Original Article

DOI: 10.5582/ddt.2014.01044

Human RNA polymerase II-associated protein 2 (RPAP2) interacts directly with the RNA polymerase II subunit Rpb6 and participates in pre-mRNA 3'-end formation

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Summary **The C-terminal domain (CTD) of the largest subunit of RNA polymerase II (Pol II) is composed of tandem repeats of the heptapeptide Tyr1-Ser2-Pro3-Thr4-Ser5-Pro6-Ser7. The CTD of Pol II undergoes reversible phosphorylation during the transcription cycle, mainly at Ser2, Ser5, and Ser7. Dynamic changes in the phosphorylation patterns of the CTD are responsible for stage-specific recruitment of various factors involved in RNA processing, histone modification, and transcription elongation/termination. Human RNA polymerase II-associated protein 2 (RPAP2) was originally identified as a Pol II-associated protein and was subsequently shown to function as a novel Ser5-specific CTD phosphatase. Although a recent study suggested that RPAP2 is required for the efficient expression of small nuclear RNA genes, the role of RPAP2 in controlling the expression of protein-coding genes is unknown. Here, we demonstrate that the C-terminal region of RPAP2 interacts directly with the Pol II subunit Rpb6. Chromatin immunoprecipitation analyses of the *MYC* and *GAPDH* protein-coding genes revealed that RPAP2 occupied the coding and 3' regions. Notably, siRNA-mediated knockdown of RPAP2 caused defects in 3'-end formation of the *MYC* and *GAPDH* pre-mRNAs. These results suggest that RPAP2 controls Pol II activity through a direct interaction with Rpb6 and participates in pre-mRNA 3'-end formation.**

Keywords: RNA polymerase II-associated protein 2, pre-mRNA 3'-end formation, transcription cycle, C-terminal domain phosphatase, gene regulation

1. Introduction

Eukaryotic RNA polymerase II (Pol II) is a multi-subunit enzyme responsible for the transcription of protein-coding mRNAs and a variety of non-coding RNAs. Pol II activity is regulated by phosphorylation of the C-terminal domain (CTD) of its largest subunit (Rpb1), which is composed of multiple repeats of the evolutionarily conserved heptapeptide sequence Tyr1-Ser2-Pro3-Thr4-Ser5-Pro6-Ser7 (*1*). The repeat number varies between species, ranging from 26 in yeast to 52 in vertebrates (*1*). The CTD undergoes reversible phosphorylation during the transcription cycle, predominantly at Ser2, Ser5, and Ser7 of the repeats (*2*). Multiple kinases and phosphatases act on the CTD in a transcription stage-specific manner, thereby generating different CTD phosphorylation patterns along transcribed genes (*2*). The dynamically phosphorylated CTD temporally couples transcription with other nuclear processes by serving as a scaffold for the recruitment of various proteins involved in transcription, chromatin modification, and RNA processing (*2,3*).

Pol II consists of 12 subunits (Rpb1–12); 10 subunits form a catalytic core and the remaining subunits (Rpb4 and Rpb7) form a peripheral subcomplex. Recent proteomics studies identified Pol II assembly intermediates containing subsets of the subunits and suggested that the Pol II complex must be fully assembled in the cytoplasm before entering the nucleus (*4*). The cytoplasmic assembly and nuclear import of the Pol II complex require a number of novel factors,

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including heat shock protein 90 and its co-chaperone R2TP/Prefoldin-like complex (*4*), Pol II-associated small GTPase family members (GPN1–3) (*5-7*), and other Pol II-associated proteins (RNA Pol II-associated protein 1 (RPAP1), RPAP2, and GrinL1A) (*7,8*).

Human RPAP2 was originally identified as a major Pol II-associated protein by affinity purification and mass spectrometry (*8*). Forget *et al*. (*5*) demonstrated that RPAP2 is located mainly in the cytoplasm and shuttles between the cytoplasm and the nucleus, and that RPAP2 binds specifically to the small GTPase GPN1, which functions in Pol II nuclear import. In addition, knockdown of RPAP2 causes the cytoplasmic accumulation of Pol II, suggesting that RPAP2 participates in Pol II nuclear import (*5*). Human RPAP2 contains zinc finger-like motifs and exhibits a phosphatase activity that is specific for phosphorylated Ser5 (Ser5P) in the Pol II CTD (*9,10*). A recent study using chromatin immunoprecipitation (ChIP) analyses demonstrated that RPAP2 is recruited to snRNA genes through specific recognition of Ser7P in the Pol II CTD (*10*). Knockdown of RPAP2 causes defective snRNA expression and a concomitant increase in the level of Ser5-phosphorylated Pol II on the gene encoding U2 snRNA, suggesting that human RPAP2 participates in snRNA gene expression by dephosphorylating Ser5P of Pol II (*10*). In the ChIP study, exogenously expressed RPAP2 was recruited to the promoter and 5' region of protein-coding genes; however, Ser7 was not required for this recruitment, suggesting the involvement of another molecular interaction between Pol II and RPAP2 (*10*). Although these findings suggest that RPAP2 participates in snRNA gene expression, the role of RPAP2 in controlling the expression of protein-coding genes is unknown. Furthermore, the distribution of endogenous RPAP2 on human genes has not yet been examined.

Here, biochemical and molecular biological analyses revealed that the C-terminus of human RPAP2 interacted with the Pol II subunit Rpb6 *in vitro*. ChIP analyses showed that endogenous RPAP2 occupied the coding and 3' regions of two Pol II-transcribed genes (*MYC* and *GAPDH*), and knockdown of RPAP2 caused defects in 3'-end formation of these pre-mRNAs. These results suggest that RPAP2 participates both in Pol II assembly (by directly interacting with Rpb6) and in pre-mRNA 3'-end formation.

2. Materials and Methods

2.1. *Cell culture and transfection*

HEK 293T and HeLa-S3 cells were maintained in Dulbecco's Modified Eagle's Medium (Nissui Pharmaceutical) supplemented with 10% fetal bovine serum (HEK293T) or 5% bovine calf serum (HeLa-S3), 0.7 μg/mL penicillin, 1.5 μg/mL streptomycin, and 2 mM *L*-glutamine. The cells were cultured at 37°C in a humidified incubator containing 5% CO_2. Transfection of siRNAs into HeLa cells was performed as described previously (*11*).

2.2. *Plasmid constructs*

The full-length open reading frame (ORF) of human *RPAP2* (NCBI accession no. NM_024813.2) was amplified from HEK293T total RNAs by RT-PCR. The N-terminal (amino acids 1-175) and C-terminal (amino acids 153–612) region of *RPAP2* were amplified by PCR using the full-length cDNA as a template. All of the cDNAs cloned into plasmids were verified by DNA sequencing. To generate the pGEX-RPAP2, pCold II-RPAP2, and pcDNA3-FLAG-RPAP2 expression vectors, the *RPAP2* cDNAs were inserted into the *Eco*RI and *Bam*HI sites of the pGEX 6P-1 (GE Healthcare), pCold II (Takara), and pcDNA3-FLAG (Invitrogen) vectors, respectively. The pGEX-2TL(+) vectors containing the human Pol II subunit cDNAs (*RPB3–12*) were a gift from Dr Koji Hisatake (University of Tsukuba). To construct the His (6H)-tagged expression vectors, the *RPB6* and *RPB12* cDNAs were inserted into the *Nde*I and *Bam*HI sites of the 6H-pET11d vector. The primers used for cDNA cloning are available upon request.

2.3. *Western blotting and antibodies*

Preparation of affinity-purified anti-RPAP2 antibodies and western blotting were performed as described previously (*11*). The following antibodies were used for western blotting: anti-penta His (Qiagen), anti-FLAG (M2; Sigma), anti-Pol II (ARNA-3; Progen), anti-beta-actin (Sigma), and normal rabbit IgG (Millipore). An anti-Pol II antibody (N20; Santa Cruz Biotechnology) was used for ChIP analysis.

2.4. *Quantitative RT-PCR (RT-qPCR)*

Total RNAs were isolated from siRNA-treated HeLa-S3 cells using Isogen II reagent (Nippon Gene). The first-strand cDNAs were synthesized from total RNA using the PrimeScript™ 1st strand cDNA Synthesis Kit (TaKaRa) and random hexamer primers. The cDNA was quantified using SYBR Premix Ex Taq™ II (TaKaRa) and the Mx3000P real-time PCR system (Stratagene). The primers used for RT-qPCR analyses are available upon request.

2.5. *Protein expression and purification*

Cold-induced expression of 6H-tagged human RPAP2 in *Escherichia coli* was performed according to the instructions of the manufacturer of the pCold II vector (TaKaRa). Purification of His-tagged recombinant

proteins was performed using Ni-NTA agarose beads (Qiagen), according to the manufacturer's instructions. All GST-fused recombinant proteins were expressed in *E. coli* and purified as described previously (*12*). Protein concentrations were determined by the Bradford method using bovine serum albumin as a standard.

2.6. *GST pull-down assay*

GST pull-down assays were performed as described previously (*11*).

2.7. *ChIP assay*

ChIP assays were performed as described previously (*13*). The sequences of the primers used for ChIP assays are available upon request.

3. Results

3.1. *Human RPAP2 binds directly to Rpb6 in vitro*

Although one group reported that RPAP2 binds directly to Ser7-phosphorylated Pol II (*10*), another reported that the CTD of Pol II is not essential for the direct interaction between RPAP2 and the purified Pol II complex (*5*). Hence, it is unclear which subunit of Pol II is the direct binding partner of RPAP2. To address this issue, we performed GST pull-down assays using purified recombinant Pol II subunits (Rpb3-12). The purities of the recombinant proteins used in this study are shown in Supplemental Figures S1 and S2 (*http://www.ddtjournal.com/docindex.php?year=2014&kanno=6*). In the first experiment, GST-fused Pol II subunits were expressed in bacteria, purified, incubated with 6H-RPAP2, and then pulled down using glutathione-Sepharose. 6H-RPAP2 bound strongly to GST-Rpb6 and GST-Rpb12 (Figure 1A, lanes 6 and 12) and weakly to GST-Rpb5 and GST-Rpb10 (Figure 1A, lanes 5 and 10). Notably, all of these RPAP2-interacting proteins are common subunits of all three classes of RNA polymerases. Next, the 6H and GST tags were exchanged and the GST pull-down assays were repeated. Although 6H-Rpb6 interacted with GST-RPAP2, 6H-Rpb12 scarcely bound to GST-RPAP2 (Figure 1B). It is possible that the *N*-terminal GST-tag masked or hindered a region of RPAP2 required for its interaction with 6H-Rpb12, but not 6H-Rpb6.

3.2. *The C-terminal region of RPAP2 is required for efficient binding to Rpb6*

Human RPAP2 comprises 612 amino acids and contains a zinc finger-like motif at its N-terminal region, but does not possess any other specific protein motifs (Figure 1C) (*10*). To determine which region of RPAP2 is involved in direct binding to Rpb6, we prepared two deletion mutant proteins; the first mutant comprised the N-terminal 175 amino acids (N) and the second comprised the C-terminal 460 amino acids (C) of RPAP2 (Figure 1C). Binding assays using GST-tagged full-length wild type (WT) and mutant RPAP2 proteins demonstrated that both full-length RPAP2 and the C-terminus of the protein bound to GST-Rpb6, whereas the N-terminus did not (Figure 1D). Notably, binding of the C-terminus of RPAP2 to Rpb6 was as efficient as that of the WT protein, suggesting that

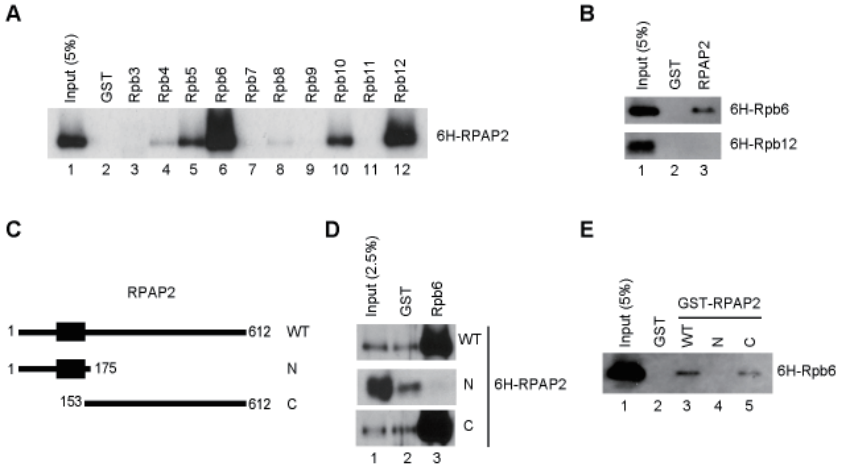

Figure 1. The C-terminus of RPAP2 binds directly to Rpb6. GST pull-down analyses of the interactions between RPAP2 and Pol II subunits (Rpb3–12). **(A)** The indicated GST-tagged Pol II subunits were incubated with 6H-RPAP2 proteins (full-length or deletion mutants) and then pulled down using glutathione-Sepharose beads. Western blotting was performed with an anti-RPAP2 antibody. **(B)** GST-tagged RPAP2 (full-length or deletion mutants) were incubated with 6H-tagged Rpb6 or Rpb12 and then pulled down using glutathione-Sepharose beads. Western blotting was performed with an anti-5His antibody. **(C)** Schematic illustration of the wild type (WT, amino acids 1–612) and deletion mutants (N, amino acids 1–175 amino acids; and C, amino acids 153–612) of RPAP2. **(D)** GST-Rpb6 was incubated with the 6H-RPAP2 proteins shown in **(C)** and then pulled down using glutathione-Sepharose beads. Western blotting was performed with an anti-RPAP2 antibody. **(E)** The GST-RPAP2 proteins shown in **(C)** were incubated with 6H-Rpb6 and then pulled down using glutathione-Sepharose beads. Western blotting was performed with an anti-5His antibody.

the C-terminus is sufficient for the interaction. Next, the 6H and GST tags were exchanged and the GST pull-down assays were repeated. Consistent with the previous result, GST-tagged full-length RPAP2 and the C-terminus mutant bound to 6H-Rpb6, but the N-terminus mutant did not (Figure 1E). However, in the second assay, the binding efficiency of the C-terminus of RPAP2 for 6H-Rpb6 was slightly lower than that of the WT protein. Taken together, these results suggest that the C-terminus of RPAP2 binds directly to the Pol II subunit Rpb6 *in vitro*.

3.3. *Characterization of a polyclonal antibody against human RPAP2*

Recently, Egloff *et al*. (*10*) used ChIP assays to analyze

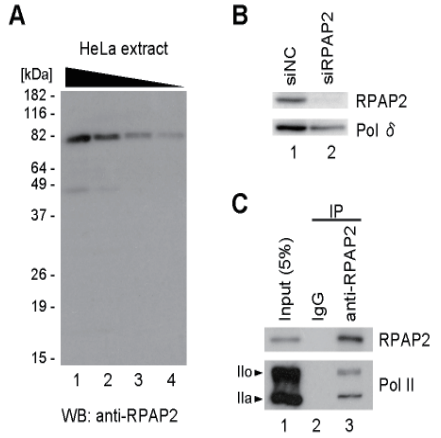

Figure 2. Characterization of a purified polyclonal antibody against human RPAP2. (A) Western blot analysis of HeLa cell total extracts using a purified polyclonal antibody against RPAP2. Lanes 1, 2, 3, and 4 contained 32, 16, 8, and 4 µg of total protein, respectively. **(B)** Western blot analyses of lysates from HeLa cells treated with a negative control siRNA (siNC) or a RPAP2-specific siRNA (siRPAP2) for 2.5 days. Western blotting was performed with purified anti-RPAP2 and anti-Pol δ (loading control) antibodies. **(C)** Immunoprecipitation of endogenous RPAP2 and Pol II using the purified anti-RPAP2 antibody. The antibody was immobilized on protein G beads and incubated with HEK-293T cell extracts. Immunoprecipitated proteins and the input extract (5%) were analyzed by western blotting with the anti-RPAP2 and anti-Pol II (ARNA-3) antibodies. IIo indicates hyperphosphorylated Rpb1 and IIa indicates hypophosphorylated Rpb1.

the distribution of exogenously expressed RPAP2 on several Pol II-transcribed genes in cultured human cells; however, the distribution of endogenous RPAP2 on these genes remains unclear. To examine its possible roles in controlling gene expression, we analyzed the distribution of endogenous RPAP2 on Pol II-transcribed genes using ChIP assays. A rabbit polyclonal antibody was raised against human RPAP2. Western blot analysis of HeLa whole cell lysates demonstrated that the affinity-purified antibody predominantly recognized a band of approximately 80 kDa, which corresponds to the predicted size of human RPAP2 (Figure 2A). Treatment of HeLa cells with a RPAP2-specific siRNA, but not a non-targeting control siRNA, depleted the 80 kDa antibody-reactive band, confirming that the purified antibody specifically recognized endogenous RPAP2 (Figure 2B). The affinity-purified antibody also selectively immunoprecipitated endogenous RPAP2 and Pol II (Figure 2C). Taken together, these findings demonstrate that the purified anti-RPAP2 antibody was specific and suitable for ChIP analyses.

3.4. *RPAP2 occupies the gene body and 3′ region of Pol II-transcribed genes*

To examine the distribution of RPAP2 on representative protein-coding genes (*MYC* and *GAPDH*), we performed ChIP assays using the purified anti-RPAP2 antibody and an anti-Pol II (N-20) antibody for comparison. The ChIP signals at several locations within each gene were quantified by real-time PCR (Figures 3A and 3B, upper panels). Consistent with previous reports, Pol II was located predominantly at the regions proximal to the *MYC* and *GAPDH* promoters (Figure 3) (*14*). On the other hand, RPAP2 was distributed throughout the gene body and 3' region of both genes, without an obvious peak near the promoter (Figure 3). Notably, RPAP2 was also located downstream of the 3'-end processing sites. An intense RPAP2 signal was identified upstream of the *GADPH* transcription start site (position -2056); this signal may have been caused by Pol II-mediated transcription of the 3' region of the *NCAPD2* gene, which is located adjacent to and in the same direction as the *GAPDH* gene.

RPAP2 is tightly associated with Pol II; hence, we assumed that the ratio of the RPAP2 ChIP signal to the Pol II ChIP signal represented the ratio of RPAP2-associated Pol II to total Pol II. The RPAP2/Pol II ratio increased towards the center of the gene bodies and peaked at the poly(A) signal sequence of *MYC* (Figure 3A) or an internal region of *GAPDH* (Figure 3B). These results suggest that RPAP2 is increasingly recruited to Pol II towards the 3'-end of at least some protein-coding genes.

3.5. *RPAP2 is required for efficient pre-mRNA 3'-end formation*

Because the ChIP results showed that RPAP2 occupies the 3' region of protein-coding genes, we hypothesized that it plays a role not only in the early stage of transcription, but also the later stages of this process, including elongation and termination, as well as transcription-associated processes such as 3'-end processing of pre-mRNAs. To address this possibility, we examined the effect of siRNA-mediated knockdown of RPAP2 on pre-mRNA 3'-end formation. Knockdown of RPAP2 by two independent siRNAs was verified by western blotting (Figure 4A) and RT-qPCR (Figure 4B) analyses. To quantitatively evaluate the efficiency of 3'-end formation, the ratio of unprocessed to total (precursor plus mature) *MYC* and *GAPDH*

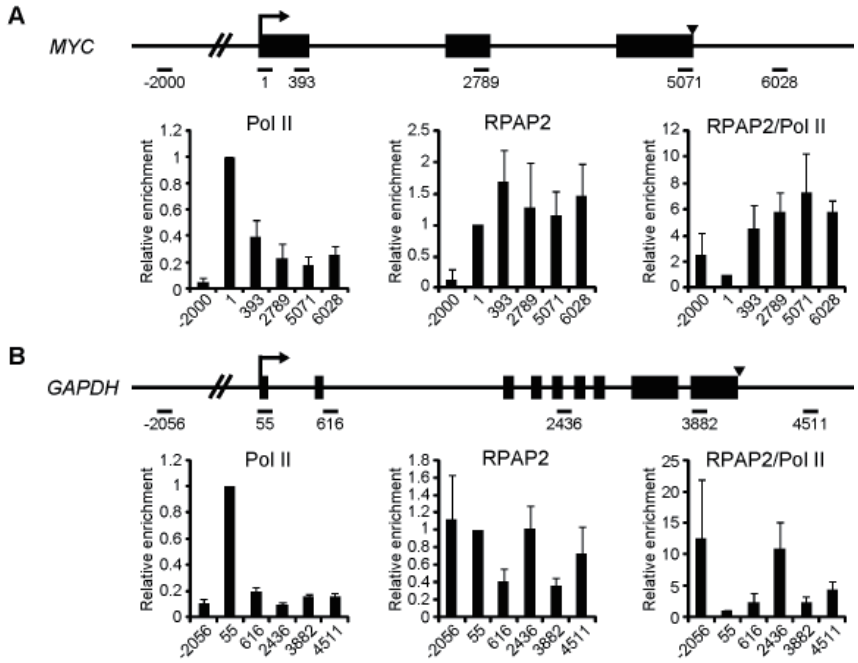

Figure 3. ChIP analyses of the distribution of human RPAP2 on Pol II-transcribed genes. ChIP analyses of the *MYC* **(A)** and *GAPDH* **(B)** genes. The upper panels show schematic illustrations of the genes: the transcription start sites are indicated by arrows, the exons are shown as black boxes, and the polyadenylation signals are indicated by arrowheads. The positions of the primer pairs used for RT-qPCR (relative to the the transcription start site) are shown below each diagram. The lower panels show the results of ChIP analyses of the indicated regions of the *MYC* and *GAPDH* genes in HEK 293T cells using antibodies against Pol II (N20) or RPAP2. The ratios of the RPAP2 signals to the Pol II signals (RPAP2/Pol II) are also shown. The data were normalized to the region at which the Pol II signal was strongest. Data are expressed as the mean ± standard deviation of four (*MYC*) or three (*GAPDH*) independent experiments.

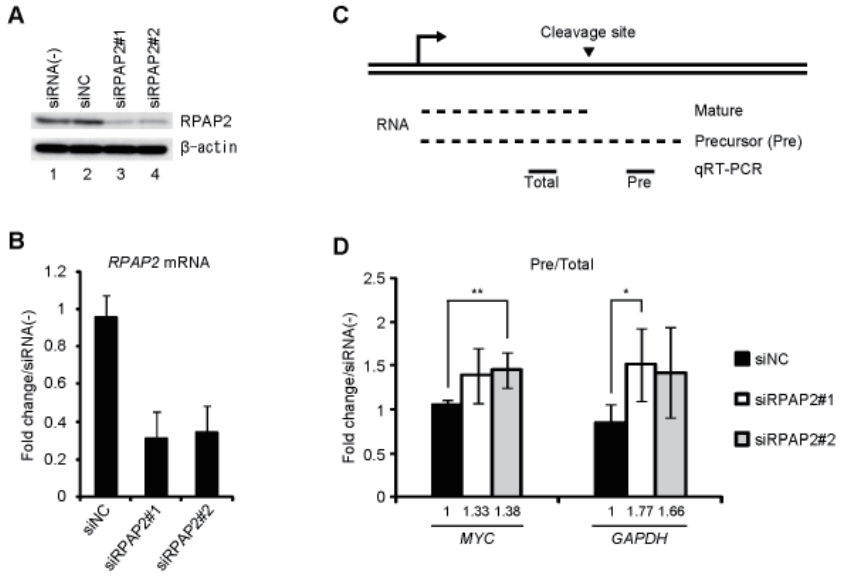

Figure 4. RPAP2 is required for efficient 3'-end formation of the *MYC* and *GAPDH* pre-mRNAs. HeLa cells were transfected with a negative control siRNA (siNC) or with one of two independent RPAP2-specific siRNAs (#1, #2). **(A)** Western blot analyses of total proteins extracted from the untreated (siRNA(-)) or siRNA-treated cells using antibodies against RPAP2 or β-actin (loading control). **(B)** RT-qPCR analyses of the efficiency of knockdown of *RPAP2* mRNA by the specific siRNAs. *RPAP2* expression levels were normalized to those of 18S ribosomal RNA. Fold changes were determined relative to the level of RPAP in siRNA(-) cells. Data are expressed as the mean ± standard deviation of four independent experiments. **(C)** Schematic illustration of the evaluation of 3'-end formation of pre-mRNAs. DNA is depicted as a double line, the transcription start site is depicted as an arrow, and the cleavage site is denoted by an arrowhead. Transcribed mature and precursor (Pre) RNAs are shown as dashed lines. The short horizontal lines ("Total" and "Pre") indicate the RT-qPCR amplicons used to quantify the total or pre-mRNA transcript. **(D)** RT-qPCR analyses of the expression levels of total (precursor plus mature) and unprocessed (precursor) *MYC* and *GAPDH* RNAs in siRNA-treated HeLa cells. The ratios of unprocessed to total RNAs (Pre/Total) are shown. Relative expression levels were determined by normalizing the data to the corresponding expression levels in siRNA(-) cells. The numbers below the graphs indicate the –fold increase in the Pre/Total ratio of siRNA-treated cells compared with siNC-treated cells. Data are expressed as the mean ± standard deviation of four independent experiments. Statistical significance was determined using Student's *t*-test (* $p < 0.05$ and ** $p < 0.01$).

RNAs was determined using RT-qPCR analyses. To detect unprocessed RNAs, first-strand cDNAs were synthesized using random hexamer primers and the cDNAs were amplified by PCR using primer pairs located downstream of the processing sites (Figure 4C). Total RNA levels were detected by amplification of the coding regions. For the *MYC* and *GAPDH* genes, the ratios of unprocessed to total mRNAs in cells treated with the RPAP2-specific siRNAs were approximately 1.3-fold and 1.7-fold higher than those in cells treated with a control siRNA, respectively (Figure 4D). Together with the results of the ChIP analyses, these findings suggest that RPAP2 participates in 3'-end formation of at least some pre-mRNAs.

4. Discussion

This study demonstrates that (*i*) the C-terminus of human RPAP2 interacts directly with the Pol II subunit Rpb6 *in vitro*, (*ii*) RPAP2 occupies not only the promoter but also the coding and 3' regions of protein-coding genes, and (*iii*) knockdown of RPAP2 results in inefficient 3'-end formation of some pre-mRNAs.

The Pol II complex comprises a ten-subunit catalytic core and an Rbp4/Rbp7 subcomplex (*15*). X-ray analyses of the yeast core Pol II revealed that the two large subunits, Rpb1 and Rpb2, form opposite sides of the active center "cleft" and the Rpb1 side of the cleft forms a mobile "clamp" (*16*). Rpb6 is positioned at the base of the clamp, close to where the Rpb4/7 subcomplex "stalk" attaches, and where an Rpb1 "linker", which connects to the CTD, emerges from the core surface (*15-17*). Insertion of the Rpb4/7 stalk into the clamp region affects the clamp movement (*15,18*). Moreover, newly transcribed RNAs exit the Pol II core *via* the stalk, and the 5' ends of the RNAs contact Rpb7 (*19*). Therefore, the stalk region of Pol II is thought to function in every stage of the transcription cycle by regulating the clamp motion, as well as in efficient coupling of transcription with RNA processing (*19,20*). Rpb6 critically contributes to the association of the stalk with the core by interacting directly with Rpb7 (*21,22*); hence, we speculate that RPAP2 may regulate transcription through a direct interaction with Rpb6.

In contrast to our finding that the C-terminus of human RPAP2 interacts directly with Rpb6, a recent study by Forget *et al*. (*5*) demonstrated that the N-terminus, but not the C-terminus, of human RPAP2 is required for stable binding to the Pol II complex. Because we did not examine the RPAP2-binding abilities of the two larger subunits of Pol II (Rpb1 or Rpb2), it is possible that the N-terminus of RPAP2 binds stably to the Pol II complex by directly interacting with these subunits. The discrepancy between our results and those of Forget *et al*. might be also explained by other possibilities. For example, the C-terminus of RPAP2 may bind to free Rpb6, but not Rpb6, within the Pol II complex. Alternatively, the interaction between Rpb6 and the C-terminus of RPAP2 may be transient and may depend on a specific change in the conformation of the Pol II complex. We speculate that a structural change in the Pol II complex may occur at a specific stage of transcription, such as pausing or termination.

At present, the molecular basis of the defective pre-mRNA 3'-end formation caused by knockdown of RPAP2 is unclear. Because there are no reports that RPAP2 interacts directly with 3'-end processing factors, it is possible that RPAP2 indirectly participates in pre-mRNA 3'-end formation. Like its yeast counterpart Rtr1, RPAP2 may function as a phosphatase that is specific for Ser5P in the CTD of Pol II (*9,10,23*). Recently, our group demonstrated that Ssu72, another Ser5P-specific phosphatase, is required for efficient pre-mRNA 3'-end formation of protein-coding genes in vertebrate cells (*13*). Based on these observations, we speculate that RPAP2 may regulate pre-mRNA 3'-end formation by controlling the phosphorylation status of the CTD of Pol II because these processes are intimately linked (*3*). Notably, a recent study demonstrated that Rtr1 also exhibits phosphatase activity that is specific for Tyr1P in the CTD of Pol II (*24*). Removing the Tyr1P marker from the CTD of Pol II is a prerequisite for the efficient recruitment of 3'-end formation factors to the elongating Pol II complex (*25*); therefore, RPAP2 may also affect the efficiency of pre-mRNA 3'-end formation by dephosphorylating Tyr1P.

The distributions of RPAP2 on protein-coding genes identified by ChIP analyses (Figure 3) resemble those of Ser2-phosphorylated Pol II (*14*). A recent study by Ni *et al*. (*9*) demonstrated that the Pol II-interacting proteins, RPRD1A and RPRD1B/CREPT, both of which contain CTD-interaction domains, interact directly with RPAP2 and serve as scaffolds that coordinate the dephosphorylation of Ser5P. Ni *et al*. (*9*) also showed that RPRD1A and RPRD1B interact directly with the Ser2P-containing CTD of Pol II and occupy protein-coding genes from the promoter to the 3'-end. Notably, Lu *et al*. (*26*) demonstrated that RPRD1B/CREPT accelerates tumorigenesis by regulating transcription termination to control the expression of cell-cycle-related genes. Together with the results presented here, these previous findings suggest that RPAP2 participates in transcription elongation and/or termination processes by interacting with Rpb6, or by acting on Ser5P of the CTD of Pol II in collaboration with RPRD1A/B. We are now using ChIP analyses and run-on assays in RPAP2-silenced cells to analyze the Pol II occupancy downstream of the 3'-end processing sites to determine whether RPAP2 participates in transcription termination.

Acknowledgements

We thank Dr. Koji Hisatake (University of Tsukuba) for providing plasmids. We also thank all of our

lab members for helpful discussion. This work was supported by Grants-in-Aid for Scientific Research on Innovative Areas (Y.O.; MEXT KAKENHI grant numbers 24118003 and 25131704) from the Ministry of Education, Science.

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(Received November 14, 2014; Accepted November 17, 2014)