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SHORT COMMUNICATIONS

A Minor Isoform of the Human RNA Polymerase II Subunit hRPB11 (POLR2J) Interacts with Several Components of the Translation Initiation Factor eIF3

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Abstract—Using the yeast two-hybrid (YTH) system we have uncovered interaction of the hRPB11cα minor isoform of *Homo sapiens* RNA polymerase II hRPB11 (POLR2J) subunit with three different subunits of the human translation initiation factor eIF3 (hEIF3): eIF3a, eIF3i, and eIF3m. One variant of eIF3m identified in the study is the product of translation of alternatively spliced mRNA. We have named a novel isoform of this subunit eIF3mβ. By means of the YTH system we also have shown that the new eIF3mβ isoform interacts with the eIF3a subunit. Whereas previously described subunit eIF3mα (GA17) has clear cytoplasmic localization, the novel eIF3mβ isoform is detected predominantly in the cell nucleus. The discovered interactions of the hRPB11cα isoform with several hEIF3 subunits demonstrate a new type coordination between transcription and the following (downstream) stages of gene expression (such as mRNA transport from nucleus to the active ribosomes in cytoplasm) in *Homo sapiens* and point out the possibility of existence of nuclear hEIF3 subcomplexes.

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Key words: *Homo sapiens*, RNA polymerase II, hRPB11 (POLR2J), yeast two-hybrid system, hEIF3, transcription, translation

All steps of gene expression are tightly coordinated *in vivo*, and the basis of this coupling is physical contacts between numerous protein factors participating in different cell processes. Indeed, RNA polymerase II transcription is physically and functionally linked with mRNA processing [1, 2]. The C-terminal domain (CTD) of the largest subunit of RNA polymerase II, Rpb1, plays a key role in the coordination of these two stages, acting as a platform for recruiting of various mRNA transcription and processing factors [3, 4]. Dynamic changes in the CTD phosphorylation pattern during transcription are accompanied by changes in the composition of the elongation complex. As a result, the nascent mRNA associates co-transcriptionally with proteins involved in mRNA splicing, nuclear export, subcellular localization, translation, and stability. The ultimate complexes are mature messenger ribonucleoprotein particles (mRNPs) competent in nuclear export. Thus, active transcription promotes more effective mRNA processing.

On the other hand, processing influences transcription. For instance, mRNA 3′ end maturation is coupled with transcription termination that occurs after mRNA endonucleolytic cleavage [5, 6]. The detailed mechanism of RNA polymerase II termination is not yet determined, as well as the complete set of essential factors that are engaged in this process [7]. Besides CTD of Rpb1, two other RNA polymerase II subunits, Rpb3 and Rpb11, have been shown to play an important role in termination. These two subunits (hRPB3 (POLR2C) and hRPB11 (POLR2J) in the case of human RNA polymerase II) form a heterodimer analogous to the α subunit homodimer of bacterial RNA polymerase [8], and, possibly, are allosterically involved in the termination signal transduction. Indeed, a genetic selection for *trans*-acting mutations that induce readthrough of two different terminators of the budding yeast RNA polymerase II yielded mutations in the Rpb11 and Rpb3 subunits [9]. The location of these amino acids substitutions on the discrete surface on the trailing end of the enzyme suggests that they can interfere with the interaction of RNA polymerase II with an extrinsic termination factor.

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Previously, it has been discovered that the hRPB11 subunit of human RNA polymerase II is encoded by the *POLR2J* multigene family, in contrast to single-copy genes encoding Rpb11 counterpart in the yeast, plant, mouse, macaque, and orangutan genomes [10-12]. Four independent genes, *POLR2J1-POLR2J4*, were revealed in human chromosome 7, upon the expression of which at least 11 types of mature mRNAs encoding different hRPB11 isoforms can be synthesized [11]. The functional role of these human RNA polymerase II hRPB11 isoforms is unknown. It has been shown that cDNA of the hRPB11bα isoform complements the disruption of the homologous single-copy yeast gene *RPB11*, which means that hRPB11bα can successfully incorporate into RNA polymerase II enzyme [10]. Possibly, under certain conditions or stages of cellular differentiation there is a pool of specialized RNA polymerases that transcribes a special set of genes. Such RNA polymerases can recruit appropriate protein factors necessary for the expression of these genes.

In this work we have used the yeast two-hybrid system approach to find interaction partners of the minor subunit hRPB11cα, which is the product of the *POLR2J3* gene [11]. To this end the full-length hRPB11cα cDNA was amplified from the plasmid pGEN-hRPB11c3 [10, 11] with oligonucleotides oGVS403 (5′-ccgaattcatgaacgcccctccagcc) and oGVS405 (5′-ccggatcctcacggcagaaggcgaag) and cloned into pMW103 vector (Km^r *HIS3*), which enables synthesis of a bait protein of interest as a fusion to the bacterial DNA binding protein LexA. The resulting plasmid pMW103-hRPB11cα was co-transformed with plasmid pDR8 (Km^r *URA3*) into the yeast SKY191 strain (MATα *trp1 his3 ura3 2lexAop-LEU2 3cIop-LYS2*) containing chromosomally integrated auxotrophic reporter gene *LEU2* under the control of two LexA operators. The plasmid pDR8 carries the *lacZ* reporter gene under the control of eight copies of the LexA operator [13]. The resulting yeast transformants did not grow on medium lacking leucine and produced white colored colonies in the presence of X-Gal, i.e. LexA-hRPB11cα fusion protein was not able to directly activate the *LEU2* and *lacZ* reporter genes, and it was possible to use this strain in the yeast two-hybrid screening. Since mRNA of the minor isoforms hRPB11bα and hRPB11cα (which differ in a single Ile34Met substitution only [11]) are most abundant in brain [10], we used a cDNA library prepared from human fetal brain (Clontech) and incorporated into the pJG4-5 (Ap^r *TRP1*) prey vector [14]. The library expression vector utilizes the galactose-inducible *Saccharomyces cerevisiae GAL1* promoter to express cloned cDNAs as translational fusions to a cassette consisting of the SV40 nuclear localization sequence (9 a.a.), the 82 residue B42 artificial acidic activating domain, and the hemagglutinin epitope tag (10 a.a.).

Twenty-five positive clones capable of simultaneously activating both reporters (*LEU2* and *lacZ*) in galactose-specific manner were obtained. Four independent clones contained the plasmids encoding the hRPB3 (POLR2C) subunit of RNA polymerase II. Finding of hRPB3 as an interaction partner of hRPB11, which form a dimer complex in the context of RNA polymerase II, was a good confirmation of the specificity of the screening performed. Two isolated clones (No. 1-19 and No. 162) contained cDNA fragments of eIF3a and eIF3i subunits of translation initiation factor hEIF3, and two others (No. 24 and No. 2-15) represented two different forms of cDNAs encoding variants of GA17 protein (Fig. 1a; see color insert). The other clones isolated during this experiment are at this moment under further analysis and will be described separately.

Initially GA17 mRNA was isolated from human dendritic cells (GenBank number AF064603.1). Later this protein was described as a receptor for herpes simplex virus [15]. It has been shown subsequently that GA17 (PCID1) protein is a component of translation initiation factor eIF3 (hEIF3) – in addition to 12 previously described eIF3a-eIF3l subunits. GA17 was renamed eIF3m, accordingly [16, 17]. To date hEIF3 is the largest complex (molecular mass of about 800 kDa) among human translation complexes. It consists of 13 proteins (from eIF3a to eIF3m), plays a central role in the formation of 48S initiation complex, and serves as a scaffold for binding multiple translation initiation factors [18]. The cDNA of clone No. 2-15 consists of 11 exons and corresponds to the complete form of a previously described GA17 mRNA. On the other hand, we found that in clone No. 24 cDNA the first exon is followed directly by the fifth one. Obviously, this new (not previously described) variant is derived from alternative splicing of corresponding mRNA (Fig. 1b). The full-length cDNA isolated encodes a protein of 242 a.a. with calculated molecular mass of 27.9 kDa. We named the new isoform of human eIF3m subunit eIF3mβ and will use further on in the paper the eIF3mα designation for a previously described GA17 (eIF3m) isoform (374 a.a., 42.5 kDa).

In similar YTH experiments we investigated whether eIF3 subunits discussed in this paper can interact with the major isoform hRPB11a. It was found that hRPB11a interacts well with eIF3mβ, weakly with eIF3a, and apparently does not interact at all with eIF3mα and eIF3i (Fig. 1a).

Using the yeast two-hybrid approach, we also tested whether the new isoform of human eIF3m interacts with eIF3a subunit. To test it, isolated cDNA fragment of eIF3a was cloned into the pMW103 vector as an *Eco*RI/*Bam*HI insert. The resulting plasmid pMW103-3a was co-transformed with the pDR8 plasmid into strain SKY191. Then yeast cells were transformed with the library-derived plasmid encoding eIF3mβ (clone No. 24), or with pJG4-5 vector as a negative control. We were able to detect *LacZ* reporter gene activation in galactose-specific manner only for the cells that harbored cDNA of

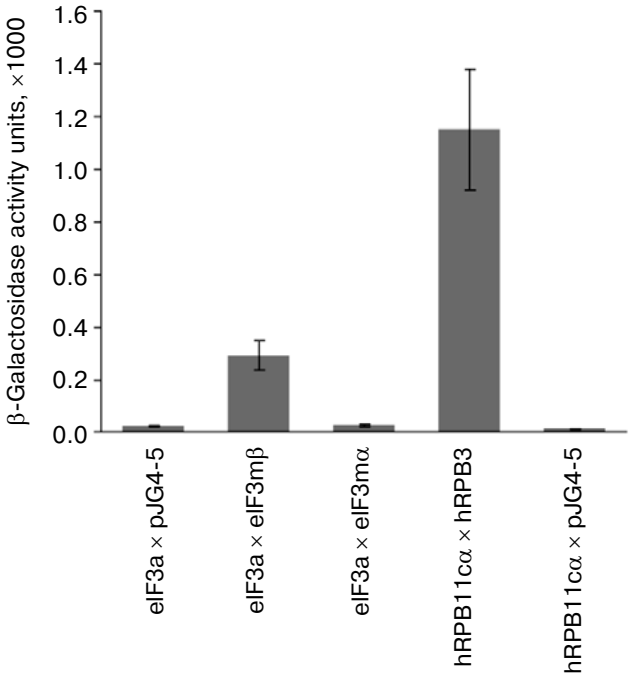

Fig. 2. Two-hybrid interaction of eIF3m isoforms and eIF3a. Yeast SKY191 strain harboring pDR8 plasmid was co-transformed with the constructs indicated and assayed for β-galactosidase activity in the liquid medium by means of the Miller method [19]. The pair of hRPB11cα and hRPB3 interacting proteins was taken as a positive control.

the eIF3mβ subunit, but not for the cells with pJG4-5 vector (Fig. 2). Thus, the new eIF3mβ isoform can possibly interact with the largest subunit of hEIF3 translation factor. In contrast to eIF3mβ, subunit eIF3mα does not interact in this test with the eIF3a subunit (Fig. 2), which is in good agreement with recent data of mapping purified hEIF3 complexes by means of cryoelectron microscopy and mass spectrometry [18].

Interaction of hRPB11cα with eIF3mα and eIF3mβ isoforms of human eIF3m was also checked *in vitro*. Initially, we tried to overexpress eIF3m subunits in *E. coli* using pET21d vector. Unfortunately, the expression level of the target proteins was very low, and the subunits were insoluble under native conditions. To solve these problems, cDNAs of eIF3mα and eIF3mβ were cloned into pET32c as *Bam*HI/*Sac*I-inserts using primers oGVS437 (5′-ccggatccccatgagcgtcccggcct) and oGVS439 (5′-ataagagctcaggtatcagaaagactc). The pET32c vector enables expression of the target protein as an N-terminus fusion with thioredoxin (Trx) and with six consecutive histidine residues. As a result, the expression level of the proteins of interest was much higher and the proteins were partly soluble. The cDNA of hRPB11cα was cloned in pET21d vector as an *Nhe*I/*Bam*HI-insert using specific primers oGVS520 (5′-ggtccatggctagcaacgcccctccagcctt) and oGVS405, and it was expressed as untagged protein (without 6His tag). The hybrid Trx-6His-eIF3m proteins were immobilized on Co^{2+}-agarose (TALON) and incubated with *E. coli* cell lysates expressing hRPB11cα. Bound proteins were eluted with imidazole and analyzed by electrophoresis in polyacrylamide gel. The Trx protein was used as a negative control. We found that hRPB11cα minor isoform was retained on Co^{2+}-agarose in detectable amount only after incubation with immobilized hybrid Trx-6His-eIF3mβ protein, but not with Trx alone (Fig. 3). The previously described eIF3mα isoform of human eIF3m subunit retains hRPB11cα under this experimental condition to a much lesser extent than the novel eIF3mβ isoform does. Thus, the eIF3mβ isoform interacts specifically with hRPB11cα *in vitro*, which is in good agreement with our YTH *in vivo* data.

In the description of GA17 protein as a receptor for herpes simplex virus, the authors reported its cell surface location [15]. Being a component of hEIF3 translation factor, GA17 should present also in cytoplasm [16, 17]. Notably, the novel eIF3mβ isoform described here does not contain a polypeptide fragment predicted as a transmembrane domain. To determine subcellular localization of human eIF3m isoforms, we cloned the corresponding cDNAs into pEGFP-N1 vector (Clontech) using primers oGVS506 (5′-gccctgctagcatgagcgtcccggccttc) and oGVS507 (5′-ccggatccgaggtatcagaaagactcaaaa). This vector provides expression of proteins fused with green fluorescent protein EGFP. HeLa cells were transfected with these constructs, and the localization of the hybrid proteins was monitored by fluorescence microscopy. We found that whereas previously known eIF3mα isoform is located solely in the cytoplasm, the novel eIF3mβ iso-

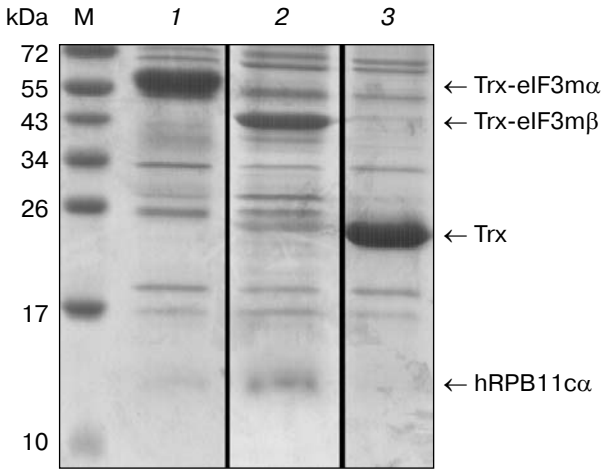

Fig. 3. *In vitro* interaction of hRPB11cα and eIF3m isoforms. 15% SDS-PAGE analysis of the proteins retained on TALON agarose. Recombinant proteins Trx-6His-eIF3mα (lane *1*), Trx-6His-eIF3mβ (lane *2*), or thioredoxin (lane *3*) were immobilized on TALON agarose. Untagged hRPB11cα subunit retains on the agarose if it binds immobilized proteins. M, molecular weight marker.

form is actively transported into the nucleus (Fig. 4; see color insert).

For a long time the different intracellular processes have been studied separately, and most of our knowledge is based on biochemical studies of individual factors. But now there is increasing evidence that all stages of gene expression are interlinked and tightly coordinated. For example, mRNA 3′ processing is necessary for both transcription termination and mRNA export, though the detailed molecular mechanisms of this process remain to be determined. Recently the human mRNA 3′ processing complex was purified and characterized. It was shown that this complex contains ~85 proteins. In addition to known 3′ processing factors, new factors participating in this process were also identified. It has been found that over 50 of these proteins some way or another mediate coupling of 3′ processing with other stages of gene expression [20]. Such sophisticated architecture of the 3′ processing complex reflects the necessity of fine coordination with different steps of gene expression. Remarkably, it was found that human mRNA 3′ processing complex, besides four RNA polymerase II subunits (hRPB1, hRPB2, hRPB5 and hRPB11), contains six subunits of hEIF3 translation factor including eIF3a and eIF3i, which is in agreement with our results about direct interactions of hEIF3 with RNA polymerase II. Previously, an unusual role of eIF3f subunit in 3′-termini maturation of HIV-1 pre-mRNA was shown [21]. The authors showed that eIF3f specifically interacts with SR protein 9G8 and cyclin-dependent kinase 11 (CDK11). Together these proteins influence the mRNA 3′-end processing.

Recently, it was found that in the budding yeast *Saccharomyces cerevisiae* the RNA polymerase II Rpb4/Rpb7 heterodimer interacts physically and functionally with components of the translation initiation factor eIF3, and it is required for efficient translation initiation [22]. It is known that Rpb4/Rpb7 heterodimer loosely associates with RNA polymerase II in yeast and shuttles between the nucleus, where it functions in transcription, and the cytoplasm, where it participates in the major mRNA decay pathways [23]. It was found that efficient translation in the cytoplasm depends on association of Rpb4/Rpb7 with RNA polymerase II in the nucleus, leading to a model in which RNA polymerase II remotely controls translation [22].

Our data demonstrate a link between the minor isoform of human RNA polymerase II subunit hRPB11 (POLR2J) and three subunits (a, i, and m) of the translation initiation factor hEIF3. Related to human hRPB11 α subunits of bacterial RNA polymerase or Rpb11 subunit of yeast RNA polymerase II play a structural role in assembly of transcription enzymes and do not dissociate from them in every round of transcription. Thus, it is possible that in humans at least several subunits of eIF3 are recruited to mRNA during transcription, though it is still generally assumed that in eukaryotic cells transcription and translation are spatially separated. Such cross talk between transcription and translation may be necessary for mRNAs quality control, which ensures that only mRNAs that have been properly terminated and processed are translated. At the same time, it is also possible that subunits of eIF3 possess functions additional to translation. For example, translation factor eIF4E is involved in the mRNA transport from cell nucleus to the cytoplasm [24, 25]. Several subunits of eIF3 have been found to have nuclear localization, which is in agreement with their putative role in the nucleus. Indeed, eIF3a fragments produced by the action of specific cleavage factors or by limited hEIF3 proteolysis have been found in the nucleus. It has been suggested that these eIF3a fragments can possibly participate in regulation of gene expression [26]. CDK11 and eIF3f are localized in the nucleus also [21]. Remarkably, the novel isoform of human eIF3m, eIF3mβ, as we show in this study, easily penetrates into the nucleus, in contrast to previously described eIF3mα (GA17) isoform that has solely cytoplasmic localization. Further experiments will show whether eIF3mβ isoform is present in at least some hEIF3 complexes.

It has been established that in fission yeast *Schizosaccharomyces pombe* there are two distinct specialized eIF3 complexes that share six common core subunits (eIF3a, b, c, f, g, i) but are distinguishing one from the other by the presence of eIF3e and eIF3d (in one complex) or eIF3m and eIF3h (in the other complex) subunits [27]. These complexes associate with different sets of mRNAs. Interaction between eIF3mβ and eIF3a subunits, which we have established during this work, may indicate the existence of nuclear subcomplexes of hEIF3 factor. Possibly, such subcomplexes are involved in "protection" and transport of some specialized sets of mRNAs synthesized by RNA polymerase II containing minor hRPB11cα and/or hRPB11bα isoforms instead of the predominantly one hRPB11a subunit.

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