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Research article

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 hRPB11bα and hRPB11bβ 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 hRPB11bα 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 *hRPB11b* 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], *hRPB10α* (Ac N° AJ252078), *hRPB10β,* (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 show that human homologs of RPB11 are encoded by a multi-gene family. We shall refer to the previously identified human gene and cDNA encoding a protein homologous to yeast RPB11, as *hRPB11a*[23–25]. We have characterised additional members of this family and discuss their properties.

Results

Characterisation of human genomic sequences encoding RPB11-related proteins a and b

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.

Table 1: Accession numbers of *RPB11* sequences

GENES	CDNA
hRPB11a	*hRPB11a*
AJ277932 (exon 1)	**X98433**
AJ277928 (exon 2)	**X82385**
AJ277929 (exon 3)	
AJ277930 (exon 4)	
hRPB11b	*hRPB11bα*
AJ277931 (exons 1–4)	**H52765**
AJ277736 (intron 4)	**AA077481**
AJ277737 (intron 4)	**AJ277739**
AJ277738 (intron 4)	*hRPB11bβ*
	AJ277740
mRPB11	*mRPB11*
AC087420 (exons 1–4)	**D85818**
	W91247
	BG046264

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 *hRPB11a* 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*. *hRPB11a* and *b* genomic sequences diverged within intron 3 (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 *hRPB11bα* and *hRPB11bβ,* respectively (Fig. 1B, Table 1). The absence of exon 3 switches the reading frame of exon 4, thereby extending the coding sequence (CDS) of *hRPB11bβ 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 *hRPB11bβ* 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 hRPB11a protein which was previously identified as a subunit of the human RPB complexes in Western-blots of immunoprecipitated RPB ([26] and our unpublished data). We have presently identified two additional cDNAs, *hRPB11bα* and *hRPB11bβ,* as distinct members of the same family.

Strikingly, as predicted from their sequences, the hRPB11a, bα and bβ polypeptides have similar sizes: 117, 115 and 116 residues, with calculated M.W. of 13.3, 13, 12.7 kDa, respectively (Fig. 1C). The N-terminal part of hRPB11a subunit differs only from the hRPB11b 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 *hRPB11a* encodes a hydrophilic 11-residue peptide, it generates a rather hydrophobic 10-residue peptide in the case of hRPB11bα (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.

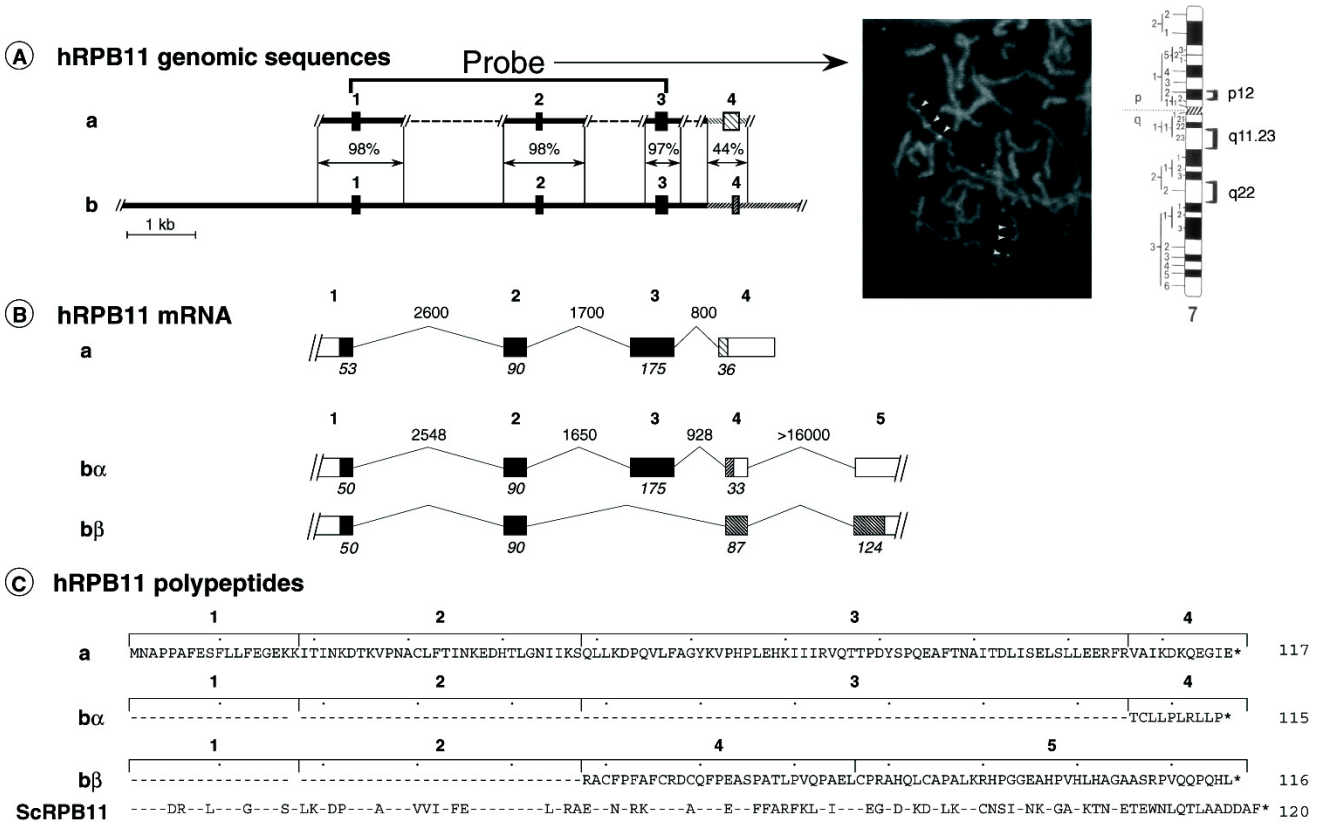

Figure 1

Structure of *hRPB11* genes, mRNAs and proteins.A) Comparison of the structures of *hRPB11a* and *b* genomic sequences. Horizontal lines represent the human genomic sequences. The identified exons are indicated by boxes. The conserved 5' sequences encompassing exons 1–3 are in black. The homologies between subtypes a and b are indicated. The divergent 3' regions are hatched. A representative metaphase that has been hybridised with a *hRPB11a* genomic probe (bracket) is shown on the right. The white arrowheads point to the position of the specifically bound loci. Chromosome 7 is represented with brackets pointing to the identified localisations. **B) Structure of *hRPB11a, bα* and *bβ* mRNAs.** Exons are indicated by boxes. Carets represent the spliced introns approximate 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) Aminoacid sequences of hRPB11a, bα and bβ polypeptides.** The translated CDS of the mRNA identified for the two genes shown above are aligned with their identity and size (aminoacids) 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 hRPB11a being taken as a reference, only the divergent residues are shown underneath. *Saccharomyces cerevisiae* RPB11 (ScRPB11) sequence is shown below.

hRPB11 maps to three distinct loci on human chromosome 7

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).

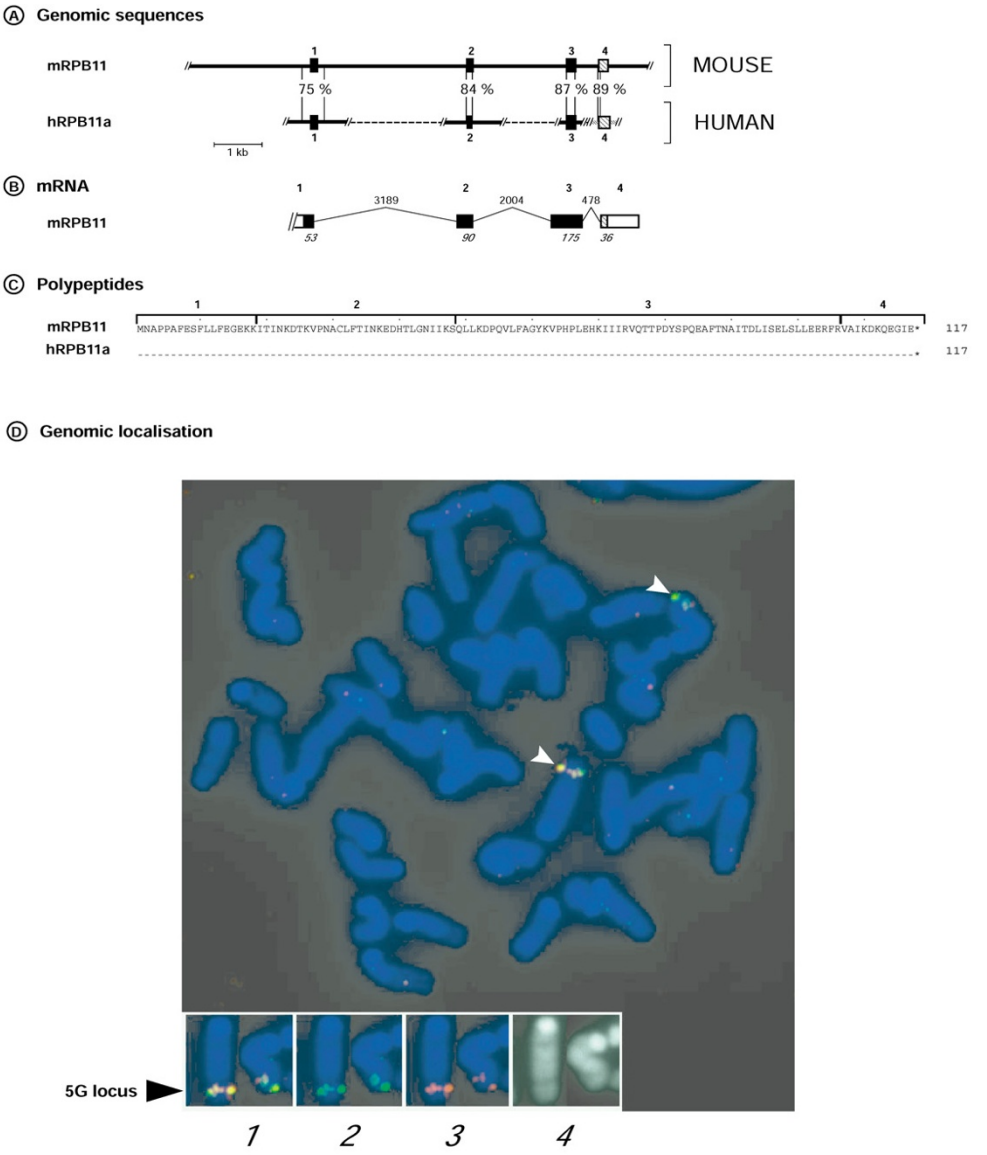

Figure 2

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 whith 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 (aminoacids) 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.

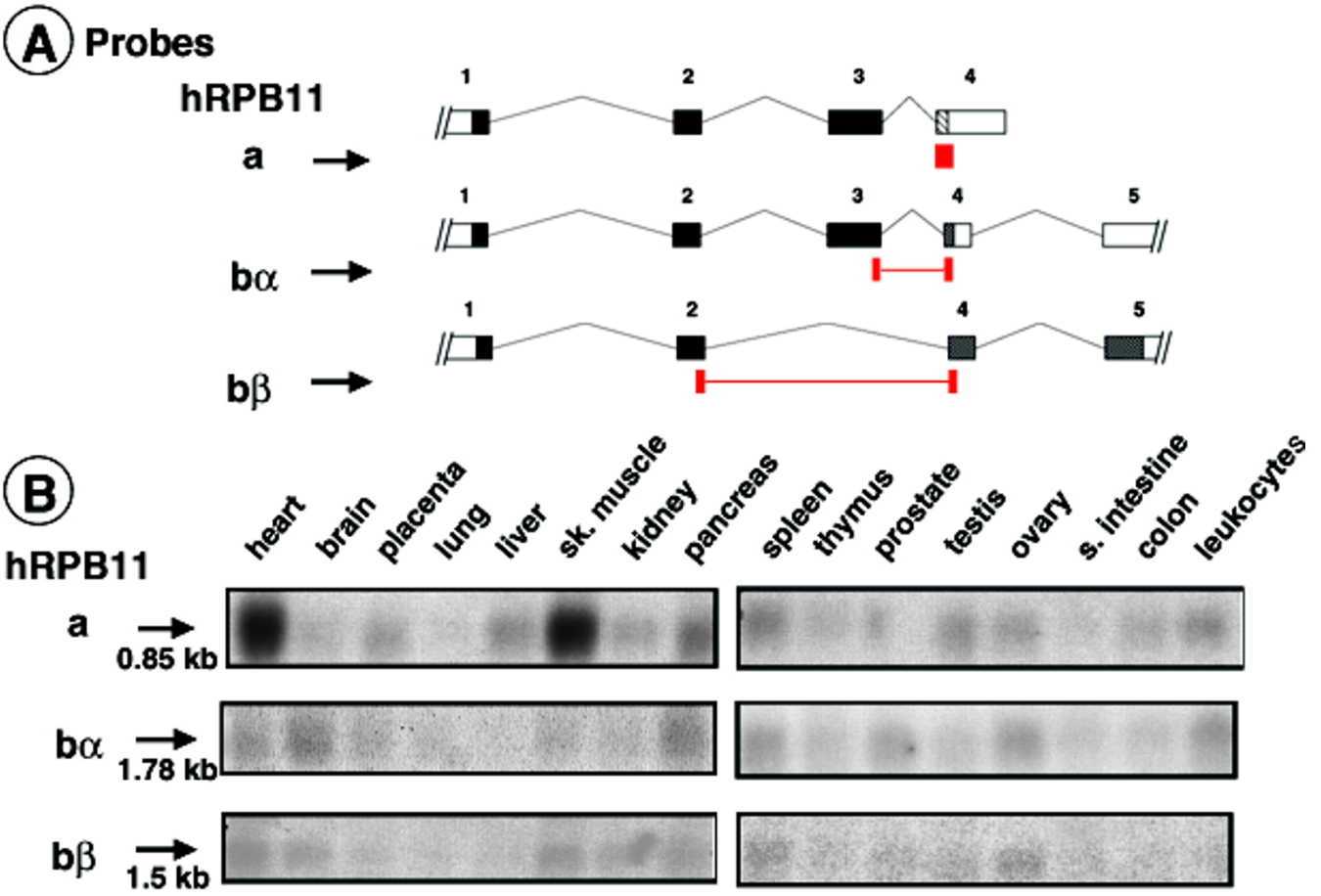

Figure 3
Nothern-blot analysis of *hRPB11* expression.A) The *hRPB11a, bα* and *bβ* transcripts are represented as in Fig 1B. The probes designed to reveal selectively each mRNA are indicated below in red. In the case of hRPB11b probes a single oligonucleotide was derived from the adjacent exons. **B)** PolyA$^+$ mRNA from 16 human normal tissues, as indicated on the top, were analysed. The *hRPB11a, bα* and *bβ* transcripts were revealed by hybridisation with specific oligonucleotidic probes and after extensive washing, the filters were exposed for 1, 2 and 1 month, respectively. The size (kb) of each mRNA is indicated on the left.

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, hRPB11bα RNA 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 hRPB11bα 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*

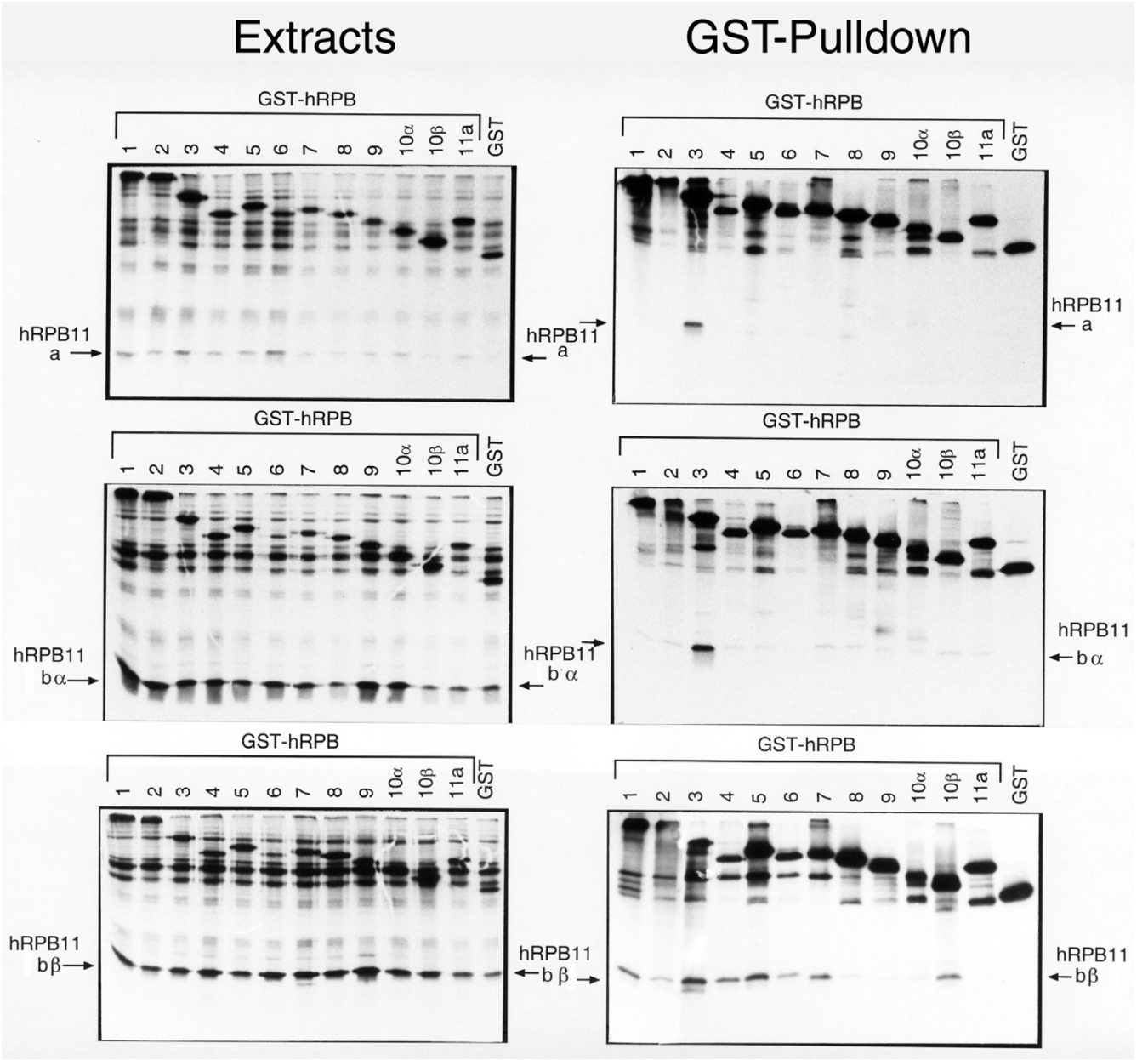

Figure 4
Interactions between hRPB11a, hRPB11bα and hRPB11bβ proteins with the twelve GST-hRPB subunits. Sf9 cells were coinfected with two recombinant baculoviruses, the first expressing one of the twelve GST-fused subunits or GST alone, the second expressing the untagged hRPB11a, bα or bβ subunits. After metabolic labelling of proteins using ^{35}S 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 hRPB11a, bα or bβ subunits.

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), *hRPB11a* or *bβ,* did not rescue the *ScRPB11* null allele (Fig. 5, lines 2 and 4). By contrast, *hRPB11bα* restored cell proliferation, although with a slower growth rate (Fig. 5, lines 3).

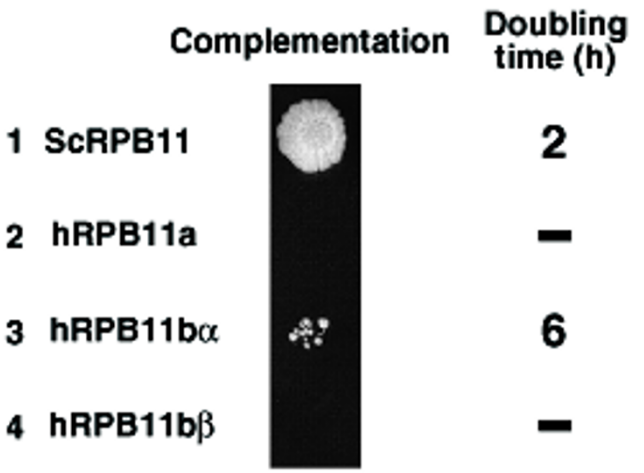

Figure 5
Complementation of *rpb11::His3* yeast strains. The cDNAs assayed for complementation are listed on the left (lines 1–4). 30 cells of the complemented yeast strains were incubated at 28°C for 2 weeks on supplemented SD minimal medium, in the presence of 5-FOA. Doubling times (h) were measured at 28°C on liquid YPD medium.

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 hRPB11bα and bβ, 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°s 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 synthenic 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 *hRPB11bα* and *bβ* CDS result from a differential splicing mechanism which we have not observed in any *hRPB11a* 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 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 bα 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 alpha-like 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 hRPB11a 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].

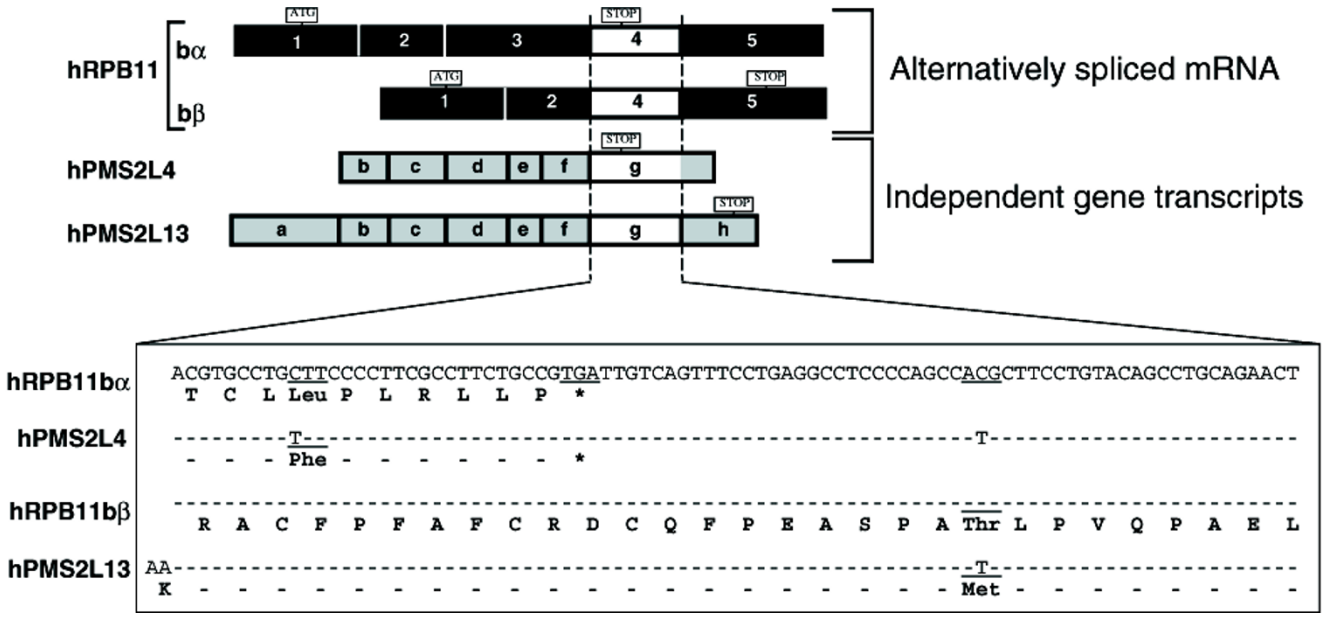

Figure 6
Similar peptides are produced via independent mechanisms in *hRPB11b, hPMS2L4* and *hPMS2L13* transcripts. The mRNAs are depicted by boxes that represent the exons. The black and grey areas represent the sequences that are specific to *hRPB11b* and *hPMS2L*, respectively. The strongly homologous sequences are represented by the open boxes. The sequence of exon 4 from *hRPB11bα* and bβ are shown together with the conserved 5' part of exon **g** from *hPMS2L4* and the complete *hPMS2L13* exon **g** with their translated peptides. Only the divergent residues are indicated in *hPMS2L, hRPB11b* being taken as a reference.

Why only hRPB11bα 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 hRPB11a. The hRPB11bα protein may therefore be able to make, although weakly, critical contacts that the hRPB11a 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 hRPB11bβ 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 hRPB11bβ establishes multiple but transient contacts with various subunits during theRPB assembly and that these interactions are revealed in our binary protein binding assay.

How did evolution create the hRPB11b genomic sequences?

The *b* types of *RPB11* genes may result from recombination events between a *hRPB11a* 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 *hRPB11b* is present in human *PMS2L* genes [31,32] that have no known murine homolog. Although the function of these *hPMS2L* 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 *hRPB11b* 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 mice 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 (*hRPB11a*) encoding subunit 11 of the hRPB complex. Strikingly, such a family does not exist in

Table 2: Strains and plasmids

Strains	Genotype
Yeast WY-11	# *MATa/MATα ura3-52 his3-Δ200 leu2-3, leu2-112 lys2-Δ201 ade2-101 RPB11/rpb11-Δ1::HIS*[39]
Yeast YGVS-074	# *MATa/MATα ura3-52 his3-Δ200 leu2-3, leu2-112 lys2-Δ201 ade2-101 RPB11/rpb11-Δ1::HIS 3trp1-Δ63*
Yeast YGVS-072	# *MATa ura3-52 his3-Δ200 leu2-3, leu2-112 lys2-Δ201 ade2-101 trp1-Δ63 rpb11-Δ1::HIS3* [pRP11/8-RPB11] (Offspring of YGVS-074 used for complementation assays)
Plasmids	**Description**
pRP11/8-RPB11	# *URA3 CEN ARS ScRPB11* EcoRI/SacI into pRS416 [39]
pBSK-hRPB11a-gen	# Partial Sau3AI genomic fragment in pBSK (Stratagene), containing exon 1 and 2 from *hRPB11a* gene
pBSK-hRPB11b-gen	# Partial Sau3AI genomic fragment (19.6 kb) in pBSK (Stratagene), containing exons 1 to 4 from *hRPB11b* gene
pBSK-mRPB11-gen1	# Partial Sau3AI genomic fragment (16.5 kb) in pBSK (Stratagene), containing exons 1 to 4 from *mRPB11* gene
pBSK-mRPB11-gen2	# Partial Sau3AI genomic fragment (17.7 kb) in pBSK (Stratagene), containing exons 1 to 4 from *mRPB11* gene
pBSK-hRPB11a	# RT-PCR cloning of *hRPB11a* CDS in pBSK. The CDS can be excised using the unique *Nhe*I and *Spe*I sites
pCRII-hRPB11bα	# RT-PCR cloning of *hRPB11bα* CDS in pCRII (Invitrogen). The CDS can be excised using *Nhe*I and *Spe*I
pCRII-hRPB11bβ	# RT-PCR cloning of *hRPB11bβ* CDS in pCRII. The CDS can be excised using the flanking EcoRI sites
pCRII-ScRPB11	# PCR cloning of *ScRPB11* CDS from pRP11/8-RPB11 in pCRII. The CDS can be excised using *Nhe*I and *Spe*I
pGEN	# 2μ*ORI, TRP1, PGK* promoter [4]
pGEN-ScRPB11	# Cloning of the *Eco*RI fragment of pCRII-ScRPB11 into the *Eco*RI site of pGEN
pGEN-hRPB11a	# Cloning of the *Nhe*I-*Xba*I fragment of pBSK-hRPB11a into the *Nhe*I site of pGEN
pGEN-hRPB11bα	# Cloning of the *Nhe*I-*Spe*I fragment of pCRII-hRPB11bα into the *Nhe*I site of pGEN
pGEN-hRPB11bβ	# Cloning of the *Eco*RI fragment of pCRII-hRPB11bβ into the *Eco*RI site of pGEN
pVL1393-hRPB11a	# Cloning of the *Nhe*I-*Xba*I fragment of pBSK-hRPB11a into the *Xba*I site of pVL1393 (PharMingen)
pVL1393-hRPB11bα	# Cloning of the *Nhe*I-*Spe*I fragment of pCRII-hRPB11bα into the *Xba*I site of pVL1393 (PharMingen)
pVL1393-hRPB11bβ	# Cloning of the *Nhe*I-*Spe*I fragment of pCRII-hRPB11bβ into the *Xba*I 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.

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 ^{32}P-labelled NheI-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 Sau3AI 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 *Not*I restriction sites and subcloned in the unique *Not*I site of pBSK yielding pBSK-mRPB11-gen1 and pBSK-mRPB11-gen2, respectively (Table 2). Both of these genomic sequences were identical to the sequence that is present in the database (Ac N° AC087420).

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 nucleotide derivatives respectively, and mixed for hybridization [38].

Recombinant baculoviruses and GST-pulldown

pVL1393-hRPB11bα and -hRPB11bβ transfer vectors (Table 2) were recombined with linearized baculovirus DNA (BaculoGold DNA, PharMingen) in Sf9 cells. The recombinant viruses were plaque-purified and expression of the proteins was verified by Western-blot analysis using specific mouse monoclonal antibodies. The other recombinant baculoviruses and the conditions for GST-pulldown assays have been described previously [8]. The glutathione-sepharose beads were washed with PBS buffer containing 0.65 M NaCI and 1% Nonidet P-40.

Northern-blot analysis

Three ^{32}P-end-labelled oligonucleotides specific to *hRPB11*a, *bα* and *bβ* mRNAs, respectively, were used to probe MTN human blots I and II (Clonetech) of poly A$^+$ mRNA from 16 normal human tissues (2 μg of each). The probe for *hRPB11a* was derived from the corresponding exon 4. The probe for *hRPB11bα* was derived from the junction between the corresponding exons 3 and 4. The probe for *hRPB11bβ* was derived from the junction between exons 2 and 4 of the *hRPB11b* gene.

Complementation in Saccharomyces cerevisiae

Yeast was grown on YPD or SD standard media. The ability of pGEN derivatives, expressing various proteins, to rescue the lethal phenotype conferred by the *rpb11::HIS3* allele was assayed by plasmid shuffling. The YGVS-072 strain (Table 2) was transformed with the pGEN derivatives using a DMSO treatment protocol and plated on SD medium supplemented with adenine (20 mg/l), leucine (30 mg/l) and lysine (30 mg/l). Trp$^+$ transformants were transferred twice to 5-fluoro-orotic acid plates and monitored for their ability to grow at 28°C. The viable clones were then grown on YPD liquid medium and the doubling time during exponential growth was determined from absorbance at 600 nm.

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