Vol. 194, No. 1, 1993 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 15, 1993 Pages 552-559

ISOLATION OF cDNA CLONES AND TISSUE EXPRESSION OF RAT RAL A AND RAL B GTP-BINDING PROTEINS

Gary M. Wildey, Manjula Viggeswarapu, Sharon Rim and Julie K. Denker

Department of Cardiovascular Biology, Research Institute,
Cleveland Clinic Foundation, 9500 Euclid Ave,
Cleveland, OH 44195

Received June 1, 1993

SUMMARY: cDNA clones encoding the low molecular weight GTP-binding proteins ral A (951 bp) and ral B (2073 bp), including the entire coding region (618 bp), were isolated from a rat PC12 pheochromocytoma library. Northern analyses demonstrated that both ral A and ral B are widely expressed in rat tissues. Two ral A transcripts of 1.1 and 2.9 kb were observed in most tissues in varying proportions. The 1.1 kb ral A band of testes was further shown to be composed of two closely migrating species. In contrast to these findings, a single ral B transcript of 2.3 kb was detected in most tissues. Steady-state levels of ral A transcripts appear greater than ral B. Quantitatively, the testes exhibited the highest ral A and ral B mRNA levels, with lower levels observed in the brain, adrenal and pituitary glands, kidney and ovary. Ral mRNA levels were lowest in muscle tissue, particularly skeletal muscle.

The ras gene family consists of three members, H-, K- and N-ras, which encode low molecular weight (LMW) GTP-binding proteins in mammals (1, 2). An exciting development over the last several years has been the discovery that ras represents only one of many LMW GTP-binding proteins that are present in mammalian cells (2-4). Interestingly, none of these LMW GTP-binding proteins has been demonstrated to be a proto-oncogene, although one, called K_{rev} or rap 1A, can reverse the transforming activity of activated ras (5). The GTP-binding domains of these newly-discovered LMW GTP-binding proteins are strongly conserved and identical to those of ras proteins. Thus, it has been suggested that these LMW GTP-binding proteins be collectively referred to as the ras superfamily of GTP-binding proteins, with ras being the prototype (6). The ras superfamily has been divided into three families, the ras-, rho- and rab-related proteins, based on more extensive amino acid sequence homologies found outside of the GTP-binding domains (7).

Ral is a member of the ras-related subfamily of GTP-binding proteins. A cDNA clone encoding ral was initially isolated from a simian β-lymphocyte library because of its similarity to ras (8). Subsequently, ral cDNA clones were isolated from human placental (9), human pheochromocytoma (10) and marine ray electric lobe (11) cDNA libraries. Two different human ral cDNA sequences have been identified, termed ral A and ral B (10). The deduced amino acid sequence identity between ral A and ral B is 85%, while the amino acid sequence identity

0006-291X/93 $4.00

between ral and ras is about 50% (8, 10). Whereas both ral A and ral B encode for proteins of 206 amino acids with a predicted molecular weight of approximately 24,000, ral A purified from human platelets migrates by SDS-PAGE as a 26-28 kDa protein (9). Northern blot analysis of ral A gene expression in adult mouse tissues revealed two transcripts of 1.2 kb and 2.8 kb (12). The highest message levels were detected in the brain, ovary and testes, with little or no detectable message in the liver, spleen, kidney, thymus, salivary gland and heart. Interestingly, brain expressed predominantly the larger ral A transcript, whereas testes and ovary expressed predominantly the smaller ral A transcript. To date, ral B has not been purified from any tissue source and its expression by various mammalian tissues has not been reported.

In this study we report for the first time the isolation of cDNA clones encoding rat ral A and ral B proteins. In addition, we quantitatively examine the expression of both ral A and ral B in various rat tissues, and report a novel ral A mRNA transcript.

EXPERIMENTAL METHODS

Isolation and sequencing of ral cDNA clones: Ral cDNA clones were isolated from a rat PC12 λgt10 cDNA library (Clonetech, Palo Alto, CA) using ral A- and ral B-specific probes generated from PCR reactions. The design of the PCR probes capitalized on the finding that human ral A and ral B amino acid sequences are most divergent from each other and from ras at their carboxy-terminal regions (amino acids 106-185)(10). Within this region, human ral A and ral B are only 70% identical. Therefore, degenerate oligonucleotide primers were synthesized based upon the amino acid sequences of human ral A and ral B immediately flanking this region [amino acid sequences ESF(A/T)ATA and (E/D)KNGKK]. These primers were used in a PCR reaction using rat brain and rat testes cDNA as templates. cDNA was synthesized using M-MLV reverse transcriptase (USB, Cleveland, OH) and random hexamer primers essentially following the manufacturer's instructions. A PCR product of the appropriate size (about 250 bp) was generated after 30 cycles using standard cycling (melt for 1 min at 94°C, anneal for 2 min at 55°C and extend for 3 min at 72°C) and buffer conditions. The PCR product was purified by agarose gel chromatography, ligated into pBS (Stratagene, La Jolla, CA) via Eco RI linkers added onto the PCR oligonucleotides and used to transform DH5α cells. Nucleotide sequencing of plasmid inserts prepared from individual transformed DH5α colonies revealed sequences which were 91% and 84% identical to human ral A and ral B, respectively (PC Gene, IntelliGenetics, Mountain View, CA).

The 250 bp PCR products encoding rat ral A or ral B were labelled by random priming with [^{32}P]dCTP (6000 Ci/mmol, Dupont/NEN, Boston, MA) using a kit from Boehringer Mannheim (Indianapolis, IN) and used to screen 500,000 plaques of the PC12 library blotted onto Colony/Plaque Screen (Dupont/NEN). The blots were hybridized (about 10^6 cpm probe/ml) overnight at 42°C in Church and Gilbert high stringency buffer (200 mM sodium phosphate, pH 7.2, 15% formamide, 7% SDS, 1 mM EDTA and 1% BSA)(13) and washed the next day 3 x 20 min in 0.5X SSC/0.1% SDS (20X SSC: 3 M NaCl, 0.3 M Na citrate, pH 7) at 65°C. Five ral A clones and four ral B clones were isolated to homogeneity. Inserts contained in clones were amplified by PCR as described above using commercially-supplied oligonucleotides (Amplimers, Clonetech). The inserts were purified by agarose gel chromatography and either blunt-end ligated into the Sma site of the pBS polylinker (ral A clones) or ligated into the Eco RI site (ral B clones) of the pBS polylinker. The ligated plasmid was used to transform DH5α cells. Inserts purified from individual transformed colonies were restriction mapped and restriction fragments were subcloned into pBS for nucleotide sequencing (Sequenase, USB, Cleveland, OH).

RNA preparation and Northern blot analysis: Total RNA was prepared using the one-step acid-extraction procedure of Chomczynski and Sacchi (14) from rat tissues previously quick-frozen in liquid nitrogen. Typically one gram of frozen tissue was used to prepare total RNA. The OD 260/280 of total RNA prepared this way was usually 1.8-2.1. Poly A+ RNA was isolated from total RNA using a Poly Attract kit from Promega (Madison, WI). RNA was size-

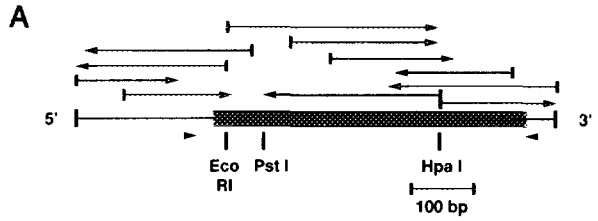
fractionated on a 1% agarose/formaldehyde gel and transferred onto nylon membranes (GeneScreen Plus, Dupont/NEN) by capillary action. The resulting blots were hybridized overnight at 42°C in Church & Gilbert high-stringency buffer as described above. Probe labelling and blot washes were also performed as described above. To quantitate the mRNA levels and ratios of ral A and ral B in various tissues, the Northern blots were analyzed using a Molecular Dynamics PhosphorImage analysis system (Sunnyvale, CA). Typically, the blots were exposed to the imager cassette overnight. The amount of RNA loaded onto each gel lane was normalized using a full-length cDNA probe to glyceraldehyde 3-phosphate dehydrogenase (15). The RNA ladder to determine transcript size was obtained from Gibco/BRL (Gaithersburg, MD).

RESULTS AND DISCUSSION

Isolation of Rat Ral A and Ral B cDNA Clones. Northern blot analysis of mRNA isolated from rat PC12 cells using the ral PCR products as probes demonstrated that two ral A transcripts (1.1 and 2.9 kb) as well as a ral B message are present in these cells. Thus, the same PCR probes were used to screen a rat PC12 cDNA library for clones encoding ral A and ral B.

Two ral A clones with similar restriction maps were sequenced and revealed that the insert of one clone (8A) was truncated in the 5'-coding region compared to the other (6A). The sequence of the insert of clone 6A is shown in Figure 1. This insert contains the entire coding region for ral A (618 bp) plus 273 bp of 5'-noncoding sequence and a short (60 bp) 3'-untranslated region which includes a polyadenylation signal (16) 10 bp upstream of a poly A^+ region. The nucleotide sequence of the 6A insert is 93% identical to human ral A in the coding region. Accordingly, the deduced amino acid sequence (206 amino acids) encoded by the 6A insert is 99% identical to human ral A, containing only a single conservative substitution at residue 147, in which a glutamate encoded by human ral A is replaced by an aspartate residue in the rat protein (Figure 3). It is interesting to note that the 5'-untranslated region of the 6A insert contains four upstream ATG start sites which are in-frame with the initiating methionine and are not interrupted by an intervening stop codon. However, none of the four start sites is supported by a Kozak consensus sequence (17) as strong as that of the initiating methionine.

Two ral B clones with the largest inserts (1.6 and 2.2 kb) and similar restriction maps were sequenced. Initial nucleotide sequencing of the insert of the smaller clone (2B) revealed that it was similar to that of human ral B but was truncated in the 5'-coding region. Thus, the insert of clone 6B was sequenced in its entirety and is shown in Figure 2. The 6B insert contains a complete coding sequence (618 bp) encoding a 206 amino acid protein plus a short 5'-untranslated region (63 bp) and a long 3'-untranslated tail (1392 bp) containing a common variant polyadenylation signal 16 bp upstream of a poly A^+ region. The nucleotide sequence of insert 6B is 85% identical to human ral B in the coding region. This results in nine amino acid differences between the deduced amino acid sequences of the rat and human ral B proteins, representing 95% identity (Figure 3). One of these substitutions is a conservative arginine for lysine substitution (residue 190) while four others involve glycine for serine substitutions (residues 6, 8, 10 and 193). Unlike the ral 6A insert, there are no ATG start sites upstream of the initiating ATG in the 6B insert. The start site in the ral 6B insert is found in the context of a strong Kozak consensus sequence.



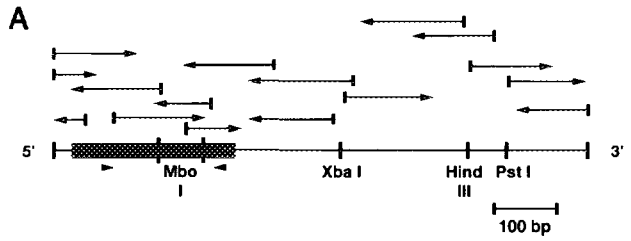
B

```
                                                    cggccaggttgacagttgggcagaagctcttggttcctcttcaagtggtaatgc
  55  cttcatgccaactttgccgaagtaacctggatgatatttgtcaaagttgatcctgtggtg
 115  atgcatgcctccagcattcccgcggcctcctgggtgcttgcggtgcttaccgatgcgacc
 175  gtggccgtggctcacgtggccccggagtttccgtcttcctaccagtctggatggcatggc
 235  ggtgcagattcttttcagtcctctgaagactgcacacaggATGGCTGCAAACAAGCCCAA
                                                  M  A  A  N  K  P  K    7
 295  GGGTCAGAATTCTTTGGCCTTACACAAAGTCATCATGGTGGGCAGTGGTGGTGTGGGCAA
        G  Q  N  S  L  A  L  H  K  V  I  M  V  G  S  G  G  V  G  K   27
 355  GTCTGCTCTGACTCTGCAGTTCATGTATGATGAGTTTGTAGAAGACTATGAACCTACCAA
        S  A  L  T  L  Q  F  M  Y  D  E  F  V  E  D  Y  E  P  T  K   47
 415  AGCAGACAGCTACAGGAAGAAGGTAGTGCTGGATGGGGAGGAAGTGCAGATCGACATCTT
        A  D  S  Y  R  K  K  V  V  L  D  G  E  E  V  Q  I  D  I  L   67
 475  AGATACAGCAGGGCAGGAAGACTACGCTGCAATTAGAGACAACTACTTCCGAAGTGGGGA
        D  T  A  G  Q  E  D  Y  A  A  I  R  D  N  Y  F  R  S  G  E   87
 535  AGGATTCCTCTGTGTCTTCTCTATCACAGAGATGGAGTCCTTTGCAGCTACAGCGGACTT
        G  F  L  C  V  F  S  I  T  E  M  E  S  F  A  A  T  A  D  F  107
 595  CAGGGAACAGATTTTAAGAGTAAAAGAAGATGAGAATGTCCCATTTCTCCTGGTTGGTAA
        R  E  Q  I  L  R  V  K  E  D  E  N  V  P  F  L  L  V  G  N  127
 655  CAAATCAGATTTAGAAGATAAAAGGCAGGTTTCTGTAGAAGAGGCAAAAAACAGAGCTGA
        K  S  D  L  E  D  K  R  Q  V  S  V  E  E  A  K  N  R  A  D  147
 715  CCAGTGGAACGTTAACTATGTGGAGACGTCTGCTAAAACGCGCGCCAACGTTGACAAGGT
        Q  W  N  V  N  Y  V  E  T  S  A  K  T  R  A  N  V  D  K  V  167
 775  ATTTTTTGATTTAATGAGGGAAATACGAGCCAGAAAGATGGAAGACAGCAAAGAAAAAAA
        F  F  D  L  M  R  E  I  R  A  R  K  M  E  D  S  K  E  K  N  187
 835  TGGAAAAAAGAAGAGGAAAAGTTTAGCCAAGAGAATCAGAGAAAGATGCTGCATTTTAta
        G  K  K  K  R  K  S  L  A  K  R  I  R  E  R  C  C  I  L    206
 895  atcaaagcccaaactcctttcttatcctgacctgaccatactaataaatataatttat(a)n
```

Figure 1. Nucleotide and amino acid sequence of rat ral A. Panel A. Sequencing strategy and partial restriction map of the ral 6A cDNA insert. The location of the coding region within the clone is shown as the dark bar. The arrows above figure show the direction and length of sequencing reactions. The arrowheads below figure show the location of oligonucleotide primers used in PCR reactions. **Panel B.** Nucleotide sequence and deduced amino acid sequence of the ral 6A cDNA clone. The deduced amino acid sequence is given below the nucleotide sequence in bold capital letters. The deduced amino acid sequence of the ral A PCR product used to probe the PC12 cDNA library and Northern blots is underlined. ATG sequences which are upstream and in-frame with the initiating methionine are also shown in bold print. The polyadenylation signal is underlined. Numbering of the nucleotide sequence and the deduced amino acid sequence is given on the left and right, respectively.

The deduced amino acid sequence of the ral 6B insert is 82% identical to that of the ral 6A insert (Figure 3). As expected, most of the sequence dissimilarity is located in the C-terminal half of the protein. Within the region of the PCR products which were used as probes, the deduced amino acid sequences are only 74% identical. The nucleotide sequences are only 71% identical in this region.

PCR was used to demonstrate that the inserts in the 6A and 6B ral clones were present in tissue mRNA pools. When primers based upon nucleotide sequences found in the 5'- and 3'-untranslated regions of the ral 6A clone (see arrowheads, Figure 1A) were used in PCR reactions



B

```
   1  cggcggctccctcagctcctcatctgggccatctgtccgtgtgtcgctgcctcagaccag
  61  caggATGGCTGCCAACAAAGGCAAGAGCCAGGGCTCCCTGGTCCTTCACAAGGTCATCAT
        M  A  A  N  K  G  K  S  Q  G  S  L  V  L  H  K  V  I  M   19
 121  GGTCGGCAGTGGGGGAGTCGGCAAGTCAGCCCTGACGCTCCAGTTCATGTATGACGAGTT
        V  G  S  G  G  V  G  K  S  A  L  T  L  Q  F  M  Y  D  E  F   39
 181  TGTAGAAGACTATGAGCCCACCAAAGCCGACAGTTACAGAAAGAAGGTGGTTCTCGACGG
        V  E  D  Y  E  P  T  K  A  D  S  Y  R  K  K  V  V  L  D  G   59
 241  AGAAGAGGTTCAGATAGACATCCTGGACACAGCCGGCCAGGAGGACTATGCGGCCATCCG
        E  E  V  Q  I  D  I  L  D  T  A  G  Q  E  D  Y  A  A  I  R   79
 301  TGACAACTACTTCCGAAGCGGAGAAGGCTTTCTGCTGGTGTTCTCCATCACGGAGCACGA
        D  N  Y  F  R  S  G  E  G  F  L  L  V  F  S  I  T  E  H  E   99
 361  GTCTTTCACCGCCACAGCCGAGTTCAGGGAACAGATCCTCCGAGTCAAGGCCGAGGAGGA
        S  F  T  A  T  A  E  F  R  E  Q  I  L  R  V  K  A  E  E  D  119
 421  CAAAATCCCTCTGCTGGTCGTGGGCAACAAGTCTGACCTGGAGGAGCGGAGGCAGGTGCC
        K  I  P  L  L  V  V  G  N  K  S  D  L  E  E  R  R  Q  V  P  139
 481  CGTGGAGGAGGCCCGAGGCAAAGCGGAAGAGTGGGGTGTGCAGTATGTGGAGACATCCGC
        V  E  E  A  R  G  K  A  E  E  W  G  V  Q  Y  V  E  T  S  A  159
 541  AAAGACCCGAGCCAACGTGGACAAGGTGTTCTTTGATCTGATGAGAGAAATCCGGGCAAA
        K  T  R  A  N  V  D  K  V  F  F  D  L  M  R  E  I  R  A  K  179
 601  GAAGATGTCGGAAAACAAGGACAAGAACGGCAGGAAAAGCGGCAAGAGCAAGAAGAGTTT
        K  M  S  E  N  K  D  K  N  G  R  K  S  G  K  S  K  K  S  F  199
 661  CAAAGAGCGGTGCTGCTTGCTGtgagcgggccgcggacagagcgagcggagaaggccaca
        K  E  R  C  C  L  L
 721  ttgcagcgcgccgagcccaggtggcctcccctggctccccgtctccagcagctgtaatt
 781  cactatcacttcttgaagatggacagatacctgtgcaaaccagtggctggcaggagaaag
 841  acgcacttcgtctgtgcgtctgtgcgatgcatctgctaacgttgccaggagctttgagag
 901  tctgccccctcccaccctccctgaaccgtgactgtcaccagactctagggtcaggaatct
 961  gagttgtgttgacaagaaagaggattgccacggcggcttgtcccccctgttctaggaca
1021  tggtcatcttttgttggaaaatcaaaatgcttcacattagcagggcgtgctggtgcaccc
1061  ctgtagtcccagaactctagaaggtaaggcagggagggtagcctgagcccaaggccccaa
1121  ggcctgggctacctagcaagaccctgtctctaaaaggaaaaagaactaacatcttatttt
1181  cttagatttaaaaagagggtctctggcaagacccctcgttagttcttatcagtttagaag
1241  tagacaggggggcggaaaacctaagctcaggtaatactgaaaaagccaaaacaatttgttt
1301  ttttttttttttctgttttaaaagataaacttctgtgacttggaagcagaattagaaag
1361  atgaacttcacttggtccagataggctgaaccaagcctcctggcgtttgtagagaagtgg
1421  gacgctgttcccttcatacttaaatgccagccaggtgctggctgtgggcttccctccta
1481  acacccttacttagtagtgaggaaactttgtgtgggggagatgtagcctgtgccccagaa
1541  caagctttctttttttctgactggatcatctcacctttttctggttcctcccactccagcc
1601  ataggcctgttctttttcttcctttctgaaaaatgtgaccagttcaaattatgtcccagg
1661  acacgggccagcgccagctacctctgcagactctacaagcggaagaggggaaggctcaga
1721  agggagggccatctcctgtagctcccggcagttggttctggtgacactgacatgaaccag
1781  gttccagaggctcacacccactctctgttaagcctcctctcggggaaggggactctggga
1841  ctgggggctgccactgagcacacactcctcccatttttccagaagtgaacatttcacttt
1901  ttggtgccgaatgtgaatctgggtgggaagagcgggagcccttctaatggatttttttt
1961  acagagttatggaatccaaggtctgatccctggaaacttgtgtcacttccaaagaatcat
2041  gggctttttcgaattaaaaaaaattacactttc(a)n
```

Figure 2. Nucleotide and amino acid sequence of rat ral B. Panel A. Sequencing strategy and partial restriction map of the ral 6B cDNA insert. The location of the coding region within the clone is shown as the dark bar. The arrows above figure show the direction and length of sequencing reactions. The arrowheads below figure show the location of oligonucleotide primers used in PCR reactions. **Panel B.** Nucleotide sequence and deduced amino acid sequence of the ral 6B cDNA clone. The deduced amino acid sequence is given below the nucleotide sequence in bold capital letters. The deduced amino acid sequence of the ral B PCR product used to probe the PC12 library and Northern blots is underlined. The polyadenylation signal is underlined. Numbering of the nucleotide sequence and the deduced amino acid sequence is given on the left and right, respectively.

```
HA   MAANKPKGQNSLALHKVIMVGSGGVGKSALTLQFMYDEFVEDYEPTKADSYRKKVVLDGE   60
RA   MAANKPKGQNSLALHKVIMVGSGGVGKSALTLQFMYDEFVEDYEPTKADSYRKKVVLDGE   60
RB   MAANKGKSQGSLVLHKVIMVGSGGVGKSALTLQFMYDEFVEDYEPTKADSYRKKVVLDGE   60
HB   MAANKSKGQSSLALHKVIMVGSGGVGKSALTLQFMYDEFVEDYEPTKADSYRKKVVLDGE   60

HA   EVQIDILDTAGQEDYAAIRDNYFRSGEGFLCVFSITEMESFAATADFREQILRVKEDEN-   119
RA   EVQIDILDTAGQEDYAAIRDNYFRSGEGFLCVFSITEMESFAATADFREQILRVKEDEN-   119
RB   EVQIDILDTAGQEDYAAIRDNYFRSGEGFLLVFSITEHESFTATAEFREQILRVKAEEDK   120
HB   EVQIDILDTAGQEDYAAIRDNYFRSGEGFLLVFSITEHESFTATAEFREQILRVKAEEDK   120

HA   VPFLLVGNKSDLEDKRQVSVEEAKNRAEQWNVNYVETSAKTRANVDKVFFDLMREIRARK   179
RA   VPFLLVGNKSDLEDKRQVSVEEAKNRADQWNVNYVETSAKTRANVDKVFFDLMREIRARK   179
RB   IPLLVVGNKSDLEERRQVPVEEARGKAEEWGVQYVETSAKTRANVDKVFFDLMREIRAKK   180
HB   IPLLVVGNKSDLEERRQVPVEEARSKAEEWGVQYVETSAKTRANVDKVFFDLMREIRTKK   180

HA   MEDSKEKNGKKKRKSLAKRIRERCCIL   206
RA   MEDSKEKNGKKKRKSLAKRIRERCCIL   206
RB   MSENKDKNGRKSGKS-KKSFKERCCLL   206
HB   MSENKDKNGKKSSKN-KKSFKERCCLL   206
```

Figure 3. Comparison of rat and human ral sequences. The deduced amino acid sequences of human ral A (HA) and ral B (HB) and rat ral A (RA) and ral B (RB) are aligned to show maximum identity. Locations where individual amino acid residues are different among any of the four sequences are shown in bold print. Numbering of the deduced amino acid sequence is given on the right. Spaces (-) are inserted in the sequences to allow maximum sequence identity.

using testes and brain cDNA as templates, a product of the appropriate size was produced which was cleavable by digestion with Eco RI and Pst I. Similarly, primers based upon the coding region of the ral 6B clone (see arrowheads, Figure 2A) generated an appropriately-sized product in PCR reactions which was cleavable by digestion with Mbo I (data not shown).

Ral mRNA Levels in Rat Tissues. The PCR products used to isolate the ral cDNA clones were also used as probes to determine the expression of ral A and ral B in a variety of rat tissues. Two ral A mRNA transcripts of 1.1 kb and 2.9 kb were observed in most tissues (Figure 4A). The larger mRNA species was 2-5 fold more abundant than the smaller mRNA species in most tissues. Notable exceptions were the brain, in which the 2.9 kb message was very predominant (7-10 fold), and the testes, in which the 1.1 kb message predominated (3-5 fold). These findings agree with those previously reported for mouse ral A expression (12). Interestingly, when longer agarose gels were used to fractionate testes mRNA it was discovered that the predominant 1.1 kb message contained two mRNA species, with the larger of the two messages being testes-specific. When the total amount of ral A mRNA was normalized against a housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), ral A mRNA levels were found to be highest in the testes, with brain, adrenal gland, pituitary gland, ovary, liver and kidney expressing 5-10 fold lower levels. Ral mRNA levels were lowest in muscle tissue, being only 1% (psoas) and 5% (atrium, ventricle) of testes levels.

Steady-state ral B mRNA levels are likely lower than ral A mRNA levels in rat tissues, as indicated by the need to use poly A^+ RNA to obtain ral B, but not ral A, mRNA transcript

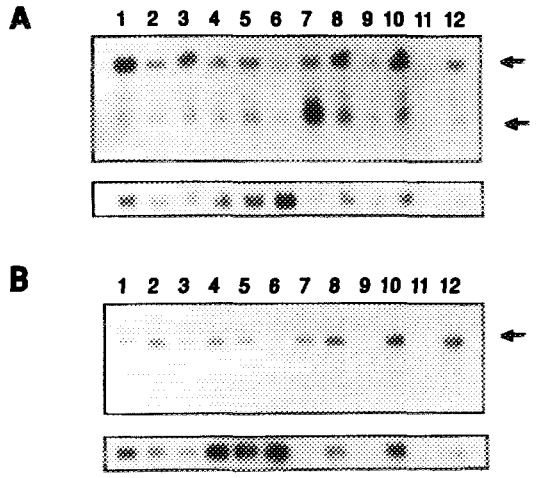


Figure 4. Northern blot analysis of ral mRNA levels in rat tissues. Panel A. Top, 10 μg of total RNA was prepared from the indicated rat tissues (lane 1, brain, 2, adrenal gland, 3, pituitary, 4, atria, 5, ventricle, 6, psoas muscle, 7, testes, 8, ovary, 9, liver, 10, kidney, 11, pancreas and 12, spleen) and analyzed by Northern blot analysis using the ral A PCR probe, as described in METHODS. Bottom, blot used for ral A Northern analysis was 'stripped' for 15 min in boiling 0.1% SSC/1% SDS and rehybridized with GAPDH. **Panel B.** Top, same order of tissues as in panel A except that approximately 5-10 μg of poly A^+ mRNA was analyzed using the ral B PCR probe. Bottom, blot used for ral B Northern analysis was 'stripped' as described above and rehybridized with GAPDH. X-ray films were developed after one week for the ral A and ral B Northerns and after 1-4 hours for the GAPDH Northerns. Similar results were obtained when Northern analyses were performed on a second set of tissue RNA extracts.

signals by Northern blotting. All tissues examined expressed a single, 2.3 kb transcript of ral B (Figure 4B). Quantitatively, the testes again contained the highest levels of ral B mRNA when normalized against GAPDH mRNA levels, however adrenal gland, pituitary gland, ovary and kidney ral B levels were about 50% that of the testes. The brain, atrium, ventricle and liver ral B mRNA levels were 5-10-fold lower than testes, with psoas ral B mRNA levels being barely detectable.

The size of the ral B mRNA transcript detected on Northern blots suggested that the insert of our ral clone 6B was a full- or near full-length clone. As for ral A, it has been reported previously that the difference between the two ral A transcripts of 1.2 and 2.8 kb observed in mouse tissues probably lies in the 3'-untranslated sequences (12). Thus, it is likely that the insert of our ral 6A clone represents either a near full-length sequence or a partial sequence corresponding to the 1.1 or 2.9 kb ral mRNA transcripts, respectively. The relationship of the two ral A mRNA transcripts which compose the 1.1 kb band observed on Northerns in testes is presently unknown. It is likely that the different ral A transcripts arise by alternative splicing or through the use of multiple polyadenylation signals, as both of these mechanisms are utilized to generate multiple ras mRNA transcripts (18). We are currently examining whether similar mechanisms generate the multiple ral A transcripts observed by Northern blotting.

Although ral proteins have been localized immunohistochemically to presynaptic nerve terminals (11) and renal cortical and medullary collecting ducts (19), the function(s) of ral

proteins within the cell is unknown at the present time. Knowledge gained from the present study on the tissue expression of ral proteins and the deduced amino acid sequences will allow the development of antisera and molecular biological probes to help address this question.

Acknowledgments: We thank Drs. Robert M. Graham, Diane Perez and Charles F. McTiernan for their advice and encouragement during the course of these studies. This work was supported by an NIH SCOR grant in Hypertension Grant (# HL33713).

REFERENCES

1. Barbacid, M. (1987) Ann. Rev. Biochem. 56, 779-827.
2. Grand, R.J.A., and Owen, D. (1991) Biochem. J. 279, 609-631.
3. Hall, A. (1990) Science 249, 635-640.
4. Bourne, H.R., Sanders, D.A., and McCormick, F. (1990) Nature 348, 125-132.
5. Kitayama, H., Sugimoto, Y., Matsuzaki, T., Ikawa, Y., and Noda, M. (1989) Cell 56, 77-84.
6. Kahn, R.A., Der, C.J., and Bokoch, G.M. (1992) FASEB J., 2512-2513.
7. Valencia, A., Chardin, P., Wittinghofer, A., and Sander, C. (1991) Biochemistry 30, 4637-4648.
8. Chardin, P., and Tavitian, A. (1986) EMBO J. 5, 2203-2208.
9. Polakis, P.G., Weber, R.F., Nevins, B., Didsbury, J.R., Evans, T., and Snyderman, R. (1989) J. Biol. Chem. 264, 16383-16389.
10. Chardin, P., and Tavitian, A. (1989) Nuc. Acid. Res. 17, 4380.
11. Ngsee, J.K., Elferink, L.A., and Scheller, R.H. (1991) J. Biol. Chem. 266, 2675-2680.
12. Olofsson, B., Chardin, P., Touchot, N., Zahraoui, A., and Tavitian, A. (1988) Oncogene 3, 231-234.
13. Church, G., and Gilbert, W. (1984) Proc. Natl. Acad. Sci. U.S.A. 81, 1991-1995.
14. Chomczynski, P, and Sacchi, N. (1987) Anal. Biochem. 162, 156-159.
15. Fort, P., Marty, L., Piechaczyk, M., El Sabrouty, S., Dani, C., Jeanteur, P., and Blanchard, J.M. (1985) Nuc. Acid Res. 13, 1431-1442.
16. Wahle, E., and Keller, W. (1992) Ann. Rev. Biochem. 61, 419-440.
17. Kozak, M. (1987) Nuc. Acid Res. 15, 8125-8132.
18. Leon, J., Guerrero, I., and Pellicer, A. (1987). Mol. Cell. Biol. 7, 1535-1540.
19. Gupta, A., Bastani, B., Chardin, P., and Hruska, K.A. (1991) Am. J. Physiol. 261, F1063-F1070.