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Source: Zoological Science, 14(6) : 917-921

Published By: Zoological Society of Japan

URL: <https://doi.org/10.2108/zsj.14.917>

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[Short Communication]

Molecular Cloning and Expression of the KIF3A Gene in the Frog Brain and Testis

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ABSTRACT—KIF3A is a member of the kinesin superfamily proteins (KIFs), but its gene has been cloned only in mouse and sea urchin. We have cloned a homolog of KIF3A from the frog, *Rana rugosa* (*rrKIF3A*). The sequence encoded a 699 amino acid protein that shares 93% similarity with mouse KIF3A (*mKIF3A*) and 69% with sea urchin kinesin-related protein (*SpKRP85*). The putative ATP-binding domain was completely identical to that of *mKIF3A* and *SpKRP85*. The level of *rrKIF3A* mRNA appeared to be high in the brain and testis of adult frogs, but low in the heart, lung and kidney. The results suggest that the *rrKIF3A* gene is expressed in the brain and testis more than other tissues of adult frogs examined, and that KIF3A is widely distributed in eukaryotic organisms.

INTRODUCTION

Cytoskeletal proteins may be important for cortical rotation (Elinson and Browning, 1988), localization of maternally encoded RNAs to the vegetal hemisphere of oocytes (Yisraeli *et al.*, 1990), and germ plasm aggregation (Savage and Danilchik, 1993) in *Xenopus laevis*. According to Robb *et al.* (1996), a particular cytoskeletal protein, a kinesin-like protein (*Xklp1*) is required for germ plasm aggregation in early *X. laevis* embryos. Recently, Vernos *et al.* (1995) found that *Xklp1* is essential for spindle organization and chromosome positioning in *Xenopus* oocytes. Kinesin was originally identified in squid giant axons and bovine brain as a motor protein which was linked with axonal transport (Vale *et al.*, 1985b). Motor proteins generate motile force by cyclic cross-bridge interactions with microtubules or actin filaments (Vale *et al.*, 1985a). These interactions are thought to be coupled to conformational changes of the motors as a result of hydrolysis of a single ATP molecule per 8-nm advance (Yang *et al.*, 1990; Schnitzer and Block, 1997). So far five mouse kinesins (*mKIF1* to 5) have been cloned (Hirokawa, 1993). *Xklp1* shows 74% similarity with *mKIF4* at the amino acid sequence level, but only 60% with *mKIF3A* (Vernos *et al.*, 1995). Thus, KIFs may be involved in many physiological processes in frog cells. In order to elucidate whether they are involved in such processes that occur in many types of cells in frogs, and whether they have been conserved through evolution, it is important to clone

cDNAs of KIFs in frogs. In the process of cloning other genes in frogs, we cloned a homolog of *mKIF3A* accidentally. In this paper, we report the nucleotide sequence and expression of the KIF3A homolog in tissues of the adult frog, *R. rugosa*.

MATERIALS AND METHODS

Animals

The frog, *Rana rugosa* was used for all experiments. At 20 hr before obtaining unfertilized eggs, frogs were primed by injection of the extract of pituitaries of *Rana catesbeiana* into the body cavity as described elsewhere (Kashiwagi and Kashiwagi, 1993). Tadpoles were staged according to Shumway (1940), and Taylor and Kollros (1946).

Cloning of the frog KIF3A cDNA

Two primers (P1, 5'-CAGGCCAACAGGAGCAAACAT-3' and P2, 25 mer oligo dT) were used for RT-PCR which was performed with 40 cycles of 94°C, 40 sec; 68°C, 2 min; 72°C, 3 min. These primers were originally designed for identifying the 3' end of mRNA of other gene in the frog, *R. rugosa* by the method of 3'-RACE (Sheflin *et al.*, 1995). Total RNA was prepared from whole tadpoles at stage 25 by the method of Chomczynski and Sacchi (1987), and was used as the initial templates for RT-PCR after treatment of DNase I (6 units per 30 µg of total RNA; Promega) at 37°C for 20 min. The fragment obtained by RT-PCR was cloned into the pUC19 plasmid vector and sequenced on both DNA strands using ABI 373A automated DNA sequencer by the manufacturer's guide (Perkin Elmer).

Southern blot analysis of RT-PCR products

Total RNAs prepared from different tissues of adult frogs were treated first with 6 units of DNase I (Promega) per 30 µg of total RNA at 37°C for 20 min and then used as the initial templates for RT-PCR. RT-PCR was carried out with 10 cycles of 94°C, 40 sec; 67°C, 2 min; 72°C, 2.5 min using the primers corresponding to nucleotides 4-26

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(F) and 1727-1748 (R) (F, 5'-CCGATCAACAAAGTAGAGAAACC-3'; R, 5'-TCGACTTAGCTGCCTGATGTTTC-3'). The PCR product (1.75 kbp) was electrophoresed on 0.8% agarose gel and electrophoretically transferred to nylon membranes (GeneScreen™; NEN Research Products). The DNA was then hybridized with the DIG-labelled origi-

nal PCR product (3.3 kbp) as a probe. The DIG DNA labeling kit and DIG luminescent detection kit (Boehringer Mannheim) were used for this analysis, following the manufacturer's protocol.

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-120 TTATGCTGGTGTGCGAAGCGGAGGTGCTGGTCCAGAGATTTGGGGTCTCCGTTCTAGCGGTGTGACGGGTATCCGGGCTCACCTCTACCCCTCAGGGCTCGCCCGGAACAGTGCAAAC
1 ATGCCGATCAACAAAGTAGAGAAACCTGAATCCTGTGATAATGTGAAGTTGTAGTTCGATGTCGCCATTGAATGATCGAGAGAAAGCCATGAGCAGCAGGATGGCTGTAATGTTGAT
1 M P I N K V E K P E S C D N V K V V V R C R P L N D R E K A M S S R M A V N V D
121 GAAATAAGGGGAACATTGCTGTTCACAAAGTAGACTCTATGAATGAGCCCCAAAGACTTTTACCTTTGACACAGTTTTTGAATAGACAGTAACCAAGTGGATGTCTACAATTTAAACA
41 E I R G T I A V H K V D S M N E P P K T F T F D T V F G I D S N Q L D V Y N L T
241 GCCAGCCAAATTATTGACTCTGTGCTGGAAGGCTACAATGGTACTATATTGCATATGGACAGACAGGCACTGGTAAAACATTTACCATTGGAGGGTGTTCGAGCTGTTCCAGAGCTCAGA
81 A R P I I D S V L E G Y N G T I F A Y G Q T G T G K T F T M E G V R A V P E L R
361 GGAATCATCCATAATTCAATTTGCTCACAATTTGGTCATATTGCTAAAGCAGAGGGAGATACAAGTTTTTGGTCAGAGTCTTATTTGAAATTTATAATGAGGAAGTACGGGACTG
121 G I I P N S F A H I F G H I A K A E G D T R F L V R V S Y L E I Y N E E V R D L
481 CTCGGTAAAGCAGACAGAGACTGGAGGTTAAAGAAAGACAGATGTGGGAGTTTACATCAAGGATTTATCGGGTTATGTAGTAAACAATGAGATGATGACCGGATTATGACT
161 L G K D Q T Q R L E V K E R P D V G V Y I K D L S G Y V V N N A D D M D R I M T
601 TTGGTCAATAAAATCGGTCTGTTGGAGCCACAAACATGAATGAGCACAGTCTCGTCCCATGCAATCTCACTATCACTATAGAGTGCAGGAGAGGGTGTGATGGCAATATGCAC
201 L G H K N R S V G A T N M N E H S S R S H A I F T I T I E C S E K G A D G N M H
721 GTACGAATGGGAAACTGCATCTTTGATGATCTTGGGGTCTGAAAGCAAGCAAGACAGGAGCAACCGGACAAAGACTGAAAGAACCCAAAAATTAATCTGTCACTTTCCACCTG
241 V R M G K L H L V D L A G S E R Q A K T G A T G Q R L K E A T K I N L S L S T L
841 GGAACGTCAATCCGCCCTGGTGTGATGGAAGGAGCACTCACGTGCCATATAGGAACCTCAAACTTACCGGACTGTTGAGGATTCACTGGAGGGAATTCAGAGCAATGATGTGTGCA
281 G N V I S A L V D G R S T H V P Y R N S K L T R L L Q D S L G G N S K T M M C A
961 AACATCGTCTCGAGATTACAATATGATGAGACAATCAGCACCTCCGCTATGCAAAACGAGCAAAAAATATCAAAAAAAGGCCAGAATCAATGAAGATCTAAAGATGCCCTTTTG
321 N I G P A D Y N Y D E T I S T L R Y A N R A K N I K N K A R I N E D P K D A L L
1081 CGCCAGTTTCAGAAAGAAATTAAGAACTCAAAAAAGAACTTAAGAAAGGAGAAAGAAATTTCTGGTCCGAAAGATAGTGGATCAGATGAGGATGATGATGAAGATGGAGAAATTTGAGAG
361 R Q F Q K E I E E L K K K L E E G E E I S G S E D S G S D E D D D E D G E I G E
1201 GTGGGAGAAAAGAAAAAGGCGAAGAGGCAAGAAAAAGGTATCCCTGATAAAATGGCAGAGATGCAAGCAGGATGATGAAGAAAGGAGGGCTCTTGAAGCAAACTGATATGGAG
401 D G E K K K R R R G K K K V S P D K M A E M Q A R I D E E R R A L E A K L D M E
1321 GAAGAAGAAAGAAATAAGCGAGAGCAGAACTGGAGAAGAGAGAAAGACCTGCTAAAAGCAACAAGAACCCAGTCCCTTCTAGAGAACTTTCTGCTTGGAGAAGAGGTCATT
441 E E E R N K A R A E L E K R E K D L L K A Q Q E H Q S L L E K L S A L E K K V I
1441 GTTGTGGGGTGGATTTACTGGTAAAGCAGAAGAACAAGACGGCTTTTAGCAATCAAAATGCTGAACTTGAAGAAGCTAGAAGGAGAGCAGAGAAGCTTCGAGGGAAGTACAGGAG
481 V G G V D L L A K A E E Q E R L L D E S N A E L E E R R R R R A E K L R R E L E E
1561 AAGGAGCAAGACGGTTAGATATTGAAGAAAAGTACACAAGCTTACAGGAGGAGGACAGGGTAAAATCAAAAAGTTGAAAAAGTGTGGACATGCTAATGGCAGCCAAAGTCTGAGATG
521 K E Q E R L D I E E K Y T S L Q E E A Q G K I K K L K K V W T M L M A A K S E M
1681 GCCGATCTCGACGAGAGCACCAAGAGAAATCGAGGGGCTATTGGAGAATCAGGACAGTAAAGTCTGAGAGCTTTGCTTTCAGATGATCATTATTGATAATTTATTCCCAAGCAT
561 A D L Q Q E H Q R E I E G L L E N I R Q L S R E L C L Q M I I I D N F I P Q D Y
1801 CAGGAATGATTGAAATTTACGTACACTGGAATGAAGATATTGGTGAATGGCAATGAAATGTGTTCATACACTGGAACAATATGAGAAAGCAAAACCCCTATACCGGACAAGAAAGAG
601 Q E M I E N Y V H W N E D I G E W Q L K C V A Y T G N N M R K Q T P I P D K K E
1921 AAGGACCTTTTGAAGTTGACCTGTCTCATGTATTTGGCTCACTGAGGAGACTGCGGCGAGTCCCTGATGAAGCTTGAAGACCTAGAACATCAAAAGGAAATCAAGACCCAAA
641 K D P F E V D L S H V Y L A Y T E S L R Q S L M K L E R P R T S K G K S R P K
2041 ACTGGTCGAAGAAGCGTTCTGCGAAACCCAGAGCTGTAATAGACTCATTATTACAGTAACCTGATCGTTCTACTGGATTATGAAGAAATTTCTCTTTCCGTTTAAAGATATGAATATAA
681 T G R R K R S A K P E A V I D S L L Q *
2161 CTGACTTTCGATTTTCAGTCAATGTTAATGCCAGTGGTCCGTTGGAGGATGCTGTTAAGTTTCAGTGTCTACTGTTCTACTCACATTTCACTCTCTCTCATGGATATAATGTAGCAAG
2281 TCACCTAGAGTAGAGTCTCTACCAGATATGTCTAATATTTCAACACTTTTCCAAATTTCTTATTAATCTGCTTGTGTTCAAAAAGTCAATGCTCAGTAAAGCACAGATGAATAAACAAA
2401 AGTATTTGTTTCATGCACCAATATAATGATGTTTGTATGTTTGTACTTTTGTGACAGGCTGAGTTTTTGTGACCTAAAAGATCTTGCCATTTGAAATTTGGCCATAATCATTCT
2521 ATAGGATTAAGTGGACAGGAAAGTCCATGTCAAGCTCTAGTCAAAAATTTGTTTGGATAGGATGAATAGTGTGGAACACTGTTAACACAGTTTATTGCTGTCTGCATCCAGCTGGTG
2641 AGTTCTCTCTCACTCTCTGCTCCAGTGTAGTGGTCACTCGGAGCAGTAAGTGAATGGAAGCTGAACCACTGAGACATGGACAGTAATTAAGAAAGTAAACAAAGATCTCATTTTTAAACA
2761 GTTTAGCTGATTTCTAAATTTTAAATTTCTGATGAGATCCCAATTTTACATATATCCATGGATGTTAGTAAATTTTGTATATACATTTCCCTGTTTATTCTTCTCATAGTGTAT
2881 TAATACATATAAATTTCTTTTGAACACTTGGCATGTTAAAAAACCTCTGTACATTAATAATGGGCTGCAATAATGATTGATGAATAGCATTAAATCTGATTTCCAGGGCTGATT
3001 CATTACAACCTGAAAAGTGGTGTCTGTTGCCATTGCTGCAATTTGGGTTTTTAAACAGAAAGCTTAAATTTTGGTAGCAGTGAAGCAGAAATCCACTATAGTTCTCATTATTCTTT
3121 AGTTATAATGAATTTGGTTCCCTGAATTAAGTACTGGAACATGTACAAAGAGAATACGAAAGGCTACCATTTGTCAGCCTTTCTCTGAAAATGTTAGTACCAGGGCTGATTGAGTCTTCCAG
3241 TATTTTATGATGACAGAACAGGATTTCTGCTTCCCACTAATTTCTAATCTGATGTTTGGCTCTCTGTTGGCTG

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Fig. 1. Nucleotide and deduced amino acid sequences of the *rrKIF3A* gene from the frog, *R. rugosa*. The nucleotide and amino acid sequences are numbered from the first nucleotide and the initiator methionine codon on the left of each line, respectively. Asterisk indicates the stop codon. The putative ATP-binding domain is boxed. Putative annealing sites are heavy-underlined for the P1 primer used for the amplification by the method of 3'-RACE (Sheflin *et al.*, 1995). Sequencing was completed using the dideoxy sequencing method (Sanger *et al.*, 1977). The sequence has been deposited in the EMBL data base (accession number AB001595).

RESULTS AND DISCUSSION

We first obtained an intense 3.3 kbp band amplified by RT-PCR. Sequence analysis of the fragment showed that it contained 3,340 nucleotides. The nucleotide sequence of the PCR product had 77% similarity with that of *mKIF3A* (Aizawa *et al.*, 1992). The 5'-end nucleotide sequence of the 3.3-kb PCR product was the same as the 3'-end nucleotide sequence (data not shown). However, the last two 3'-end nucleotides (AT) of the sequence coincided with the first two nucleotides of the initiation codon (ATG) for methionine. Therefore, the 5'-RACE (Maruyama *et al.*, 1995) was employed with the 3 backward primers corresponding to nucleotides 196-216 (K1), 221-242 (K2) and 246-266 (K3) of the *rrKIF3A* cDNA [(K1), 5'-GCTCATGGCTTTCTCTCGATC-3'; (K2), 5'-TCATCAACATT-

TACAGCCATCC-3'; (K3), 5'-TGAACAGCAATAGTCCCCTT-3'] in order to examine whether this putative nucleotide sequence (ATG) was true. By this, we extended 120 bp upstream of the 5' end of *rrKIF3A* cDNA. The nucleotide sequence shown in Fig. 1 had 75% similarity with that of *mKIF3A*, though 68% with *SpKRP85* (Rashid *et al.*, 1995) and only 47% with *Xklp1* (Vernos *et al.*, 1995). Comparison with the sequence of the *mKIF3A* gene suggested that ATG at the nucleotide position of 1 to be the initiation codon (Fig. 1). In addition, the last 7 nucleotides of putative annealing sites for the P1 primer (5'-CAAACAT-3') appeared to be identical (see the heavy underline in Fig. 1). Therefore, they may have worked as both forward and backward primers to generate the original 3.3-kb fragment.

The cDNA encoded a protein of 699 amino acids with a

<i>rrKIF3A</i>	MPINKVEKPEKSCDVKVVRCPRLNDRKAMSSRMVNVDEIRGTIAVHKVDSMN-EPPK	59
<i>mKIF3A</i>	MPINKSEKPEKSCDVKVVRCPRLNERKSMCYRQAVSVDEMIRGTITVHKITDSSN-EPPK	59
<i>SpKRP85</i>	MP---GGSSGN-DNVRVVVRCPRLNKRITGQGFKSVKMDEMIRGTIVQVTPNPAPSGEPPK	56
<i>rrKIF3A</i>	TFTFDTVFGIDSNLDVYNYLTARPIIDSVLEGYNGTIFAYGQTGTGKTFTMEGVRAVPEL	119
<i>mKIF3A</i>	TFTFDTVFGPEKSNLDVYNYLTARPIIDSVLEGYNGTIFAYGQTGTGKTFTMEGVRAVPEL	119
<i>SpKRP85</i>	SFTFDTVFAPGAKQTDVYNYLTARPIVDAITTEGYNGTIFAYGQTGTGKTFTMEGVRSQPEL	116
<i>rrKIF3A</i>	RGIIIPNSFAHIFGHIAKAEGDTRFLVRVSYLEIYNEEVRDLLGKDQTORLEVKERPDVGV	179
<i>mKIF3A</i>	RGVIIPNSFAHIFGHIAKAEGDTRFLVRVSYLEIYNEEVRDLLGKDQTORLEVKERPDVGV	179
<i>SpKRP85</i>	RGIIIPNSFAHIFGHIAKAEQENVRFVLRVSYLEIYNEEVRDLLGKDQQRLEVKERPDVGV	176
<i>rrKIF3A</i>	YIKDLSAYVVNNADDMDRIMTLGHKNRSVGTATNMNEHSSRSHAIIFTITIECSEKGDAGNM	239
<i>mKIF3A</i>	YIKDLSAYVVNNADDMDRIMTLGHKNRSVGTATNMNEHSSRSHAIIFTITIECSEKGVADNM	239
<i>SpKRP85</i>	YMKDLSAYVVNNADDMDRIMTLGNKNRSVGTATNMNESSSRSHAIIFTITIEKSDMGLDKEQ	236
<i>rrKIF3A</i>	HVRMGKHLHVDLAGSERQAKTGATGQRLKEATKINLSLSTLGNVISALVDGKSTHVPYRN	299
<i>mKIF3A</i>	HVRMGKHLHVDLAGSERQAKTGATGQRLKEATKINLSLSTLGNVISALVDGKSTHVPYRN	299
<i>SpKRP85</i>	HVRMGKHLHVDLAGSERQAKTGATGQRLKEATKINLSLSTLGNVISALVDGKSTHVPYRN	296
<i>rrKIF3A</i>	SKLTRLQDSLGGNSKTMCCANIGPADYNYDETISTLRYANRAKNIKKNKARINEDPKDAL	359
<i>mKIF3A</i>	SKLTRLQDSLGGNSKTMCCANIGPADYNYDETISTLRYANRAKNIKKNKARINEDPKDAL	359
<i>SpKRP85</i>	SKLTRLQDSLGGNAKTMCCANIGPADYNYDETISTLRYANRAKNIKKNKARINEDPKDAL	356
<i>rrKIF3A</i>	LRQFQKEIEELKKKL-EEGEEVSGSDISGSDDEEDDEGEIGEDGEK-KKRR-R-GKKKVS	415
<i>mKIF3A</i>	LRQFQKEIEELKKKL-EEGEEVSGSDISGSDDEEDDEGEIGEDGEKRRKRRDQAGKKKVS	417
<i>SpKRP85</i>	LRQFQKEIEELKKKQISESGEGLDDDEESGSEESGDE--EAGEGGMK-KKRRKGNPKRKLIS	413
<i>rrKIF3A</i>	PKMAEMQARIDEERRALEAKLDMEEEEERNKARAELEKREKDLLKAQQEHQSLLLEKLSAL	475
<i>mKIF3A</i>	PKMMEEMQAKIDEERRALEAKLDMEEEEERNKARAELEKREKDLLKAQQEHQSLLLEKLSAL	477
<i>SpKRP85</i>	PETMAMQKKIDEERKALEEKIDMVEEQRNIVHRELQRRESELHKAQDDQKTLNLEKLNAT	473
<i>rrKIF3A</i>	EKKVIVGGVDLLAKAEEQERLLDESNAELEERRRRAEKLRRELEEKQERLDIEEKYTSL	535
<i>mKIF3A</i>	EKKVIVGGVDLLAKAEEQERLLDESNAELEERRRRAEQLRRELEEKQERLDIEEKYTSL	537
<i>SpKRP85</i>	QKLLIVGGVDLLAKSEEQERLLDESNAELEERRRRAEQLRRELEEKQERLDIEEKYTSL	533
<i>rrKIF3A</i>	QEEAQGKTKKLLKKVWTMLMAAKSEMADLQEHQREIEGLENIRQLSRELCLQMIIDNF	595
<i>mKIF3A</i>	QEEAQGKTKKLLKKVWTMLMAAKSEMADLQEHQREIEGLENIRQLSRELRLQMLIIDNF	597
<i>SpKRP85</i>	QEEAQGKTKKLLKKVWTMLMAAKSEMADLQEHQREIEGLENIRQLSRELRLQMLIIDNF	593
<i>rrKIF3A</i>	IPQDYQEMIENYVHWNEDI GEWQLKCVAYTGNMRKQTPVADPKKEDPF-EVDLSHVYLA	654
<i>mKIF3A</i>	IPQDYQEMIENYVHWNEDI GEWQLKCVAYTGNMRKQTPVADPKKERDPF-EVDLSHVYLA	656
<i>SpKRP85</i>	IPQDYQEMIENYVHWNEDI GEWQLKCVAYTGNMRKQTPVADPKKSLAYGEADLSMFLT	653
<i>rrKIF3A</i>	YTI-E-ESLRQSLMKLERPRTSKGKSRPKTGRRKRSAPKPEAVIDSLLO	699
<i>mKIF3A</i>	YTI-E-ESLRQSLMKLERPRTSKGKSRPKTGRRKRSAPKPEAVIDSLLO	701
<i>SpKRP85</i>	MNLEGGGMKYKPSQGSQSRPKTSSGRPKTKKQASMASS-IDALLQ	699

Fig. 2. Amino acid sequence similarities of different KIF3As. Comparison of *rr*- and *mKIF3A*s, and *SpKRP85* is shown. The amino acid sequences were deduced from the nucleotide sequences of different cDNAs encoding the proteins [see references (Rashid *et al.*, 1995; Vernos *et al.*, 1995)]. Regions of identity are boxed. To maximize homologies, gaps represented by hyphens are introduced in the three sequences.

molecular weight (M_r) of 79,876 Da. When full length amino acid sequences were deduced for *rr*- and *mKIF3As*, and *SpKRP85* from the nucleotide sequences of cDNA, all amino acid sequences were found to be very similar (Fig. 2). *rrKIF3A* had 93% similarity with *mKIF3A*, and 70% with *SpKRP85*, but only 27% with *Xklp1* throughout their whole length (Fig. 2). In addition, there are two types of *mKIF3*, or *mKIF3A* and 3B (Yamazaki *et al.*, 1995). *mKIF3A* forms a complex with *mKIF3B* to work as a microtubule plus end-directed motor for membrane organelle transport (Yamazaki *et al.*, 1995). *rrKIF3A*, however, had only 49% similarity with *mKIF3B* when comparison of amino acid sequences of these two proteins was made. Judged from high similarities (>70%) among *rr*- and *mKIF3As*, and *SpKRP85*; in particular the ATP-binding domain of *rrKIF3A* is completely identical to those of *mKIF3A* and *SpKRP85*, it is reasonable to assume that *KIF3A* has been highly conserved through evolution.

rrKIF3A mRNA could not be detected by Northern blot analysis in various tissues of frogs when total RNA was used (data not shown), leading us to perform RT-PCR. The number for cycles was minimized to see the difference in the *rrKIF3A* mRNA level in various tissues of frogs. After agarose gel electrophoresis, the amplified transcripts were detected by the Southern blotting. As it can be seen in Fig. 3, the level of *rrKIF3A* mRNA appeared to be high in the brain and testis, and low in the heart, lung and kidney. By contrast, other tissues such as spleen, liver, pancreas, ovary and muscle had little *rrKIF3A* mRNA. According to Aizawa *et al.* (1992), *mKIF3A* mRNA was found abundantly in the brain among murine tissues examined, which is compatible with the results obtained in this study. Although accurate amounts of *rrKIF3A* mRNA in different tissues were not determined, the results in Fig. 3 probably show the relative amount of its mRNA in various tissues because the PCR was performed with only 10 cycles. The difference in the mRNA levels probably comes from neither poor qualities nor quantities of total RNAs, because the RNAs used as the initial templates for RT-PCR did not appear to be

degraded on 0.8% agarose gel electrophoresis (data not shown).

Finally, this study has not provided any evidence for the function(s) of *rrKIF3A* in the frog brain and testis. However, *mKIF3A* itself, which is a two-headed motor protein as well as other *KIFs*, but its tail is the second shortest among them (Hirokawa, 1993), is sufficient for supporting microtubule motility *in vitro* (Kondo *et al.*, 1994). It could be, therefore, easily speculated that frog *KIF3A* also functions in the brain as mouse *KIF3A* does, but its function in the testis is not clear yet. *KIF3A* may also work as a motor protein in the flagella of frog sperm. Further investigations will be required to elucidate the function(s) in these tissues of frogs.

REFERENCES

- Aizawa H, Sekiya Y, Takemura R, Zhang Z, Nangak M, Hirokawa N (1992) Kinesin family in murine central nervous system. *J Cell Biol* 119: 1287–1296
- Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162: 156–159
- Elinson RP, Browning R (1988) A transient array of parallel microtubules in frog eggs: potential tracks for a cytoplasmic rotation that specifies the dorso-ventral axis. *Dev Biol* 128: 185–197
- Hirokawa N (1993) Axonal transport and the cytoskeleton. *Curr Opin Neurobiol* 3: 724–731
- Kashiwagi A, Kashiwagi K (1993) Artificially produced chimeras between haploids and diploids in Japanese anurans. *Sci Rep Lab Amphibian Biol Hiroshima Univ* 12: 37–53
- Kondo S, Sato-Yoshitake R, Noda Y, Aizawa H, Nakata T, Matsuura Y, Hirokawa N (1994) *KIF3A* is a new microtubule-based anterograde motor in the nerve axon. *J Cell Biol* 125: 1095–1107
- Maruyama IN, Rakow TL, Maruyama HI (1995) cRACE: a simple method for identification of the 5' end of mRNAs. *Nucleic Acid Res* 23: 3796–3797
- Rashid DJ, Wedaman KP, Scholey JM (1995) Heterodimerization of the two motor subunits of the heterotrimeric kinesin, *KRP_{85/95}*. *J Mol Biol* 252: 157–162
- Robb DL, Heasman J, Raats J, Wylie C (1996) A kinesin-like protein is required for germ plasm aggregation in *Xenopus*. *Cell* 87: 823–831
- Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* 74: 5463–5467
- Savage RM, Danilchik MV (1993) Dynamics of germ plasm localization and its inhibition by ultraviolet irradiation in early cleavage *Xenopus* embryos. *Dev Biol* 157: 371–382
- Schnitzer MJ, Block SM (1997) Kinesin hydrolyses one ATP per 8-nm step. *Nature* 388: 386–390
- Sheflin LG, Brook EM, Spaulding SW (1995) Assessment of transcript polyadenylation by 3' RACE: the response of epidermal growth factor messenger ribonucleic acid to thyroid hormone in the thyroid and submaxillary glands. *Endocrinology* 136: 5666–5676
- Shumway W (1940) Stages in the normal development of *Rana pipiens*. I. External form. *Anat Rec* 78: 139–147
- Taylor A, Kollros JJ (1946) Stages in the normal development of *Rana pipiens* larvae. *Anat Rec* 94: 7–23
- Vale RD, Schnapp BJ, Reese TS, Sheetz MP (1985a) Organelle, based and microtubule translocations promoted by soluble factors from the squid giant axon. *Cell* 40: 449–454
- Vale RD, Reese TS, Sheetz MP (1985b) Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility. *Cell* 42: 39–50

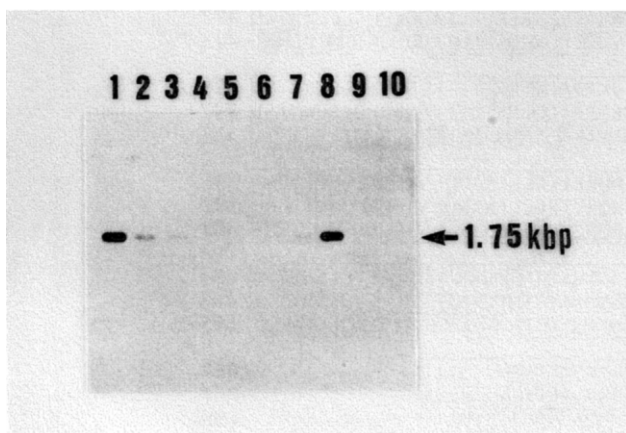


Fig. 3. Southern blot analysis of RT-PCR products. Each lane represents as follows; 1, brain; 2, heart; 3, lung; 4, liver; 5, pancreas; 6, spleen; 7, kidney; 8, testis; 9, ovary and 10, muscle.

- Vernos I, Raats J, Hirano T, Heasman J, Karsenti E, Wylie C (1995) Xklp1, a chromosomal *Xenopus* kinesin-like protein essential for spindle organization and chromosome positioning. *Cell* 81: 117–127
- Yamazaki H, Nakata T, Okada Y, Hirokawa N (1995) KIF3A/B: a heterodimeric kinesin superfamily protein that works as a microtubule plus end-directed motor for membrane organelle transport. *J Cell Biol* 130: 1387–1399
- Yang JT, Saxton WM, Stewart RJ, Raff EC, Goldstein LSB (1990) Evidence that the head of kinesin is sufficient for force generation and motility *in vitro*. *Science* 249: 42–47
- Yisraeli J, Sokol S, Melton D (1990) A two-step model for the localization of maternal mRNA in *Xenopus* oocytes: involvement of microtubules and microfilaments in the translocation and anchoring of Vgl mRNA. *Development* 108: 289–298

(Received May 22, 1997 / Accepted August 29, 1997)