Human testis cDNA for the regulatory subunit RII$_\alpha$ of cAMP-dependent protein kinase encodes an alternate amino-terminal region

Ole Øyen*[+], Frode Myklebust*, John D. Scott, Vidar Hansson[+] and Tore Jahnsen*[+]

*Institute of Pathology, Rikshospitalet, Oslo 1, [+]Institute of Medical Biochemistry, University of Oslo, Blindern, Oslo 3,
Norway and Department of Physiology and Biophysics and Department of Biological Chemistry,
Unversity of California Irvine, Irvine, CA 92717, USA

Phosphorylations catalyzed by cAMP-dependent protein kinase are essential for sperm motility, and type II cAMP-dependent protein kinase in mature sperm has been shown to be firmly bound to the flagellum via the regulatory subunit, RII. The present study documents high-levelled expression of a human, testis-specific RII$_x$ mRNA (2.0 kb) analogous to the rat mRNA which is induced in haploid germ cells [(1988) FEBS Lett. 229, 391–394]. We report the molecular cloning of a full-length human cDNA corresponding to this unique testis mRNA, and the presence of an alternate amino-terminal region (amino acids 45–75) of the predicted RII$_x$ protein (404 amino acids) compared with the previously published mouse and rat sequences. However, this alternate region is also shown to be present in RII$_x$ mRNA (7.0 kb) of human somatic cells. Our data indicate the divergent amino-terminal sequence to be due to species differences, suggesting an active evolutionary pressure on this particular region, which could be involved in subcellular attachment of RII$_\alpha$ and thereby localization of kinase activity to certain targets within the cell.

Cyclic AMP-dependent protein kinase; Spermatogenesis; Sperm motility

1. INTRODUCTION

Previous studies have demonstrated that the essential role played by cyclic AMP (cAMP) in stimulating sperm motility is mediated by phosphorylations catalyzed by cAMP-dependent protein kinase [1]. A 56 kDa protein called axokinin has been identified [2], and evidence presented that axokinin phosphorylation by cAMP-dependent protein kinase is required and sufficient for flagellar motility [3].

Four different regulatory subunits (RI$_\alpha$ [4], RI$_\beta$ [5], RII$_\alpha$ [6] and RII$_\beta$ [7,8]) and three different catalytic subunits (C$_\alpha$ [9], C$_\beta$ [10,11] and C$_\gamma$ [Beebe, S., Øyen, O. and Jahnsen, T., in preparation]) for cAMP-dependent protein kinases have now been identified at the gene/mRNA level. RI

subunits give rise to type I cAMP-dependent protein kinase, whereas RII subunits give rise to type II. We have previously isolated human testis cDNA clones for RI$_\alpha$ [12], RII$_\beta$ [8] and the three known catalytic subunits (C$_\alpha$, C$_\beta$, C$_\gamma$; in preparation).

Type II cAMP-dependent protein kinase in mature sperm has been shown to be firmly bound to the flagellum via the regulatory subunit, RII [13,14]. Presumably, this would serve to anchor the kinase activity to the flagella, the locomotive element of the spermatozoa. In brain a type II cAMP-dependent protein kinase has been shown to be bound to microtubules via a microtubule-associated protein (MAP-2) [15,16]. Furthermore, immunohistochemical studies have indicated that MAP-2 only colocalizes with a subpopulation of RII [17].

In agreement with the important role proposed for type II cAMP-dependent protein kinase in mature spermatozoa, we have recently reported the

Correspondence address: O. Øyen, Institute of Pathology, Rikshospitalet, 0027 Oslo 1, Norway

high-levelled expression of a unique mRNA species (2.2 kb; rat) for the regulatory subunit RII_α of cAMP-dependent protein kinase at late stages of spermatogenesis, during spermatid elongation [18]. We wanted to investigate if this germ cell-specific mRNA contained the same coding region as the RII_α mRNA (6.0 kb; rat) found in most somatic cells [6]. Furthermore, we would like to explore the intriguing possibility that a specific, altered isoform of the RII_α molecule could be responsible for the documented flagellar attachment of RII [14].

In the present study we report the molecular cloning of a human RII_α cDNA, analogous to the abundant germ cell-specific rat mRNA, and we document the presence of a unique amino-terminal region compared with the published rat/mouse RII_α sequences [6]. However, this alternate region appears to be species-specific rather than cell-specific.

2. MATERIALS AND METHODS

2.1. Screening the cDNA library

A human testis λ gt11 cDNA library was purchased from Clontech (Palo Alto, CA, USA; Cat.no. HL 1010). Approximately one million clones were screened on nitrocellulose filters, as previously described [7], using a nick-translated rat RII_α cDNA probe (0.7 kb SalI-BglII fragment; all within the open reading frame [6]). Twenty-three positive clones were obtained, of which 3 were selected (based on hybridization intensity and length), purified and amplified for further characterization.

2.2. DNA sequencing

The cDNA inserts were excised from λ gt11 by EcoRI digestion. DNA fragments were prepared for sequencing in M13mp18/19 by conventional restriction site subcloning, and sequencing was performed with the dideoxy chain termination method using the modified T7 bacteriophage DNA polymerase [18].

2.3. Computer analysis

Nucleotide and protein sequence data was analysed using the program package from the University of Wisconsin Genetics Computer Group [20]. Sequence homology searches were based on algorithms introducing gaps to obtain the best alignment [21,22].

2.4. Preparation of rat testis and various human tissues

Sprague-Dawley rats of various ages (15, 30, 50, 60 and 80 days of age) were decapitated, and testes were immediately removed.

Human tissue samples from testis, skeletal muscle, myocardium, spleen, lung, kidney and adrenal gland were obtained at

surgery soon after removal from the patient. All tissue samples were frozen at once in liquid N_2, and stored at $-75°C$ for later RNA extraction.

2.5. Preparation of total RNA

RNA extraction from rat testis and various human tissues was performed as previously described [23] by homogenization in guanidinium isothiocyanate. Total RNA was isolated by centrifugation through a cesium chloride gradient and purified by phenol/chloroform extractions.

2.6. Northern analysis

The samples were electrophoresed on a 1.5% agarose gel containing formaldehyde as denaturing agent and with recirculating 20 mM sodium phosphate running buffer [23]. 20 μg total RNA was used in each lane, separated on the gel and transferred to a nylon filter (ICN, Biotrans) by capillary blotting technique. When using the nick-translated [23] cDNA for human testis RII_α (1.4 kb EcoRI fragment of the 1.6 kb clone) as probe, the following prehybridization/hybridization conditions were employed; 50% formamide, 5 × SSC [24], 5 × Denhardt's solution [24], 50 mM sodium phosphate (pH 6.5), 0.1% SDS [24], 250 μg/ml denatured salmon sperm DNA, at 42°C for 3 h (prehybridization)/overnight (hybridization). The filter was washed twice with 0.5 × SSC at 50°C for 20 min before autoradiography. mRNA sizes were estimated by comparison to RNA standards (Bethesda Research Laboratories, USA).

2.7. Hybridization with oligonucleotide probes

Oligonucleotides (oligo 1 (28-mer), oligo 2 (40-mer) and oligo 3 (40-mer)) were designed as shown in fig.3A and purchased from Genetic Designs Inc. (Houston, TX, USA). End-labelling was performed as described [25], using $[\gamma\text{-}^{32}P]ATP$ (Amersham, England; PB10218) and T_4 polynucleotide kinase. Northern nylon filters were probed using the following prehybridization/hybridization conditions: 40% formamide, (5 × SSC, 5 × Denhardt's solution, 50 mM sodium phosphate (pH 6.5), 0.1% SDS, 250 μg/ml denatured salmon sperm DNA, 50 μg/ml tRNA, at 42°C. Washing was performed using 0.5 × -1 × SSC at 50°C.

3. RESULTS

3.1. Isolation of cDNA clones and composite sequence

By screening a human testis cDNA library with a rat RII_α cDNA probe [6], 23 positive clones were obtained, of which 3 (selected on basis of hybridization signal intensity and length) were characterized further. Sequencing revealed two of these cDNAs to be overlapping clones (fig.1) corresponding to the known RII_α nucleotide and amino acid sequences [6,26,27], whereas one clone turned out to contain intron sequences (among them Alu sequences) as well, representing precursor RNA, presumably of nuclear origin. By oligo-
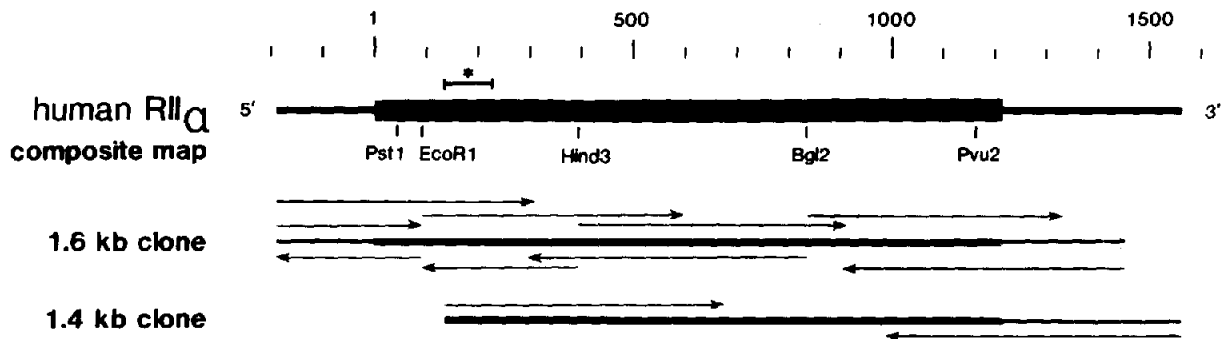
Fig.1. Composite restriction map for human testis RII$_\alpha$ cDNA. Two overlapping cDNA clones, 1.6 and 1.4 kb in size, were sequenced in M13 according to the strategy outlined (arrows). The open reading frame is indicated by solid bars, and nucleotides are numbered from the ATG. The region of the cDNA marked with a star (nts 132–224) represents the unique area lacking homology to the rat/mouse RII$_\alpha$ cDNA sequence [6]. The linker EcoRI sites at the ends of the clones have not been indicated.

nucleotide hybridization (oligo from the intron sequence; not shown) this intron-containing cDNA was shown to correspond to faint RNA bands (approx. 2.9 and 3.9 kb in size, fig.4) in between the two major human testis RII$_\alpha$ bands.

The composite human testis RII$_\alpha$ cDNA (figs 1,2) contained 189 nucleotides (nts) of 5'-noncoding sequence (75% G/C), an open reading frame (ORF) of 1212 nts (encoding 404 amino acids) and a 3'-non-translated region of 352 nts. No usual polyadenylation site signal (AATAAA or ATTAAA) preceded the 10 terminal As. However, the resembling hexanucleotide TATAAA was found 18 nts upstream of the 10 As (fig.2). Recently a germ cell-specific c-abl mRNA was shown not to contain the usual polyadenylation site signal [28], suggesting the use of alternative polyadenylation sites (favoring shorter mRNAs) in germ cells. With this in mind, the 3'-end of our sequence (10 terminal As preceded by TATAAA, fig.2) most probably corresponds to the polyadenylation site of the testis-specific 2.0 kb human mRNA for RII$_\alpha$ (fig.4).

3.2. Nucleotide and amino acid sequence comparisons

Compared with RII$_\alpha$ nucleotide sequences from mouse brain (full-length, starting at the ATG) [6] and rat skeletal muscle (partial, starting at nt 91 in the mouse sequence) [6] our human testis cDNA showed a nucleotide homology of 88% inside the ORF and 87% in the 3'-non-translated region (mouse brain). The predicted human RII$_\alpha$ amino acid sequence was 88% homologous with the deduced mouse brain sequence. The hinge region with the autophosphorylation site (Ser-92) and the stringent parts of the two cAMP-binding sites were highly conserved.

However, a specific area of the human testis RII$_\alpha$ sequence (nts 133–225, amino acids 45–75; figs 1–3) at the 5' (amino-terminal)-side of the hinge region showed a striking lack of homology compared with the mouse/rat sequence (the mouse and rat sequences are very similar also in this area). In the regions 5' (nts 1–132) and 3' (nts 226–1212) to this unique area the nucleotide/amino acid homologies were 94%/95% and 90%/93%, respectively (vs mouse sequence). When the unique human region and the corresponding region of the rat/mouse sequence were considered to be isolated, no significant nucleotide and amino acid homology was found (fig.3). The human sequence gave rise to 3 additional amino acids in this area. Furthermore, most of the amino acid changes in the divergent area were of the non-conservative type, whereas changes outside this region tended to be conservative amino acid substitutions (fig.3B).

The bovine heart RII$_\alpha$ primary amino acid sequence [26] showed an overall homology of 90% to our deduced human sequence. Again, there was a striking dissimilarity in the particular area discussed above with many non-conservative changes and 3 amino acids lacking. However, there were short stretches of amino acids within this divergent region that seemed to be conserved compared with the human sequence. Within this particular region

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-189  CCAGGTCGGCCGTGGTAGCGTAGGGTTGCGCGGCCCGGAAACGCAGAGCCGGCCAAAGAGCGGCGCGACGTGAGCCGGGGCCGTGCGCGA

 -99  AGAGACCTCGCGGGCGCGGAGCGAAAGGCCGGCGTGAGTGAGCGCGGAGACAGTGGCCGCCGGCGGCCCAACCCGTCTATCCCTTCGGCC

  -9  GCCGCCGGCATGAGCCACATCCAGATCCCGCCGGGGCTCACGGAGCTGCTGCAGGGCTACACGGTGGAGGTGCTGCGGACAGCAGCCGCCT
        MetSerHisIleGlnIleProProGlyLeuThrGluLeuLeuGlnGlyTyrThrValGluValLeuArgGlnGlnProPro  27

  82  GACCTCGTCGAATTCGCAGTGGAGTACTTCACCCGCCTGCGCGAGGCCCGCGCCCCAGCCTCAGTCCTGCCCGCCGGCCACCCCACGCCAG
        AspLeuValGluPheAlaValGluTyrPheThrArgLeuArgGluAlaArgAlaProAlaSerValLeuProAlaAlaThrProArgGln  57

 172  AGCCTGGGCCACCCCCCGCCAGAACCCGGCCCCGGACCGTGTCGCCGACGCCAAAGGGGACAGCGAGTCGGAGGAGGACGAGGACTTGGAA
        SerLeuGlyHisProProProGluProGlyProAspArgValAlaAspAlaLysGlyAspSerGluSerGluGluAspGluAspLeuGlu  87

 262  GTTCCAGTTCCTAGCAGATTTAATAGACGAGTATCAGTCTGTGCTGAGACCTATAACCCTGATGAGGAAGAGGAAGATACAGATCCAAGG
        ValProValProSerArgPheAsnArgArgValSerValCysAlaGluThrTyrAsnProAspGluGluGluGluAspThrAspProArg  117
                                      ↑    Hinge

 352  GTGATTCATCCTAAAACTGATGAACAGAGATGCAGACTTCAGGAAGCTTGCAAAGATATTCTCCTTTTCAAAAATCTTGATCAGGAACAG
        ValIleHisProLysThrAspGluGlnArgCysArgLeuGlnGluAlaCysLysAspIleLeuLeuPheLysAsnLeuAspGlnGluGln  147

 442  CTTTCTCAAGTTCTCGATGCCATGTTTGAAAGGATAGTCAAAGCTGATGAGCATGTCATTGACCAAGGAGATGATGGAGACAACTTTTAT
        LeuSerGlnValLeuAspAlaMetPheGluArgIleValLysAlaAspGluHisValIleAspGlnGlyAspAspGlyAspAsnPheTyr  177

 532  GTCATAGAACGGGGAACTTATGACATTTTAGTAACAAAAGATAATCAAACCCGCTCTGTTGGTCAATATGACAACCGTGGCAGTTTTGGA
        ValIleGluArgGlyThrTyrAspIleLeuValThrLysAspAsnGlnThrArgSerValGlyGlnTyrAspAsnArgGlySerPheGly  207

 622  GAACTAGCTCTGATGTACAACACCCcGAGAGCTGCTACCATTGTTGCTACCTCAGAAGGCTCCCTTTGGGGACTGGACCGGGTGACTTTT
        GluLeuAlaLeuMetTyrAsnThrProArgAlaAlaThrIleValAlaThrSerGluGlySerLeuTrpGlyLeuAspArgValThrPhe  237
              cAMP  A

 712  AGAAGAATCATAGTGAAAAATAATGCAAAGAAGAGGAAGATGTTTGAATCATTTATTGAGTCTGTGCCCCTCCTTAAATCACTAGAGGTG
        ArgArgIleIleValLysAsnAsnAlaLysLysArgLysMetPheGluSerPheIleGluSerValProLeuLeuLysSerLeuGluVal  267

 802  TCAGAACGAATGAAGATTGTGGATGTAATAGGAGAGAAGATCTATAAGGATGGAGAACGCATAATCACTCAGGGTGAAAAGGCTGATAGC
        SerGluArgMetLysIleValAspValIleGlyGluLysIleTyrLysAspGlyGluArgIleIleThrGlnGlyGluLysAlaAspSer  297

 892  TTTTACATCATAGAGTCTGGCGAAGTGAGCATCTTGATTAGAAGCAGGACTAAATCAAACAAGGATGGTGGGAACCAGGAGGTCGAGATT
        PheTyrIleIleGluSerGlyGluValSerIleLeuIleArgSerArgThrLysSerAsnLysAspGlyGlyAsnGlnGluValGluIle  327

 982  GCCCGCTGCCATAAGGGGCAGTACTTTGGAGAGCTTGCCCTGGTCACCAACAAACCCAGAGCTGCCTCAGCTTATGCAGTTGGAGATGTC
        AlaArgCysHisLysGlyGlnTyrPheGlyGluLeuAlaLeuValThrAsnLysProArgAlaAlaSerAlaTyrAlaValGlyAspVal  357
                                      cAMP  B

1072  AAATGCTTAGTTATGGATGTACAAGCATTCGAGAGGCTTCTGGGGCCCTGCATGGACATCATGAAGAGGAACATCTCACACTATGAGGAA
        LysCysLeuValMetAspValGlnAlaPheGluArgLeuLeuGlyProCysMetAspIleMetLysArgAsnIleSerHisTyrGluGlu  387

1162  CAGCTGGTGAAGATGTTTGGCTCCAGCGTGGATCTGGGCAACCTCGGGCAGTAGGTGTGCCACACCCCAGAGCCTTCTTAGTGTGACACC
        GlnLeuValLysMetPheGlySerSerValAspLeuGlyAsnLeuGlyGln    404

1252  AAAACCTTCTGGTCAGCCACAGAACACATACAGAAAACAGACATGACAGAACTGTTCCTGCCGTTGCCGCCACTGCTGCCATTGCTGTGG

1342  TTATGGGCATTTAGAAAACTTGAAAGTCAGCACTAAAGGATGGGCAGAGGTTCAACCCACACCTCCACTTTGCTTCTGAAGGCCCATTCA

1432  TTAGACCACTTGTAAAGATTACTCCAACCCAGTTTTTATATCTTTGGTTCAAAACGGCATGTCTCTCCAACAATTTAAGTGCCTGATACA

1522  AAGTCCAAAGTATAAACATGCTCCTTTCCTCTCAAAAAAAAAA    1564
        Poly(A)
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Fig.2. Nucleotide and deduced amino acid sequence for human testis RII$_\alpha$ cDNA. The alternate region, dissimilar to the rat/mouse RII$_\alpha$ cDNA sequence, is shown in bold types. In addition, the following are indicated: the amino acids of the hinge region (Hinge) with the autophosphorylation site (↑; Ser-92), the stringently conserved parts of the two cAMP-binding domains (cAMP A and B) and the possible alternative polyadenylation site signal (poly(A)).
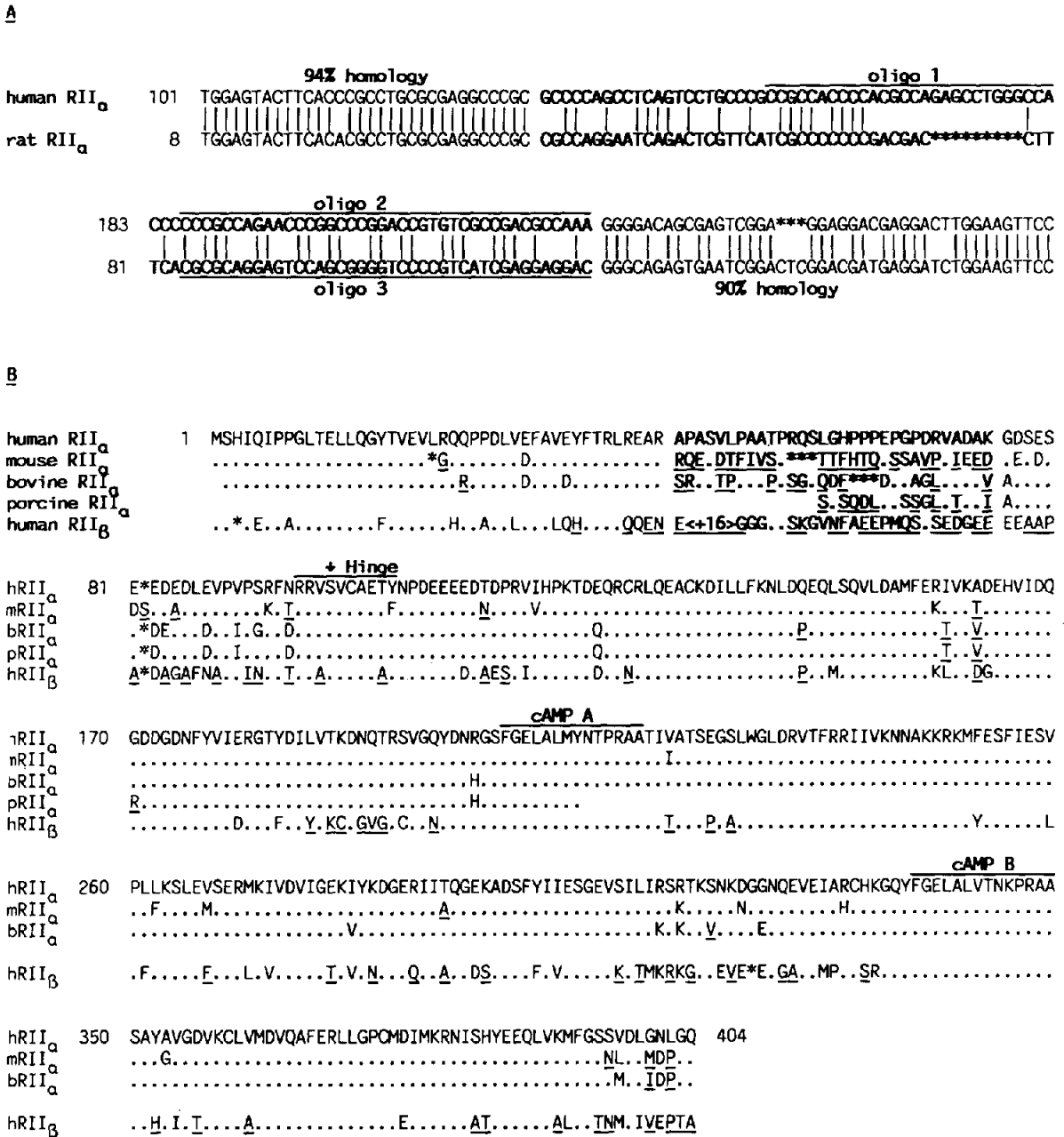
A

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                        94% homology                                             oligo 1
human RIIₐ  101  TGGAGTACTTCACCCGCCTGCGCGAGGCCCGC GCCCCAGCCTCAGTCCTGCCCGCCGCCACCCCACGCCAGAGCCTGGGCCA
                 |||||||||||| ||||| ||||||||||||||| ||  |  || |||  ||   | ||| |  |||| |||||                |
rat RIIₐ     8  TGGAGTACTTCACACGCCTGCGCGAGGCCCGC CGCCAGGAATCAGACTCGTTCATCGCCCCCCCCGACGAC*********CTT


                        oligo 2
          183  CCCCCCGCCAGAACCCGGCCCGGACCGTGTCGCCGACGCCAAA GGGGACAGCGAGTCGGA***GGAGGACGAGGACTTGGAAGTTCC
               |  ||| ||  || ||||| || ||  ||| ||| |||||| |  | |||  || |||||||  |||| || ||||| |||||||||
           81  TCACGCGCAGGAGTCCAGCGGGGTCCCCGTCATCGAGGAGGAC GGGCAGAGTGAATCGGACTCGGACGATGAGGATCTGGAAGTTCC
                        oligo 3                                           90% homology
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B

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human RIIₐ   1  MSHIQIPPGLTELLQGYTVEVLRQQPPDLVEFAVEYFTRLREAR APASVLPAATPRQSLGHPPPEPGPDRVADAK GDSES
mouse RIIₐ      ....................*G......D..........     RQE.DTFIVS.***TTFHTQ.SSAVP.IEED .E.D.
bovine RIIₐ     ....................R....D...D.........      SR..TP...P.SG.QDF***D..AGL....V A....
porcine RIIₐ                                                S.SQDL..SSGL.T..I A....
human RIIᵦ      ..*.E..A........F......H..A..L...LQH....QQEN E<+16>GGG..SKGVNFAEEPMQS.SEDGEE EEAAP


                     ↓ Hinge
hRIIₐ   81  E*EDEDLEVPVPSRFNRRVSVCAETYNPDEEEEDTDPRVIHPKTDEQRCRLQEACKDILLFKNLDQEQLSQVLDAMFERIVKADEHVIDQ
mRIIₐ       DS..A..........K.T.........F........N....V....................................K...T......
bRIIₐ       .*DE...D..I.G..D.............................Q....................P...............T..V.....
pRIIₐ       .*D....D..I....D.............................Q...................................T..V.....
hRIIᵦ       A*DAGAFNA..IN..T..A.....A........D.AES.I......D..N...................P..M.........KL..DG.....


                                              cAMP A
hRIIₐ  170  GDDGDNFYVIERGTYDILVTKDNQTRSVGQYDNRGSFGELALMYNTPRAATIVATSEGSLWGLDRVTFRRIIVKNNAKKRKMFESFIESV
mRIIₐ       ..............................................................I...........................
bRIIₐ       ......................................H...................................................
pRIIₐ       R.....................................H...........
hRIIᵦ       ..........D...F..Y.KC.GVG.C..N...............T...P.A......................Y......L


                                                                                     cAMP B
hRIIₐ  260  PLLKSLEVSERMKIVDVIGEKIYKDGERIITQGEKADSFYIIESGEVSILIRSRTKSNKDGGNQEVEIARCHKGQYFGELALVTNKPRAA
mRIIₐ       ..F....M...................................A..................K.....N........H..............
bRIIₐ       .........................V....................................K.K..V....E....................

hRIIᵦ       .F.....F...L.V.....T.V.N...Q..A..DS....F.V.....K.TMKRKG..EVE*E.GA..MP..SR...............


hRIIₐ  350  SAYAVGDVKCLVMDVQAFERLLGPCMDIMKRNISHYEEQLVKMFGSSVDLGNLGQ  404
mRIIₐ       ...G.............................................NL..MDP..
bRIIₐ       ................................................M..IDP..

hRIIᵦ       ..H.I.T....A..............E......AT......AL..TNM.IVEPTA
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Fig.3. Comparison of RIIₐ nucleotide and amino acid sequences. Gaps have been introduced (*) to obtain the best alignment. (A) Comparison of human testis RIIₐ and rat RIIₐ nucleotide sequences, focusing on the dissimilar region (bold types). The homologies outside this unique region, but inside the ORF, have been indicated. Oligonucleotides complementary to the sequences indicated (oligo 1, 2 (human) and 3 (rat)) were used for Northern analyses. (B) Comparison of RIIₐ and RIIᵦ amino acid sequences, from above; human testis RIIₐ (deduced sequence), mouse RIIₐ (deduced) [6], bovine RIIₐ (primary amino acid sequence) [26], porcine RIIₐ (deduced; partial cDNA clone) [27] and human RIIᵦ (deduced) [8]. '+16' points to an area where RIIᵦ contains 16 additional (non-homologous) amino acids compared with human testis RIIₐ. The functional regions have been indicated as in fig.2. Punctuations denote identity with the human RIIₐ amino acids. Non-conservative amino acid changes [20] have been underlined.

the bovine sequence showed no homology to the deduced mouse/rat sequence.

The deduced, partial porcine RII_α amino acid sequence [27] diverged from the human sequence within the unique area (fig.3B), but showed significant homology with the bovine sequence.

The major dissimilarities between human RII_α and human RII_β were also confined to this particular amino-terminal region with no significant homology and 16 additional amino acids in the RII_β sequence, whereas rat RII_β and human RII_β [8] were highly homologous (nucleotide homology within the ORF 91%, amino acid homology 97%) throughout the molecule, including this amino-terminal region.

3.3. Analysis of human RII_α mRNA size and distribution

Using the human testis RII_α cDNA as a probe (fig.4, upper left panel), the germ cell-specific mRNA (previously denoted 2.2 kb in rat) [18] was detected in post-pubertal rat testis. The less abundant 6.0 kb rat RII_α mRNA (found in most somatic cells) cannot be seen in this cross-species blot, or in the faint 3.1 kb band previously described [18]. In human testis an abundant 2.0 kb mRNA obviously corresponding to the germ cell-specific rat mRNA was detected, as well as a larger 7.0 kb mRNA presumably corresponding to the 'somatic' 6.0 kb rat mRNA. In addition, two faint intermediate bands (approx. 2.9 and 3.9 kb) most probably corresponding to intron-containing precursor mRNAs were seen.

Using an oligonucleotide of 28 bases (oligo 1) from the unique region of human testis RII_α cDNA (fig.3A) for hybridization, all the human RII_α-related RNA bands were detected (fig.4, upper right panel). In addition, faint cross-species bands (*) were detected in post-pubertal rat testis of about 3.1 and 2.0 kb in size. An oligonucleotide of 40 bases (oligo 2) from another part of the unique human region (fig.3A) also hybridized with all the human RII_α RNAs (fig.4, lower left panel), and this oligo clearly detected a rat RNA of about 3.1 kb (*). However, this oligo obscured the 2.0 kb
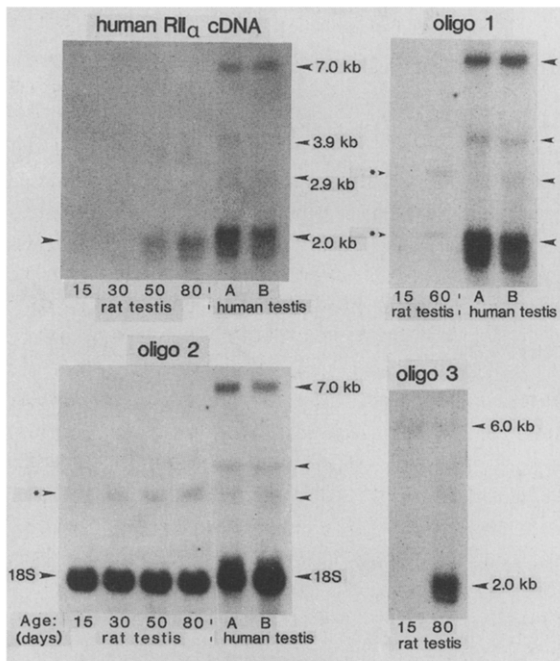

Fig.4. Northern blots showing RII_α-related mRNA bands in rat and human testis. Total RNA was extracted from testis of rats of various ages and from human testis of two different patients, 14 (A) and 65 (B) years of age. The resulting filters were hybridized with the following ^{32}P-labelled probes (see figs 1 and 3A): human testis RII_α cDNA (1.4 kb EcoRI fragment of the 1.6 kb cDNA clone); oligo 1 (28-mer from the unique region of human testis RII_α cDNA); oligo 2 (40-mer from the unique region of human testis RII_α cDNA); and oligo 3 (40-mer from the unique region of rat testis RII_α cDNA). Weak bands representing cross-species detection have been indicated (*). Oligo 2 shows cross-hybridization with human and rat 18 S ribosomal bands. Approximate mRNA sizes are indicated.
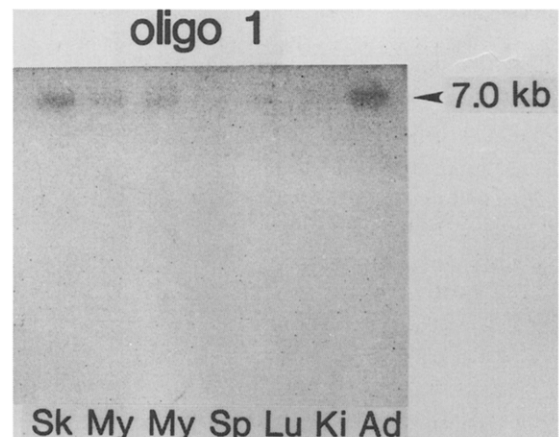

Fig.5. Northern blot showing RII_α mRNA in various human tissues. Total RNA was extracted from different human tissues: skeletal muscle (Sk), myocardium (My), spleen (Sp), lung (Lu), kidney (Ki) and adrenal gland (Ad), and subjected to Northern analysis using oligo 1 (28-mer from the unique region of human testis RII_α cDNA) as ^{32}P-labelled probe.

mRNAs (rat and partly human) by cross-hybridizing with the 18 S ribosomal band. Another 40 base oligonucleotide (oligo 3) from the corresponding unique region of the rat RII_α sequence (see fig.3A) clearly detected the germ cell-specific 2.0 kb rat mRNA, as well as the larger 6.0 kb rat band. The intermediate 3.1 kb band was not seen. This oligo did not detect any RNA signal in human testis (not shown).

Oligo 1 also detected the larger 7.0 kb human RII_α band in various other human tissues (fig.5), whereas no 2.0 kb mRNA was seen other than in testis. Hybridization with human RII_α cDNA gave rise to an identical blot (not shown).

4. DISCUSSION

By this study we document the high-levelled expression of a human testis-specific RII_α mRNA (2.0 kb; fig.4) analogous to the unique rat mRNA shown to be induced in haploid germ cells [18]. This abundant testis mRNA seems to be conserved through mammalian evolution, suggesting important functions conveyed by the encoded RII_α protein. These functions could be anchoring the kinase activity to the sperm flagella [13,14], where phosphorylation of certain substrate proteins is essential for sperm motility [1,2].

Furthermore, we report the molecular cloning of a human RII_α cDNA corresponding to the testis-specific mRNA (fig.4) and the presence of an alternate amino-terminal region (amino acids 45–75) of the predicted RII_α protein compared with the previously published mouse brain/rat skeletal muscle RII_α sequences [6]. The intriguing possibility of this unique region representing a germ cell-specific RII_α form (and only corresponding to the testis-specific 2.0 kb mRNA) was, however, proven false by oligonucleotide hybridization showing that the unique human region was also present in the 7.0 kb mRNA found in most somatic cells. Furthermore, the corresponding unique rat region was also shown to be present both in the germ cell-specific rat mRNA, as well as in the somatic 6.0 kb band. The high degree of conservation both 5′ (dimerization domain) and 3′ to the alternate area indicates the human and mouse/rat cDNAs to be derived from the same gene. If the unique human region did represent an alternate RII_α form, a likely mechanism for the generation of this heterogeneity

would be alternate splicing. However, according to previous data [6], the first coding exon of the mouse RII_α gene encodes amino acids 1–84, which includes amino acids on both sides of the alternate region.

These data suggest that the divergent area is merely due to species differences, particularly confined to the area. The partial amino acid homology found, when comparing the human and bovine sequences of this region, supports the concept of an evolutionary 'drift' confined to this region. This could indicate that the part of the RII_α protein in question has no functional significance, which would allow frequent mutations during evolution in the corresponding genome sequence. The nucleotide homology (human vs mouse/rat) within the divergent area is, however, surprisingly low (45–55%, depending on gaps introduced) compared with 3′-non-translated similarity (87%). Furthermore, the major differences between human RII_α and human RII_β are also confined to this particular area (no homology and 16 additional RII_β amino acids), but human RII_β and rat RII_β sequences are highly homologous (nucleotide and amino acid 91% and 97%, respectively) [8], and this level of homology also pertains to the particular region in question. This could point to an active evolutionary pressure on this unique area in RII_α; modulating it in a species-specific way. The functional basis for such an evolutionary mechanism would be the interaction of RII_α with certain proteins showing species specificity. This hypothesis would implicate the particular region of RII_α in question, between the dimerization domain and the hinge region, to be involved in subcellular attachment. Concerning germ cells, cell-specific coupling proteins [14] could be responsible for anchoring RII_α to the sperm flagella.

However, the possibility that there is a further heterogeneity in $RII_{(\alpha)}$ forms cannot be ruled out. The oligonucleotides (oligos 1 and 2) corresponding to different parts of the unique human RII_α region did detect a rat RNA band which presumably corresponds to the intermediate rat RNA band (3.1 kb) previously described [18] (and faintly a 2.0 kb rat RNA). The 3.1 kb rat RNA may correspond to an intron-containing precursor (analogous to the faint human intermediate bands), and contain a sequence analogous to the alternate region of human RII_α cDNA. This would

suggest the occurrence of a 'species shift' once in evolution, involving the recombination of exon and intron sequences. There is also a possibility of several different RII_α forms 'hiding' within the same mRNA species and emerging from a common gene. Or there could be several RII_α-related genes.

The final solution to these questions has to come from genomic characterization of RII_α and related genes, which is currently one of the major issues of investigation in our laboratory. The exploration of these problems would not only help answer important questions regarding RII_α and its function, but might also uncover general evolutionary mechanisms.

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