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Human phosphorylated CTD-interacting protein, PCIF1, negatively modulates gene expression by RNA polymerase II

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Abstract

Phosphorylation of the C-terminal domain (CTD) of the largest subunit of RNA polymerase II (Pol II) regulates transcription cycle and coordinates recruitment of RNA processing factors and chromatin regulators. Recently, we reported the identification of human PCIF1 as a novel protein that directly binds to the phosphorylated CTD via its WW domain, which is highly homologous to the WW domain of human peptidylprolyl isomerase Pin1. Although PCIF1 has been shown to associate with phosphorylated Pol II, functional consequence of the interaction remains unclear. Here we further characterized the cytological, structural, and functional properties of human PCIF1. Immunofluorescence microscopy revealed that endogenous PCIF1 was colocalized with the phosphorylated Pol II and the transcription elongation factor DSIF in the cell nucleus. We also found that PCIF1 WW domain inhibits the CTD phosphatase activity of SCP1 *in vitro*. By examining the effect of either PCIF1 overexpression or knockdown on the transactivation of reporter gene expression by various transcriptional activation domains, we found that PCIF1 significantly repressed the transactivation depend on its CTD binding ability. These data suggest that PCIF1 modulates phosphorylation status of the CTD and negatively regulates gene expression by Pol II.

Keywords: Transcription; WW domain; Phosphorylation; CTD; RNA polymerase II; PCIF1

The C-terminal domain (CTD) of the largest subunit of RNA polymerase II (Pol II) is composed of 26–52 tandem repeats of the evolutionary conserved consensus heptapeptide Tyr[1]-Ser[2]-Pro[3]-Thr[4]-Ser[5]-Pro[6]-Ser[7] [1]. The CTD is essential for cell viability and plays a central role in coordinating various nuclear processes required for proper gene expression by Pol II [2–4]. The CTD undergoes reversible phosphorylation during the transcription cycle predominantly at Ser2 and Ser5, and the level and pattern of phosphorylation are determined by the concerted action of various CTD kinases and phosphatases [5,6]. Prior to transcription initiation, Pol II with a hypophosphorylated CTD (Pol IIA) preferentially enters promoters and the Mediator complex is thought to associate with Pol IIA, at least in part, via the CTD. Upon initiation, phosphorylation at Ser5 occurs by TFIIH and promotes the recruitment of capping enzyme to the early transcription complex. As transcription proceeds from 5′ to 3′ direction, phosphorylation at Ser2 by P-TEFb is increased and required for efficient recruitment of 3′ end processing factors and the histone methyltransferase Set2 to the elongating hyperphosphorylated Pol II (Pol IIO). Thus, the CTD acts as a phosphorylation-regulated loading platform for various proteins involved in transcription regulation, chromatin modification, and RNA processing [3,4,6,7]. To better understand the molecular mechanisms by which the phosphorylated CTD coordinates nuclear processes required for proper gene expression, it is important to iden-

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tify and characterize factors that regulate phosphorylation status and structural change of the CTD.

We recently identified a novel human factor that can directly interact with the phosphorylated CTD, which we designated PCIF1 (Phosphorylated CTD Interacting Factor 1) [8]. PCIF1 is ubiquitously expressed in most human tissues and contains a single WW domain at the N-terminal region. PCIF1 WW domain (PCIF1-WW hereafter) is responsible for direct and specific binding to the phosphorylated CTD and exhibits the considerable homology to the WW domain of human peptidylprolyl *cis*/*trans* isomerase (PPIase) Pin1 (Pin1-WW hereafter) [8]. Pin1-WW is classified into group IV WW domain which specifically binds to the phosphorylated Ser/Thr-Pro (pS/pT-P) motif found in many phosphorylated proteins [9]. Pin1 has recently been shown to modulate phosphorylation level of the CTD and negatively regulate Pol II activity in both transcription cycle and the cell cycle [10,11]. Although PCIF1 has been shown to associate with Pol IIO *in vivo*, the functional consequences of their interaction remain unclear.

In the present study, we further characterized the cytological, structural, and functional properties of PCIF1 in regulation of both phosphorylation status and transcriptional activity of Pol II. Immunofluorescence microscopy revealed that endogenous PCIF1 was colocalized with Pol IIO and the transcription elongation factor DSIF in the cell nucleus. We found that PCIF1-WW strongly inhibited the *in vitro* CTD phosphatase activity of SCP1, which has been recently shown to preferentially dephosphorylate Ser5 within the CTD repeat [12]. We tested the effect of PCIF1 overexpression on the transactivation of a luciferase reporter gene expression by the transcriptional activation domain (TAD) of VP16 and observed that PCIF1 overexpression strongly repressed the transactivation, depending on its binding ability to the phosphorylated CTD. To confirm the inhibitory activity of PCIF1 on the transcriptional activation processes, we further tested the effect of siRNA-mediated knockdown of PCIF1 on the transactivation by various TADs, including VP16, CHOP, and Elk1. We found that depletion of endogenous PCIF1 resulted in marked augmentation of the transactivation activities of all three TADs examined. These results support the idea that PCIF1 negatively modulates Pol II activity.

Materials and methods

The Materials and methods section appears in Supplementary data.

Results

PCIF1 WW domain exhibits phosphorylation-dependent CTD binding property and structural similarity to Pin1 WW domain

In the previous studies, preferential binding of PCIF1-WW to the phosphorylated CTD was demonstrated by the experiment using GST-CTD phosphorylated by HeLa cell nuclear extracts (NE) *in vitro* [8]. To exclude the possibility that other types of posttranslational modification of the CTD might had occurred in NE and affected the binding property, we first want to confirm the phospho-specific CTD binding ability of PCIF1-WW using the GST-CTD phosphorylated by the specific CTD kinase, P-TEFb [13]. As shown in Fig. 1B, a time-dependent increase of CTD phosphorylation was observed by incubation with the baculovirus-expressed recombinant P-TEFb (lanes 1–4). Phosphorylation was judged by both reduced mobilities of silver stained bands (upper) and increased signal intensity in immunoblot probed with the phospho-dependent CTD antibody H5 (middle). Phosphorylation of the CTD was inhibited in the presence of kinase inhibitor DRB [13] (lane 5). Blot overlay assay was then performed using ^{32}P-labeled GST-PCIF1-WW probe (lower). While equivalent amount of proteins were blotted, only the fully phosphorylated GST-CTDs gave prominent signals (compare lane 1 and lane 4). This result clearly demonstrated that PCIF1-WW preferentially binds to the phosphorylated CTD compared to the unphosphorylaed CTD.

PCIF1-WW exhibits the considerable homology to Pin1-WW, which has been shown to specifically bind to the pS/pT-P motif found in many phosphorylated proteins including Pol II [9](Fig. 1A). Previous structural and mutagenesis studies have revealed that the side chains of Ser16 and Arg17 in loop 1 of Pin1-WW are crucial for recognizing the phosphorylated CTD heptapeptide [14] (Fig. 1A, indicated by bold letter). Importantly, the same residues are found in the corresponding positions (Ser54 and Arg55) in PCIF1. To test whether these residues in PCIF1-WW are also critical for specific interaction with the phosphorylated CTD, we introduced a point mutation at position 54 by substituting Ser to His (S54H) and compared its binding affinity for the phosphorylated CTD with that of the wild-type WW domains. In the case of Pin1-WW, it was previously shown that the corresponding mutation (S16H) resulted in great reduction of binding affinity for the phosphorylated CTD [14]. We first performed a blot overlay assay in which equivalent amounts of either purified full-length wild-type or mutant PCIF1 were probed with ^{32}P-labeled phosphorylated GST-CTD. As shown in Fig. 1C, S54H mutation severely lowered the binding affinity for the phosphorylated CTD (compare lane 3 and lane 4). We also performed the GST pull-down assays from 293T total cell extracts using GST fused WW domain of wild-type as well as mutant PCIF1. Whereas wild-type GST-WW efficiently pulled-down the endogenous hyperphosphorylated Pol II largest subunit (IIo), S54H mutant pulled-down the much smaller amount of IIo from the equivalent amount cell extracts (Fig. 1D, lanes 2 and 3). Thus, Ser 54 in PCIF1-WW is critical for the efficient binding to the phosphorylated CTD. These results suggest that PCIF1-WW possesses the phosphorylation-dependent CTD binding property and structural similarity to Pin1 WW domain.