIG. 3. Cell cycle-dependent expression of ARK1 and ARK2 mRNA. (A) Cell cycle analysis of NIH3T3 cells. NIH3T3 cells were rendered quiescent by incubation for 36 h in DMEM supplemented with 0.5% FCS. After the addition of FCS to 10%, cells were collected at the times indicated and small portion of the cells (approximately 1×10^6) were stained with PI and subjected to the cell cycle analysis by flow cytometry. (B) Expression of ARK1 and ARK2 mRNA during the cell cycle. Total RNA was extracted from the cells at the indicated time points. Ten μg each of RNA was subjected to 1% agarose formaldehyde gel electrophoresis and transferred onto a nylon membrane. Northern hybridization with mARK1 or mARK2 cDNA probe was performed as described in the Materials and Methods. The amounts of loaded RNAs were evaluated by hybridization with a β-actin cDNA probe. The sizes of the transcripts are indicated at the right.

cytoplasm in anaphase, the thin bridges called midbodies are formed between the cytoplasm of two daughter cells. Intense immunoreactivities against Flag-ARK2 were specifically localized to this structure before (Fig. 4B, left panel) and after (Fig. 4B, right panel) the cleavage of polar spindle microtubules. Less intense immunoreactivities were diffusely distributed (Fig. 4B), but sometimes in a granular pattern (data not shown). The similar results were obtained with 293-ARK2.2 cells (data not shown).

Chromosomal mapping of murine Ark1 and Ark2 gene. Since the expression of both ARK1 and ARK2 appears to be correlated with the cell cycle progression, it was interesting to determine the chromosomal location of these genes in relation to the loci that have been implicated in human diseases and spontaneous mutants in mice. We determined the mouse chromosomal location of *Ark1* and *Ark2* by interspecific backcross analysis using progenies derived from matings of [(C57BL/6J $\times$ *Mus spretus*)F_1 $\times$ C57BL/6J] mice. This interspecific backcross mapping panel has been typed for over 2400 loci that are well distributed among all the autosomes as well as the X chromosome (24). C57BL/6J and *M. spretus* DNAs were digested with several enzymes and analyzed by Southern blot hybridization for informative restriction fragment length polymorphisms (RFLPs) using mouse cDNA probes. The 7.0 kb *Sac*I *M. spretus* RFLP (see Materials and Methods) was used to follow the segregation of the *Ark1* locus in backcross mice. The mapping results indicated that *Ark1* is located in the distal region of mouse chromosome 2 linked to *Cd40*, *Nfatc2*, *Mc3r* and *Pck1*. Although 84 mice were analyzed for every marker and are shown in the segregation analysis (Fig. 5A), up to 166 mice were typed for some pairs of markers. Each locus was analyzed in pairwise combinations for recombination frequencies using the additional data. The ratios of the total number of mice exhibiting recombinant chromosomes to the total number of mice analyzed for each pair of loci and the most likely gene order are: centromere $-$*Cd40*$-$1/144$-$*Nfatc2*$-$6/166$-$*Mc3r*$-$0/162$-$*Ark1*$-$2/124$-$*Pck1*. The recombination frequencies [expressed as genetic distances in centiMorgans (cM) +/$-$ the standard error] are $-$*Cd40*$-$0.7+/$-$0.7$-$*Nfatc2*$-$3.6+/1.5$-$[*Mc3r, Ark1*]$-$1.6+/$-$1.1$-$*Pck1*. No recombinants were detected between *Mc3r* and *Ark1* in 162 animals typed in common, suggesting that the two loci are within 1.9 cM (upper 95% confidence limit).

A 5.4 kb *Bam*HI *M. spretus* RFLP (see Materials and Methods) was used to follow the segregation of the *Ark2* locus in backcross mice. *Ark2* mapped to the central region of mouse chromosome 11 linked to *Csfgm*, *Myhsf1*, *Glut4*, and *Myo1c*. In this case, 118 mice were

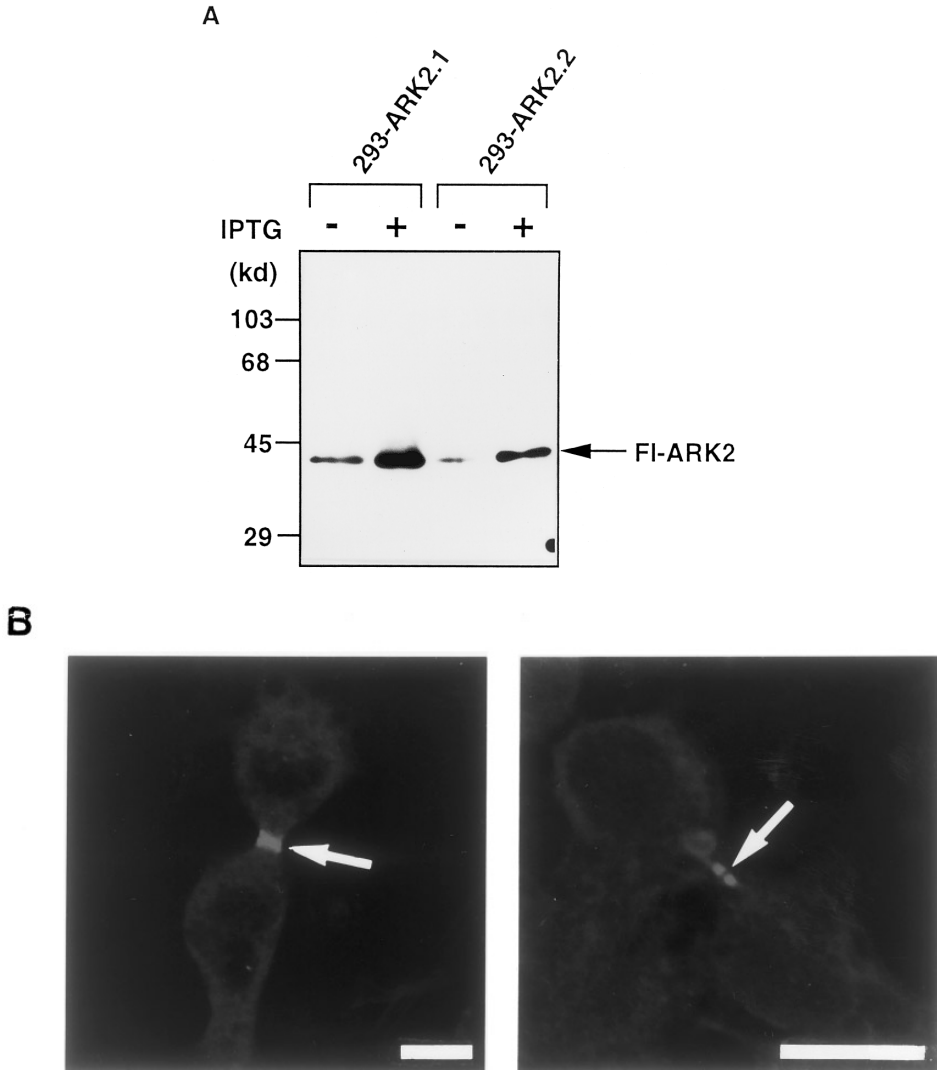

FIG. 4. Subcellular localization of ARK2. (A) Inducible expression of ARK2 in 293-ARK2. Two representative clones (293-ARK2.1 and -ARK2.2) were treated with or without 5 mM IPTG for 12 hr. The cells were lysed in the lysis buffer and immunoprecipitated with anti-Flag mAb. The immunoprecipitates were subjected to Western blotting with biotin-conjugated anti-Flag mAb. The right arrow indicates the position of Flag-ARK2. The molecular weight standards are indicated at the left. (B) Subcellular localization of ARK2 in 293-ARK2.1. After culturing with IPTG, the cells were stained with anti-Flag mAb, followed by FITC-conjugated anti-mouse Ig. ARK2 accumulated in the midbodies in early (left panel) and late (right panel) phases of cytokinesis. Scale bar = 15 μm.

analyzed for every marker and are shown in the segregation analysis (Fig. 5B) and up to 191 mice were typed for some pairs of markers. Again, each locus was analyzed in pairwise combinations for recombination frequencies using the additional data. The ratios of the total number of mice exhibiting recombinant chromosomes to the total number of mice analyzed for each pair of loci and the most likely gene order are: centromere −*Csfgm*−9/144−*Myhsf1*−5/136−*Ark2*−0/191−*Glut4*−4/176−*Myo1c*. The recombination frequencies [expressed as genetic distances in centiMorgans (cM) +/− the standard error] are *Csfgm*−6.3+/−*2.0*−*Myhsf1*−*3.7*+/−*1.6*− [*Ark2, Glut4*]−*2.3*+/−*1.1*− *Myo1c*. No recombinants were detected between *Ark2* and *Glut4* in 191 animals typed in common, suggesting that the two loci are within 1.6 cM of each other (upper 95% confidence limit).

We have compared our interspecific maps of chromosomes 2 and 11 with composite mouse linkage maps that report the map location of many uncloned mouse mutations (provided from Mouse Genome Database, a computerized database maintained at The Jackson Laboratory, Bar Harbor, ME). *Ark1* and *Ark2* mapped in regions of the composite map that lack mouse mutations with a phenotype that might be expected for an alteration in these loci (data not shown).

The distal region of mouse chromosome 2 shares a region of homology with human chromosomes 20q (Fig.

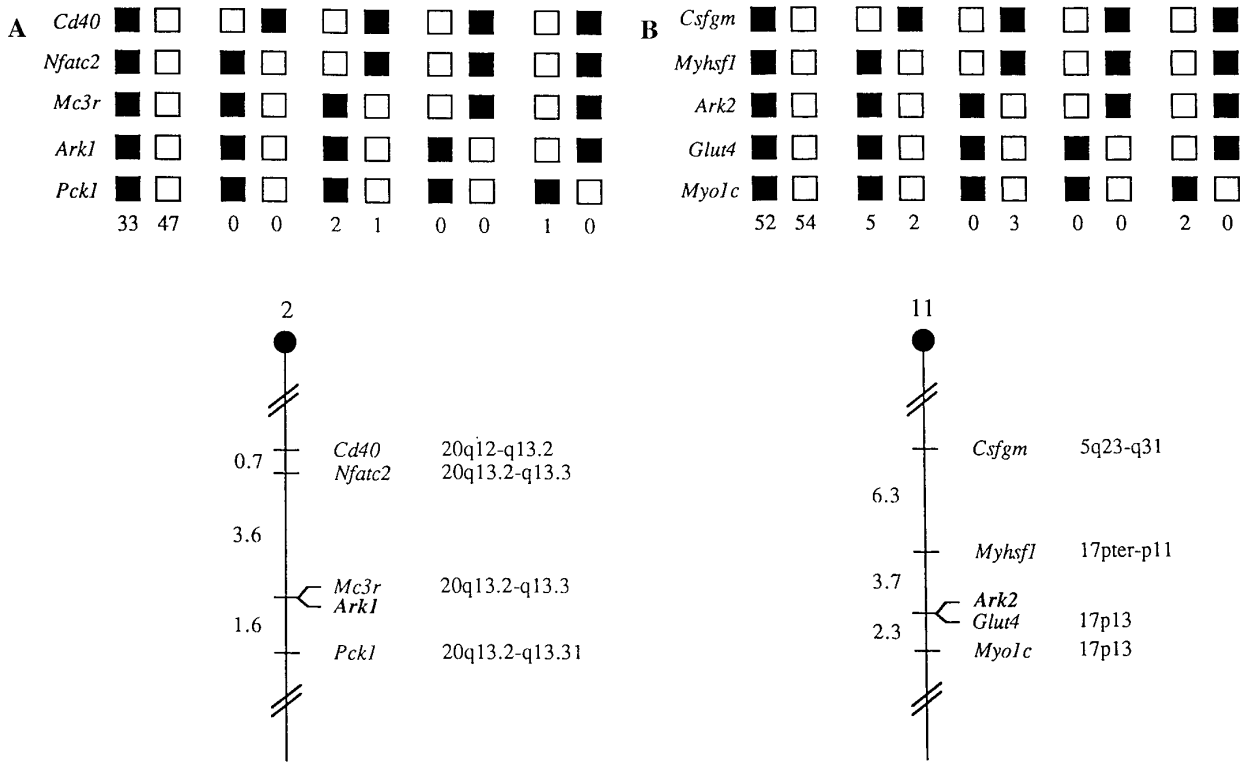

FIG. 5. Murine chromosomal location of *Ark1* and *Ark2*. The segregated patterns of *Ark1* (A) and *Ark2* (B) and their flanking genes in 84 and 118 backcross animals, respectively, that were typed for all loci are shown at the top. For individual pairs of loci, more animals were typed (see text). Each column represents the chromosome identified in the backcross progeny that was inherited from the (C57BL/6J × *M. spretus*) F_1 parent. The shaded boxes represent the presence of a C57BL/6J allele and white boxes represent the presence of a *M. spretus* allele. The number of offspring inheriting each type of chromosome is listed at the bottom of each column. Partial chromosome 2 and 11 linkage maps showing the location of *Ark1* (A) and *Ark2* (B) in relation to linked genes are shown at the bottom. Recombination distances between loci in centimorgans are shown to the left of the chromosome and the positions of loci in human chromosomes, where known, are shown to the right. References for the human map positions of loci cited in this study can be obtained from GDB (Genome Data Base), a computerized database of human linkage information maintained by The William H. Welch Medical Library of The Johns Hopkins University (Baltimore, MD).

5A). Our placement of *Ark1* in this interval suggests that *Ark1* will map to 20q in humans. The central region of mouse chromosome 11 shares regions of homology with human chromosomes 5q and 17p. The tight linkage between *Glut4*, which has been mapped to human chromosome 17p13 and *Ark2* in mouse, suggests that the human homologue of *Ark2* will reside on 17p, as well (Fig. 5B).

Our present identification and subsequent characterization of ARK2 suggested that the two closely related kinases ARK1 and ARK2 may have different roles in the cell cycle progression through different expression timing and subcellular localization. The unique accumulation of ARK2 in the midbodies during mitosis suggests that ARK2 may be directly involved in regulating the cleavage of polar spindle microtubules. Further studies will be required to address the actual roles of ARK1 and ARK2 in mitosis and meiosis.

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