A human RNA polymerase II subunit is encoded by a recently generated multigene family

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Abstract

Background: The sequences encoding the yeast RNA polymerase II (RPB) subunits are single copy genes.

Results: While those characterized so far for the human (h) RPB are also unique, we show that hRPB subunit 11 (hRPB11) is encoded by a multigene family, mapping on chromosome 7 at loci p12, q11.23 and q22. We focused on two members of this family, hRPB11a and hRPB11b: the first encodes subunit hRPB11a, which represents the major RPB11 component of the mammalian RPB complex; the second generates polypeptides hRPB11β and hRPB11β through differential splicing of its transcript and shares homologies with components of the hPMS2L multigene family related to genes involved in mismatch-repair functions (MMR). Both hRPB11a and b genes are transcribed in all human tissues tested. Using an inter-species complementation assay, we show that only hRPB11a is functional in yeast. In marked contrast, we found that the unique murine homolog of RPB11 gene maps on chromosome 5 (band G), and encodes a single polypeptide which is identical to subunit hRPB11a.

Conclusions: The type hRPB11β gene appears to result from recent genomic recombination events in the evolution of primates, involving sequence elements related to the MMR apparatus.

Background

In eukaryotes, mRNAs are transcribed by RNA polymerase II (RPB). To date, most studies have focused on the yeast polymerases. Yeast RPB consists of 12 polypeptides ranging from 220 to 6 kDa [1–3]. Much less is known about the human (h) RPB, although the sequences encoding the subunits homologous to the yeast RPB have been determined. Complementation experiments have shown that many yeast subunits may be replaced in vivo by their human counterparts indicating a remarkable
functional conservation through evolution [4–8]. This supports the view that the 3D structure of the yeast RPB [9,10] can most likely be extended to other eukaryotic nuclear RPB molecules.

We have undertaken the characterisation of the human RPB subunits. All the subunit genes identified so far are unique: hRPB1 (Ac N° X74870-74) [11], hRPB2 (Ac N° AC068261), hRPB3 (Ac N° AC004382), hRPB4 (Ac N° U89387) [7], hRPB5 (Ac N° AC004151), hRPB6 (Ac N° AF006501) [12], hRPB7 (Ac N° U52427) [13], hRPB8 (Ac N° AJ252079-80), hRPB9 (Ac N° Z23102) [14], hRPB10 (Ac N° AJ252078), hRPB10α, (Ac N° Z47728-29) [15]. The present report focuses on the hRPB11 gene which remained to be characterised.

It has been shown in many systems that the RPB11 subunit is able to heterodimerize with RPB3, evoking the alpha dimer in bacteria that directs the assembly of the two largest subunits of the RPB complex [16–22,9,10]. We shall refer to the previously characterised hRPB11a protein (Ac N° AJ277932), hRPB11b (Ac N° AJ277930) and cDNA encoding a protein homologous to yeast RPB11, as hRPB11α (Ac N° AJ277938), hRPB11β (Ac N° AJ277939), hRPB11γ (Ac N° AJ277940) (Fig. 1A).

Differential splicing of hRPB11b transcripts
We characterised two types of cDNAs from HeLa cells corresponding to hRPB11b transcripts and differing by the presence or absence of exon 3: they were named hRPB11βa and hRPB11ββ, respectively (Fig. 1B, Table 1). The absence of exon 3 switches the reading frame of exon 4, thereby extending the coding sequence (CDS) of hRPB11ββ into an additional exon 5, identified in another genomic sequence (Ac N° AC004951).

Most of the human cDNAs and ESTs in the databases (Table 1) perfectly matched the cDNAs reconstituted from the exons of both hRPB11a and b genes, indicating that these sequences are transcribed in vivo. Exon 3 being present in all the genomic clones, we conclude that the hRPB11ββ cDNA is produced by differential splicing resulting in exon 3 skipping.

Three types of proteins are encoded by the hRPB11 genes
The hRPB11a gene yields one type of mRNA that encodes the hRPB11α protein which was previously identified as a subunit of the human RPB complexes in Western-blots of immunoprecipitated RPB (Fig. 1C). The N-terminal portion of hRPB11α subunit differs only from the hRPB11β polypeptide by the presence of an additional Lys encoded at the junction between exons 1 and 2. By contrast, the C-terminal portions of these polypeptides differ drastically: while exon 4 of hRPB11α encodes a hydrophilic 10-residue peptide, it generates a rather hydrophobic 10-residue peptide in the case of hRPB11ββ (Fig. 1C); concerning hRPB11bβ, due to exon 3 skipping, an unrelated peptide, rich in Pro (16%), Ala (14.5%), Gln (9%), His (9%) and Cys (7%) residues, is produced.

The screening of our genomic DNA library yielded several clones. Analysis of lambda clone 27 (Fig. 1A), revealed four coding exons within a 5.5 kb DNA sequence that we named hRPB11a gene, according to their identity with the hRPB11α cDNA. Lambda clone 11 was distinct from hRPB11a. Three exons were identified by their strong homology with exons 1, 2 and 3 from hRPB11a (Fig. 1A, Table 1). The fourth exon was identified by comparing this genomic sequence with two cDNAs from the database (Table 1). This exon 4 sequence was specific to a subset of genomic sequences that we referred to as type b. hRPB11α and b genomic sequences diverged within intron 3 (Fig. 1A).

Table 1: Accession numbers of RPB11 sequences

| GENES   | CDNA             |
|---------|------------------|
| hRPB1   | hRPB1            |
| hRPB1a  | hRPB1a           |
| hRPB1b  | hRPB1bβ          |
| hRPB1bα | hRPB1ββ          |
| hRPB1ββ | hRPB1ββ          |
| mRPB1   | mRPB1            |
| AC087420 | AC087420        |
| (exons 1) | (exons 1–4)     |

The present report focuses on the hRPB11 gene which remained to be characterised.

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It has been shown in many systems that the RPB11 subunit is able to heterodimerize with RPB3, evoking the alpha dimer in bacteria that directs the assembly of the two largest subunits of the RPB complex [16–22,9,10]. We show that human homologs of RPB11 are encoded by a multi-genie family. We shall refer to the previously identified human gene and cDNA encoding a protein homologous to yeast RPB11, as hRPB11α[23–25]. We have characterised additional members of this family and discuss their properties.

Results
Characterisation of human genomic sequences encoding RPB11-related proteins α and β
In addition to the previously characterised hRPB11 cDNA, referred to as hRPB11a in the present work, a series of highly related human cDNAs were found in the databases ([24,25], Table 1). We show that these cDNAs were transcribed from a family of genomic sequences.

| GENES   | CDNA             |
|---------|------------------|
| hRPB1   | hRPB1            |
| hRPB1a  | hRPB1a           |
| hRPB1b  | hRPB1bβ          |
| hRPB1bα | hRPB1ββ          |
| hRPB1ββ | hRPB1ββ          |
| mRPB1   | mRPB1            |
| AC087420 | AC087420        |
| (exons 1) | (exons 1–4)     |
We localised the hRPB11 genomic sequences on metaphasic chromosomes with a fluorescent genomic probe encompassing the conserved exons 1 to 3 of hRPB11a (see Fig. 1A), thus revealing both hRPB11a and b genomic sequences. 50 metaphases were analysed: 90% showed specific signals on chromosome 7, at positions q11.23 and q22, and about 80% at position p12.

A unique mRPB11 gene maps on mouse chromosome 5

The screening of our mouse genomic library yielded a unique mRPB11 gene (Fig. 2A, Table 1) which is transcribed into a unique type of transcript (Fig. 2B, Table 1) that encodes a mRPB11 protein identical to the human hRPB11a counterpart (Fig. 2C). In marked contrast to the human system, a single locus is detected on the murine chromosome 5, at cytogenetic band G (Fig. 2D).
Figure 2
Structure of mRPB11 gene, mRNA and protein.
A) Comparison of the structures of hRPB11a and mRPB11 genomic sequences. Horizontal lines represent the genomic sequences. The identified exons are indicated by boxes. The conserved 5’ sequences encompassing exons 1–3 are in black, as in fig. 1A. The homologies between the mouse and human sequences which are restricted to the exons are indicated.

B) Structure of mRPB11 mRNA. The exons are indicated by boxes. The carets represent the spliced introns with sizes (bp). The 5’ and 3’ untranslated regions are shown as open boxes. The size (bp) of the coding sequence (CDS) present in each exon is indicated below.

C) Amino acid sequences of mRPB11 polypeptide. The translated CDS of the mRNA identified for the mRPB11 and hRPB11a genes shown above are aligned with their identity and size (amino acids) indicated on the left and right, respectively. The limits of the exons encoding each part of the sequence are indicated by brackets, with the corresponding exon numbers indicated above. The sequence of mRPB11 being taken as a reference, is aligned with hRPB11a, complete identity is indicated by the uninterrupted series of – symbols.

D) Genomic localisation of mRPB11. A representative metaphase that has been simultaneously hybridised with the pBSK-mRPB11-gen1 and pBSK-mRPB11-gen2 derived fluorescent probes, respectively green and red, is shown. The white-arrow heads point to the position of the specifically bound loci. The hybridised chromosome is identified in the bottom of the figure, 1: both pBSK-mRPB11-gen1 and 2 probes are visualised, 2: only pBSK-mRPB11-gen1 probe is visualised, 3: only pBSK-mRPB11 gen2 probe is visualised, 4: chromosome staining.
The hRPB11a and hRPB11b genomic sequences are transcribed in all human tissues tested

Expression of these cDNAs was tested in 16 independent human tissues by Northern-blot analysis (Fig. 3). One major band was detected with each probe in all tissues. Strikingly, the relative levels of expression of hRPB11a versus hRPB11b isoforms varied, depending on the tissue. While hRPB11a was the major transcript in most tissues with highest levels in heart and skeletal muscle, hRPB11bαRNA was most abundant in the brain (note the different exposure times in Fig. 3). hRPB11bβ transcripts were weak in all tissues, although more readily detected in the heart, skeletal muscle and ovary.

The proteins encoded by the three cDNAs exhibit specific interaction properties

The pairwise interaction abilities of all the hRPB subunits have previously been analysed using a GST pull-down assay [8]. Similarly, we compared the interaction properties of hRPB11a, bα and bβ with those described for hRPB11a [24] (Fig. 4). In this assay, hRPB11a and bα revealed the ability to interact only with GST-hRPB3. By contrast, hRPB11bβ not only interacted with GST-hRPB3, but also with GST-hRPB1, 2, 4, 5, 6, 7 and 10β.

Complementation experiments in budding yeast

We asked whether the human RPB11 homologues were able to compensate for the disruption of the *Saccharomyces cerevisiae* (Sc) essential RPB11 gene. In the complementation assay used, overexpression of *ScRPB11*...
rescued this lethal phenotype by restoring yeast proliferation with a doubling time of 2 h (Fig. 5, line 1), whereas the empty vector did not (not shown). Under the conditions where all the human proteins were expressed to similar levels in the transformed yeast cells (data not shown), hRPB11α or hRPB11β did not rescue the ScRPB11 null allele (Fig. 5, lines 2 and 4). By contrast, hRPB11βα restored cell proliferation, although with a slower growth rate (Fig. 5, lines 3).

Figure 4
Interactions between hRPB11α, hRPB11βα and hRPB11ββ proteins with the twelve GST-hRPB subunits. Sf9 cells were coinfected with two recombinant baculoviruses, the first expressing one of the twelve GST-fused subunits or GST alone, the second expressing the untagged hRPB11α, βα or ββ subunits. After metabolic labelling of proteins using 35S Met, extracts were prepared and GST-pulldown assays were performed. Aliquots of the total extracts (Extracts) and of the GST-bound fractions (GST-pulldown) were analysed by SDS-PAGE and revealed by autoradiography. Arrows point to the position of the non-tagged hRPB11α, βα or ββ subunits.
hRPB11b genomic sequences share a domain with hPMS2L genes

Databases were screened for sequence similarities with the hRPB11b exons 4 and 5. The sequences of hRPB11b α and b β, could be aligned with hPMS2L4 (Ac N° D38438) and hPMS2L13 (Ac N° AB017004): strikingly, the sequences of hRPB11b exon 4 and hPMS2L exon g were nearly identical (Fig. 6). The hPMS2L cDNAs are encoded by a multigene family, in which exon g can be translated in two frames, depending on the gene (Fig. 6). This is due to the presence of additional nucleotides at the 5’ end of exon g, i.e. two A residues in hPMS2L13, when compared to hPMS2L4. Hence, very similar peptides can be produced from hPMS2L and hRPB11b cDNAs by completely distinct mechanisms involving small insertions and alternative splicing, respectively.

Discussion

A multigene family encodes the hRPB11 but not the mRPB11 subunit

Our results demonstrate the existence in the human genome of a family of sequences related to the hRPB11a gene. Three distinct loci were detected using these genomic sequences as a probe on human chromosome 7 (Fig. 1A). Four distinct genomic sequences, hRPB11a, hRPB11b, and two type b-related sequences not described here (Ac N° AC004951 and AC004084), were identified. Quantitative PCR measurements of the genomic copy number of hRPB11 exon 3 suggested the presence of about twelve distinct hRPB11 sequences in the human haploid genome (not shown).

In sharp contrast, such a gene family does not exist in mouse. The mRPB11 gene is unique, maps to a unique locus at 5G which was previously identified as a region syntenic to the human locus 7q11.23 [27,28] and encodes a single murine mRPB11 protein identical to hRPB11a. The amplification of these genomic sequences may therefore represent a recent evolutionary event, that may be restricted to the primates, including human and african green monkey, as both RPB11 b-type mRNAs were present in COS-7 and CV1 cells (not shown).

These genomic sequences yield stable mRNAs

hRPB11a and hRPB11b transcripts were detected as stable mRNAs from 16 human tissues with, in some cases, a clear expression specificity, as shown by both Northern-blot (Fig. 3) and RT-PCR experiments (not shown). This is further confirmed by the fact that they have also been isolated from cDNA libraries from various tissues (see Table 1). The hRPB11α and b β CDS result from a differential splicing mechanism which we have not observed in any hRPB11α transcript. It is tempting therefore to speculate that a selective pressure maintains both isoforms of hRPB11b messenger RNAs.

Using specific antibodies, the hRPB11a protein was readily detected in extracts from either human tissues or cell lines [19]. By contrast, the hRPB11bα or b β proteins have not been detected so far, suggesting that their expression may be regulated at the translational level. We conclude that the hRPB11b proteins are either present at very low levels in these cells, or restricted to specific cell lines and/or situations that remain to be identified.

The hRPB11 proteins exhibit distinctive properties

Both hRPB11a and b β proteins were found to contact exclusively hRPB3 in coexpression assays, consistent with previous results (see Introduction). The yeast ScRPB3/ ScRPB11 heterodimer has been modelled as an alphalike dimer [29,22], in which both C-terminal domains consist of two long alpha helices that cross each other and point toward the outside of the RPB complex [9,10]. The hRPB11bα protein differs from hRPB11α at the very C-terminal end of this structure: its incorporation into the RPB complex instead of hRPB11a may therefore alter the interactions with the surrounding molecules. Despite this difference, both hRPB11a and bα can indeed integrate the RPB complex in vivo. We show that hRPB11bα is able to functionally replace ScRPB11 in the yeast RPB. Strikingly, the hRPB11a protein, known as a bona fide human RPB subunit, is not functional in yeast, whereas RPB11 of the distantly related fission yeast Schizosaccharomyces pombe can replace ScRPB11 in vivo [30].
Why only hRPB11\(\alpha\) protein is functional in yeast may be related to the fact that its C-terminal domain exhibits a higher homology to the one of ScRPB11, both being rather hydrophobic, than the hydrophilic C-terminal domain of hRPB11\(\alpha\). The hRPB11\(\beta\) protein may therefore be able to make, although weakly, critical contacts that the hRPB11\(\alpha\) protein cannot make. These data point to a critical function of this C-terminal domain, that is encoded by a separate specific exon in mammals, in vivo.

The observation that the hRPB11\(\beta\) protein exhibits a completely distinct set of interactions with the other RPB subunits is presently difficult to integrate into the available model of the yeast RPB [9]. It is possible that hRPB11\(\beta\) establishes multiple but transient contacts with various subunits during the RPB assembly and that these interactions are revealed in our binary protein binding assay.

How did evolution create the hRPB11\(b\) genomic sequences?

The \(b\) types of RPB11 genes may result from recombination events between a hRPB11\(a\) gene and at least two other genes, recruiting new exons 4 and 5, respectively. While the origin of exon 5 remains to be identified, exon 4 of hRPB11\(b\) is present in human PMS2L genes [31,32] that have no known murine homolog. Although the function of these PMS2L genes is still elusive, they share five coding exons with the PMS2 gene (\(b\) to \(f\), Fig. 6) which plays a critical role in the mismatch repair (MMR) machinery and is located on human chromosome 7p22 [32,33]. The hPMS2L and hRPB11 genes are located close to each other at positions 7p12, 7q11.23 and 7q22, supporting a recombinational origin [31,32]. The primate specific hRPB11\(b\) gene products may provide a new link between the transcription and MMR machineries, together with the hPMS2L gene products. Thus, it will be of interest to explore the potential contribution of this species-specific gene rearrangement to the phenotypical differences between human and mouse mutants which, when affected in their MMR activity, exhibit different types of tumors [34,35]. Because of the presence of these primate-specific variants, drugs which are often tested in rodents may be mis-evaluated regarding their effects on human patients. The present findings indicate that more surprises may arise from studies of fundamental cellular processes, even in closely related species.

Conclusions

The human genome contains a family of genes that includes the gene (hRPB11\(a\)) encoding subunit 11 of the hRPB complex. Strikingly, such a family does not exist in...
the murine genome which contains a unique gene (mRPB11) encoding a protein which is identical to hRPB11a. Our observations strongly suggest that the hRPB11b genes have been engineered by evolution in the primate genomes to produce proteins with novel properties, required only under specific circumstances, the nature and role of which remain to be identified.

**Materials and methods**

**Cloning of genomic sequences**

MboI partially-digested placenta DNA was inserted into the unique BamHI site of lambda GEM12, yielding, after transformation of E. coli TAP90, a library of about 1.2×10^6 independent phages, equivalent to five human genomes. This library was screened using the 32P-labelled Nhel-SpeI fragment from pBSK-hRPB11a as a probe (Table 2). One hundred positive phages were isolated and characterised by Southern blot analysis indicating the existence of several distinct restriction profiles (data not shown). For further sequence analysis, the DNA inserts of two phages, 27 and 11, were partially digested by Sau3A1 and subcloned in the unique BamHI site of pBSK yielding pBSK-hRPB11a-gen and pBSK-hRPB11b-gen, respectively (Table 2). Alternatively, DNA fragments were directly sequenced after PCR amplification from several phages.

A mouse SV129 D3 genomic library was similarly generated from mouse ES cells in lambda GEM12, yielding a library of about 2.5×10^6 independent phages, equivalent to 10 murine genomes. About 1.2×10^6 clones were screened as described above for the human genomic library. 26 positive clones were obtained. A Southern-blot analysis was performed on 12 independent clones (not shown) that revealed an identical restriction pattern indicating that they corresponded to a unique gene sequence. For further sequence analysis, the DNA inserts of two independent phages were excised using the flanking EcoRI sites and directly sequenced after PCR amplification from several phages.

**Table 2: Strains and plasmids**

| Strains | Genotype |
|---------|----------|
| Yeast WY-11 | #MATa/MATα ura3-52 his3-Δ1 leu2-3, ura2-112 lys2-Δ1201 ade2-101 rpb11-Δ1::HIS3[39] |
| Yeast YGVS-074 | #MATa/MATα ura3-52 his3-Δ1 leu2-3, ura2-112 lys2-Δ1201 ade2-101 rpb11-Δ1::HIS3 [39] |
| Yeast YGVS-072 | #MATa ura3-52 his3-Δ1 leu2-3, ura2-112 lys2-Δ121 ade2-101 rpb11-Δ1::HIS3 [39] |
| Yeast YGVS-074 used for complementation assays | #MATa ura3-52 his3-Δ1 leu2-3, ura2-112 lys2-Δ1201 ade2-101 rpb11-Δ1::HIS3 [39] |

**Plasmids**

| Plasmids | Description |
|----------|-------------|
| pRP11/8-RPB1I | # Cloning of the α3436-gene fragment in pBSK (Stratagene), containing exons 1 and 2 from rpb11 | |
| pBSK-hRPB1Ia-gen | # Partial Sau3AI genomic fragment in pBSK (Stratagene), containing exons 1 to 4 from hRPB1Ia gene |
| pBSK-hRPB1Ib-gen | # Partial Sau3AI genomic fragment (19.6 kb) in pBSK (Stratagene), containing exons 1 to 4 from hRPB1Ib gene |
| pBSK-mRPB1I-gen1 | # Partial Sau3AI genomic fragment (16.5 kb) in pBSK (Stratagene), containing exons 1 to 4 from mRPB1I gene |
| pBSK-mRPB1I-gen2 | # Partial Sau3AI genomic fragment (17.7 kb) in pBSK (Stratagene), containing exons 1 to 4 from mRPB1I gene |
| pBSK-hRPB1Ia | # RT-PCR cloning of hRPB1Ia CDS in pBSK. The CDS can be excised using the unique Nhel and SpeI sites |
| pCRII-hRPB1Iβ | # RT-PCR cloning of hRPB1Iβ CDS in pCRII (Invitrogen). The CDS can be excised using Nhel and SpeI |
| pCRII-hRPB1Iβ | # RT-PCR cloning of hRPB1Iβ CDS in pCRII. The CDS can be excised using the flanking EcoRI sites |
| pCRII-ScRPB1I | # PCR cloning of ScRPB1I CDS from pRP11/8-RPB1I in pCRII. The CDS can be excised using Nhel and SpeI |
| pGEN | # 2µORI, TRP1, PGK promoter [4] |
| pGEN-ScRPB1I | # Cloning of the EcoRI fragment of pCRII-ScRPB1I into the EcoRI site of pGEN |
| pGEN-hRPB1Ia | # Cloning of the Nhel-XbaI fragment of pBSK-hRPB1Ia into the Nhel site of pGEN |
| pGEN-hRPB1Iβ | # Cloning of the Nhel-SpeI fragment of pCRII-hRPB1Iβ into the Nhel site of pGEN |
| pGEN-hRPB1Iβ | # Cloning of the EcoRI fragment of pCRII-hRPB1Iβ into the EcoRI site of pGEN |
| pVL1393-hRPB1Ia | # Cloning of the Nhel-XbaI fragment of pBSK-hRPB1Ia into the XbaI site of pVL1393 (PharMingen) |
| pVL1393-hRPB1Iβ | # Cloning of the Nhel-SpeI fragment of pCRII-hRPB1Iβ into the XbaI site of pVL1393 (PharMingen) |
| pVL1393-hRPB1Iβ | # Cloning of the Nhel-SpeI fragment of pCRII-hRPB1Iβ into the XbaI site of pVL1393 (PharMingen) |

The murine genome which contains a unique gene (mRPB11) encoding a protein which is identical to hRPB11a. Our observations strongly suggest that the hRPB11b genes have been engineered by evolution in the primate genomes to produce proteins with novel properties, required only under specific circumstances, the nature and role of which remain to be identified.

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cDNA cloning
The cDNA fragments were amplified by RT-PCR from total HeLa cell RNA using the appropriate primers and inserted in either pBSK or PCRII vectors. In each case, unique restriction sites were introduced in front of the ATG and after the stop codons. Several independent clones of each cDNA were sequenced. Restriction fragments spanning the complete coding sequences (CDS) were then transferred to various expression vectors (Table 2).

Localisation on human chromosomes by FISH
Human metaphase spreads were hybridised using as a probe the biotinylated 4.5 kb fragment encompassing hRPB11a exons 1 to 3 that was amplified using the TaKaRa system (BIO Whittaker Europe SPRL) [36,37].

Mouse metaphase spreads were analysed as described using as probes the pBSK-mRPB11-gen1 and 2 plasmid DNAs, that were labelled using green and red fluorescent systems. G.V.S acknowledges the support from the University Louis Pasteur of Strasbourg and the Russian Foundation for Basic Research (Grant N° 01-04-49741).

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