The molecular mechanism that coordinates transcription and pre-mRNA processing

Y. Hirose, H. Fan, K. Sakuraba, I. Yunokuchi, and F. Harada

Each step of pre-mRNA processing is intimately linked to transcription by RNA polymerase II (RNAP II) mediated by the physical interaction between the carboxy-terminal domain (CTD) of the largest subunit of RNAP II and pre-mRNA processing factors. The CTD consists of multiple repeats of an evolutionary conserved heptapeptide with the consensus sequence Y-S-P-T-S-P-S. The CTD is subject to reversible phosphorylation during the transcription cycle. Phosphorylation of the CTD appears to function as an important regulatory switch for assembly and disassembly of macro-molecular complexes carrying out the synthesis and processing of pre-mRNA. To better understand the molecular mechanism that coordinates transcription and pre-mRNA process-ing, we have identified and characterized novel mammalian factors that can directly interact with the phosphorylated CTD (pCTD).

We screened a human cDNA expression library using ^{32}P-labeled CTD as a probe and identified several human WW domain-containing proteins as pCTD interacting factors. Among these, we have extensively characterized a novel human protein PCIF1 (Phosphorylated CTD Interacting Factor 1), which consists of 704 amino acids and contains a WW domain near the N-terminus. The PCIF1 WW domain directly and specifically bound to the pCTD. The binding affinity of the PCIF1 WW domain to CTD was significantly increased by phosphorylation of CTD. Co-immunoprecipitation data showed that PCIF1 bound to RNAP II with a hyperphosphorylated CTD (RNAP IIO) *in vivo*. Immunofluorescence confocal microscopy demonstrated that PCIF1 was co-localized with endogenous RNAP IIO in the nucleus. We also observed that the overexpression of PCIF1 in human cultured cells was able to repress the transactivation of luciferase reporter gene driven by various transcription activation domains and that the repression ability was dependent on its WW domain. The WW domain of the PCIF1 exhibits the considerable homology to the WW domain of human cis-trans peptidyl prolyl-isomerase Pin1, which has been shown to bind specifically to a phosphorylated Ser/Thr-Pro motif and the pCTD. In vitro data by GST pull-down experiments suggested that PCIF1 shared targets with Pin1, not only RNAP IIO but also other reported Pin1 targets.

Phosphorylation and dephosphorylation of the CTD on Ser 2 and Ser 5 position in the heptapeptide have been suggested to be dynamically and differentially regulated during transcription cycle. We, therefore, examined binding affinities of several WW domains to the differentially phosphorylated CTD peptide. Remarkably, PCIF1 WW domain could preferentially bind to a CTD peptide phosphorylated at Ser 5 compared to a CTD peptide phosphorylated at Ser 2 whereas Pin1 WW exhibited similar affinity to both CTD peptides. We speculate that PCIF1 may play a role in early stage of transcription cycle or in coupling transcription to pre-mRNA processing through the association with RNAP IIO phosphorylated at Ser 5. We are currently investigating cellular functions of PCIF1 by targeted gene disruption in the chicken B-cell line DT40.