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題目(和文)	RNAポリメラーゼIIの転写伸長段階を制御する新規ヒトタンパク質 Rtf1に関する研究
Title(English)	Study on Rtf1, an uncharacterized human protein that controls the elongation phase of RNA polymerase II transcription
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論 文 要 旨 (英 文)

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報告番号	乙 第	号	氏 名	Cao Qing-Fu (曹 青福)
(要 旨) This thesis is composed of 6 chapters. In chapter 1, general knowledge about transcription and transcription factors related to this study are introduced. Rtf1 was first identified as a suppressor of a TBP mutant in <i>Saccharomyces cerevisiae</i>. Rtf1 is an evolutionarily conserved protein that is generally considered as a component of the Paf1 complex (PAF1C) and plays a role in the regulation of histone modifications, including H2B monoubiquitination (H2Bub), in <i>S. cerevisiae</i>. Indeed, however, stable physical interaction between Rtf1 and PAF1C was only detected in <i>S. cerevisiae</i> and has not been observed in other species. Further, in humans, Rtf1 does not seem to be required for PAF1C-mediated transcription regulation. The purpose of my thesis is to understand (i) whether human Rtf1 is involved in the regulation of transcription and, if so, (ii) how human Rtf1 regulates transcription. In chapter 2, to examine whether human Rtf1 is involved in transcription regulation, experiments were performed from three aspects. First, subcellular localization of human Rtf1 in HeLa cells was examined. The result of immunofluorescence staining showed that human Rtf1 is a nuclear protein. Second, to examine whether human Rtf1 is a transcription factor, in vitro transcription assays were performed using Rtf1-depleted HeLa cell nuclear extract (NE) and naked DNA templates. Strikingly, Rtf1-depleted NE exhibited severe transcription defects compared to IgG-depleted control NE, and the defects were almost fully restored by addition of the recombinant Rtf1 protein. These results showed that human Rtf1 is required for transcription in vitro. However, it was not clear which step of transcription is controlled by human Rtf1. Third, to address this question, primer extension assays were performed. Depletion of Rtf1 from NE only decreased the transcripts longer than 98 nt. These findings clearly showed that human Rtf1 is a transcription elongation factor. In chapter 3, to understand how human Rtf1 promotes transcription elongation, various biochemical approaches were taken. First, P1.0, a phosphocellulose fraction obtained from NE, was used in in vitro transcription assays. P1.0 lacks essential transcription elongation factors and is unable to direct the synthesis of long transcripts. Addition of recombinant human Rtf1 to P1.0 did not restore the transcription defects of P1.0, but the simultaneous addition of recombinant Rtf1 and P0.3, another NE-derived fraction, to P1.0 restored the transcription defects, suggesting that an additional “Rtf1 coactivator” is required to promote transcription elongation. Next, to purify and identify the Rtf1 coactivator, several purification methods were tested, such as chromatography, glycerol gradient sedimentation analysis, immunoprecipitation, and immunodepletion. The results showed that human Rtf1-mediated transcription is independent of PAF1C, DSIF, and Tat-SF1. In chapter 4, to understand the structure-function relationship of human Rtf1, mutational analysis was performed. The level of H2Bub was decreased by the knockdown of Rtf1 and rescued by the overexpression of full length Rtf1 in HeLa cells. Several deletion mutants of human Rtf1 were prepared and tested in rescue experiments. The results showed that an N-terminal region, including the histone modification domain (HMD) identified in yeast, is required for H2Bub in humans. Next, mutant proteins were examined in in vitro transcription assays. The results demonstrated that the HMD is dispensable for transcription, whereas the so-called Plus3 domain and the C terminal region are critical for transcription. Weak physical interaction between human Rtf1 and PAF1C was also tested using deletion mutants. Binding assays showed that the C-terminal region of Rtf1 is important for its binding to PAF1C. Taken together, it was found that human Rtf1 requires distinct structural domains for H2Bub and transcriptional activation. In chapter 5, to investigate the role of human Rtf1 and PAF1C in transcription regulation in living cells, RNA-seq and chromatin immunoprecipitation were performed. Given the above findings in vitro, comparative analysis of Rtf1 and PAF1C knockdown phenotypes was performed following knockdowns of Rtf1, Paf1, and Ski8, the latter two being components of human PAF1C. There were significant overlaps among the genes affected by Rtf1, Paf1, and Ski8 knockdowns. At the same time, more than 50% of the genes affected by Rtf1 knockdown were not affected by Paf1 or Ski8 knockdown, indicating that expression of certain genes is more sensitive to the inhibition of Rtf1 than that of PAF1C. Moreover, the results of ChIP assays showed that human Paf1 is recruited to some target genes independently of Rtf1. Collectively, it was found that human Rtf1 and PAF1C have both similarities and differences in the regulation of gene expression in cells. In chapter 6, based on the results obtained from chapters 2 to 5, I discussed the functions of human Rtf1 from three aspects: (i) PAF1C-dependent and -independent functions of human Rtf1; (ii) PAF1C-controlled, Rtf1-controlled, and H2Bub-controlled genes; and (iii) functional similarities and differences of Rtf1 proteins among species. Identification of the Rtf1 coactivator will be a key step toward the distinction of PAF1C-dependent and -independent mechanisms of action of Rtf1.				