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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

ZOOLOGICAL SCIENCE 14: 917–921 (1997)

[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* (*rr*KIF3A). The sequence encoded a 699 amino acid protein that shares 93% similarity with mouse KIF3A (*m*KIF3A) and 69% with sea urchin kinesin-related protein (*Sp*KRP85). The putative ATP-binding domain was completely identical to that of *m*KIF3A and *Sp*KRP85. The level of *rr*KIF3A 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 *rr*KIF3A 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 (*X*klp1) is required for germ plasm aggregation in early *X. laevis* embryos. Recently, Vernos *et al.* (1995) found that *X*klp1 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 microtubles 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 (*m*KIF1 to 5) have been cloned (Hirokawa, 1993). *X*klp1 shows 74% similarity with *m*KIF4 at the amino acid sequence level, but only 60% with *m*KIF3A (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 *m*KIF3A accidentally. In this paper, we report the nucleotide sequence and expression of the KIF3A homolog in tissues of the adult frog, *R. rugosa*.

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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

(F) and 1727-1748 (R) (F, 5'-CCGATCAACAAAGTAGAGAAACC-3'; R, 5'-TCGACTTAGCTGCCTGATGTTC-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 original 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 TTATGCGTGGTGTGCGAAGCGGAGGTGCTGGTCCAGAGATTTGGGGTCTCCGTTCTAGCGGTGTCAGCGGGTATCCGGGCTCACCTCTACCCCTCAGGGCTCGCCCGGAACAGTGCAAAC

   1 ATGCCGATCAACAAAGTAGAGAAACCTGAATCCTGTGATAATGTGAAGGTTGTAGTTCGATGTCGCCCATTGAATGATCGAGAGAAAGCCATGAGCAGCAGGATGGCTGTAAATGTTGAT
   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 GAAATAAGGGGAACTATTGCTGTTCACAAAGTAGACTCTATGAATGAGCCCCCAAAGACTTTTACCTTTGACACAGTTTTTGGAATAGACAGTAACCAGTTGGATGTCTACAATTTAACA
  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 GCCAGGCCAATTATTGACTCTGTGCTGGAAGGCTACAATGGTACTATATTTGCATATGGACAGACAGGCACTGGTAAAACATTTACCATGGAGGGTGTTCGAGCTGTTCCAGAGCTCAGA
  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 GGAATCATCCCTAATTCATTTGCTCACATATTTGGTCATATTGCTAAAGCAGAGGGAGATACAAGGTTTTTGGTCAGAGTGTCTTATTTGGAAATTTATAATGAGGAAGTACGGGACTTG
 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 CTCGGTAAAGACCAGACACAGAGACTGGAGGTTAAAGAAAGACCAGATGTGGGAGTTTACATCAAGGATTTATCGGGTTATGTAGTAAACAATGCAGATGATATGGACCGGATTATGACT
 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 TTGGGTCATAAAAATCGGTCTGTTGGAGCCACAAACATGAATGAGCACAGTTCTCGTTCCCATGCAATCTTCACTATCACTATAGAGTGCAGCGAGAAGGGTGCTGATGGCAATATGCAC
 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 GTACGAATGGGAAAACTGCATCTTGTAGATCTTGCGGGTTCTGAAAGACAAGCAAAGACAGGAGCAACCGGACAAAGACTGAAAGAAGCCACAAAAATTAATCTGTCACTTTCCACCCTG
 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 GGAAACGTCATCTCCGCCCTGGTTGATGGAAGGAGCACTCACGTGCCATATAGGAACTCCAAACTTACGCGACTGTTGCAGGATTCACTGGGAGGGAATTCCAAGACAATGATGTGTGCA
 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 AACATCGGTCCTGCAGATTACAACTATGATGAGACAATCAGCACCCTCCGCTATGCAAACCGAGCAAAAAATATCAAAAATAAGGCCAGAATCAATGAAGATCCTAAAGATGCCCTTTTG
 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 CGCCAGTTTCAGAAAGAAATTGAAGAACTCAAAAAGAAACTTGAAGAAGGAGAAGAAATTTCTGGTTCCGAAGATAGTGGATCAGATGAGGATGATGATGAAGATGGAGAAATTGGAGAG
 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 GATGGAGAAAAGAAAAAAAGGCGAAGAGGCAAGAAAAAGGTATCCCCTGATAAAATGGCAGAGATGCAAGCACGGATTGATGAAGAAAGGAGGGCTCTTGAAGCAAAACTTGATATGGAG
 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 GAAGAAGAAAGGAATAAAGCGAGAGCAGAACTGGAGAAGAGAGAGAAAGACCTGCTAAAAGCACAACAAGAACACCAGTCCCTTCTAGAGAAACTTTCTGCCTTGGAGAAGAAGGTCATT
 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 GTTGGTGGGGTGGATTTACTGGCTAAAGCAGAAGAACAAGAACGGCTTTTAGACGAATCAAATGCTGAACTTGAAGAACGTAGAAGGAGAGCAGAGAAGCTTCGCAGGGAACTAGAGGAG
 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  A  E  K  L  R  R  E  L  E  E

1561 AAGGAGCAAGAACGGTTAGATATTGAAGAAAAGTACACAAGCTTACAGGAGGAGGCACAGGGTAAAATCAAAAAGTTGAAAAAAGTGTGGACCATGCTAATGGCAGCCAAGTCTGAGATG
 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 GCCGATCTGCAGCAAGAGCACCAAAGAGAAATCGAGGGGCTATTGGAGAACATCAGGCAGCTAAGTCGAGAGCTTTGTCTTCAGATGATCATTATTGATAATTTTATTCCCCAAGACTAT
 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 CAGGAAATGATTGAAAATTACGTACACTGGAATGAAGATATTGGTGAATGGCAATTGAAATGTGTTGCATACACTGGAAACAATATGAGAAAGCAAACCCCTATACCGGACAAGAAAGAG
 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 AAGGACCCTTTTGAAGTTGACCTGTCTCATGTATATTTGGCCTACACTGAGGAGAGTCTGCGGCAGTCCCTGATGAAGCTTGAAAGACCTAGAACATCAAAAGGAAAATCAAGACCCAAA
 641 K  D  P  F  E  V  D  L  S  H  V  Y  L  A  Y  T  E  E  S  L  R  Q  S  L  M  K  L  E  R  P  R  T  S  K  G  K  S  R  P  K

2041 ACTGGTCGAAGAAAGCGTTCTGCGAAACCAGAAGCTGTAATAGACTCATTATTACAGTAACTGATCGTTCTACTGGATTATGAAGAATTTCTCTTTTCCGTTTAAGAATATGAATTATAA
 681 T  G  R  R  K  R  S  A  K  P  E  A  V  I  D  S  L  L  Q  *

2161 CTGACTTTCGCATTTCAGTGCAATGTTAATTGCCAGGTGGTCCGTGGAGGCATGCTGTTAAGTTTCAGTTGCTACTGTTCTACTCACATTTCACTCTCCTCATGGATATAATGTAGCAAG
2281 TCACCTAGAGTAGAGCTCCTACCAGATATGTCTAATATTTCAAACATTTTGCCAATATTCTTATTAATCTGCTTGTGTTCAAAAGTGCAATGCTCAGTAAAGCACAGATGAATAAACAAA
2401 AGTATTTGTTCATGCACCAATATAATGATGTTTGATGTTGTATTTGACTTTACTTTGTGCAGGCTGTAGTTTTTGTTGACCTAAAAAGATCTTGCCATTGAAATTTGGCCATAATCATTC
2521 ATAGGATTACTGGACAGGAAGTCCATGTCAAGCTCTAGTCAAAATTATGTTTTGGATAGGATTGAATAGTGTTGGAAACACTGTTAACACAGTTTATTGCTGTCTGCATCCCAGCTGGTG
2641 AGTTCTCCTCTCACTTCCTGTCCCAGTGATAGTGGTCATCGGAGCAGTAAGTGAATGGAAGCTGAACCACTGAGACATGGACAGTAATTAAAAAGTTAACAAAGATCTCATTTTTTAACA
2761 GTTTAGCTGTATTCTTAAATTTTAAATTCTGATGAGATCCACCAATTTTTACATATATCCATGGATGTTAGTAAATTATTTTGTATATACATTCCCTTGTTATTCATTCTTCATAGTGAT
2881 TAATACATATAATAATTCTTTTTGAACTACTTGGCATGTTAAAAAAACCTCTTGTACATTAAAATGGGCTGTCAATAATGATTGATGAATAGCATTTAATCTGATTTCCCAGGGCTGATT
3001 CATTACAACTGAAAAAGTGGTGCTGTTGCCCATTGCTGCCAATTGGGTTTTATGAAACAGAAGCTTAAATTTTGGTAGCAGTGAGCAGAAATTCCACTATAGTTCTCATTTATTTTCTTT
3121 AGTTATAATGAATTGGTTTCCCTGAATTACTGGAAACATGTACAAAGAGAATACGAAGGCTACCATTGTCAGCCTTTCCTTCTGAAAATGTTAGTTACCGGGCTGATTCAGTGACTTCAG
3241 TATTTTATGCATGCAGAACAGGATTTCTGTCTTCCCACTAACTTTCTAATCTAATCTGCATGTTTGCTCCTGTTGGCCTG
```

Fig. 1. Nucleotide and deduced amino acid sequences of the *rr*KIF3A 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 *m*KIF3A (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 intiation 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 *rr*KIF3A cDNA [(K1), 5'-GCTCATGGCTTTCTCTCGATC-3'; (K2), 5'-TCATCAACATTTACAGCCATCC-3'; (K3), 5'-TGAACAGCAATAGTTCCCCTT-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 *rr*KIF3A cDNA. The nucleotide sequence shown in Fig. 1 had 75% similarity with that of *m*KIF3A, though 68% with *Sp*KRP85 (Rashid *et al.*, 1995) and only 47% with *X*klp1 (Vernos *et al.*, 1995). Comparison with the sequence of the *m*KIF3A 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

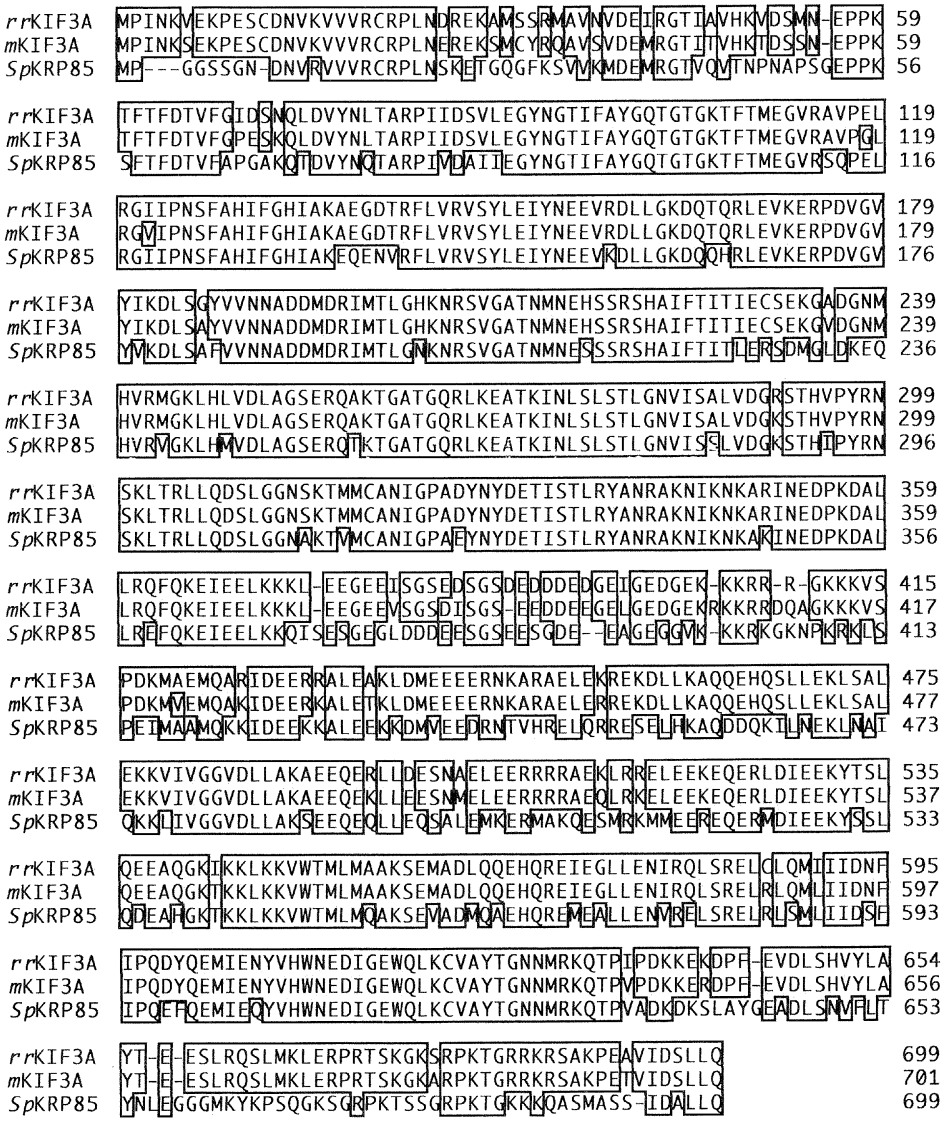

Fig. 2. Amino acid sequence similarities of different KIF3As. Comparison of *rr*- and *m*KIF3As, and *Sp*KRP85 is shown. The amino acid sequences were deduced from the nucleotide sequences of different cDNAs encoding the proteins [see references (Rashid *et al.*, 1995; Vernos *et al.*, 1995)]. Regions of identity are boxed. To maximize homologies, gaps represented by hyphenes are introduced in the three sequences.

molecular weight (Mr) of 79,876 Da. When full length amino acid sequences were deduced for *rr*- and *m*KIF3As, and *Sp*KRP85 from the nucleotide sequences of cDNA, all amino acid sequences were found to be very similar (Fig. 2). *rr*KIF3A had 93% similarity with *m*KIF3A, and 70% with *Sp*KRP85 , but only 27% with *X*klp1 throughout their whole length (Fig. 2). In addition, there are two types of *m*KIF3, or *m*KIF3A and 3B (Yamazaki *et al.*, 1995). *m*KIF3A forms a complex with *m*KIF3B to work as a microtubule plus end-directed motor for membrane organelle transport (Yamazaki *et al.*, 1995). *rr*KIF3A, however, had only 49% similarity with *m*KIF3B when comparison of amino acid sequences of these two proteins was made. Judged from high similarities (>70%) among *rr*- and *m*KIF3As, and *Sp*KRP85; in particular the ATP-binding domain of *rr*KIF3A is completely identical to those of *m*KIF3A and *Sp*KRP85, it is reasonable to assume that KIF3A has been highly conserved through evolution.

*rr*KIF3A 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 *rr*KIF3A 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 *rr*KIF3A 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 *rr*KIF3A mRNA. According to Aizawa *et al.*(1992), *m*KIF3A 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 *rr*KIF3A 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 intial templates for RT-PCR did not appear to be degraded on 0.8% agarose gel electrophoresis (data not shown).

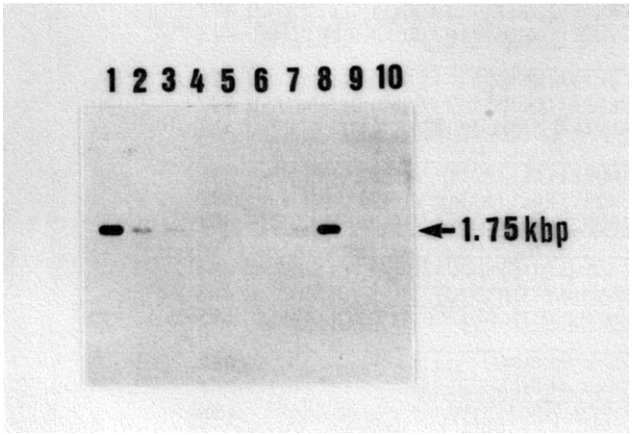

Fig. 3. Southern blot analysis of RT-PCR products. Each lane represents as follows; 1, brain; 2, heart; 3, lung; 4, liver; 5, pancreas; 6, spleen; 7, kidney; 8, testis; 9, ovary and 10, muscle.

Finally, this study has not provided any evidence for the function(s) of *rr*KIF3A in the frog brain and testis. However, *m*KIF3A 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.

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