

CTIP2 is a negative regulator of P-TEFb

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The positive transcription elongation factor b (P-TEFb) is involved in physiological and pathological events including inflammation, cancer, AIDS, and cardiac hypertrophy. The balance between its active and inactive form is tightly controlled to ensure cellular integrity. We report that the transcriptional repressor CTIP2 is a major modulator of P-TEFb activity. CTIP2 copurifies and interacts with an inactive P-TEFb complex containing the 7SK snRNA and HEXIM1. CTIP2 associates directly with HEXIM1 and, via the loop 2 of the 7SK snRNA, with P-TEFb. In this nucleoprotein complex, CTIP2 significantly represses the Cdk9 kinase activity of P-TEFb. Accordingly, we show that CTIP2 inhibits large sets of P-TEFb- and 7SK snRNA-sensitive genes. In hearts of hypertrophic cardiomyopathic mice, CTIP2 controls P-TEFb-sensitive pathways involved in the establishment of this pathology. Overexpression of the β -myosin heavy chain protein contributes to the pathological cardiac wall thickening. The inactive P-TEFb complex associates with CTIP2 at the MYH7 gene promoter to repress its activity. Taken together, our results strongly suggest that CTIP2 controls P-TEFb function in physiological and pathological conditions.

Discovered in 1995 (1), P-TEFb (CyclinT1/Cdk9) is involved in physiological and pathological transcriptionally regulated events such as cell growth, differentiation, cancer, cardiac hypertrophy, and AIDS (for review, see refs. 2 and 3). It has been suggested to be required for transcription of most RNA polymerase II-dependent genes. However, a recent study suggests that a subset of cellular genes are distinctively sensitive to Cdk9 inhibition (4). P-TEFb is dynamically regulated by both positive and negative regulators. In contrast to Brd4, which is associated with the active form of P-TEFb (5, 6), the 7SK small nuclear RNA (7SK snRNA) and HEXIM1 inhibit Cdk9 activity in the inactive P-TEFb complex (7–10). P-TEFb elongation complexes are crucial for HIV-1 gene transactivation and viral replication. Recently, new P-TEFb complexes containing the HIV-1 Tat protein have been characterized (11, 12), providing evidence for the recruitment of an inactive Tat/P-TEFb complex to the HIV-1 promoter (13). However, defining the diverse nature and functions of the different P-TEFb complexes will require further investigations. The cellular protein CTIP2 (Bcl11b) has been highlighted as a key transcription factor for thymocyte (14, 15) and neuron development (16), odontogenesis (17), cancer evolution (18), and HIV-1 gene silencing (19). Besides AIDS, hypertrophic cardiomyopathy is a well-described P-TEFb-dependent pathology (for review, see refs. 20 and 21).

Here, we report that CTIP2 represses P-TEFb function as part of an inactive P-TEFb complex. In hearts of hypertrophic cardiomyopathic mice, CTIP2 controls P-TEFb-sensitive pathways involved in the establishment of this pathology. Together with

the inactive P-TEFb complex, CTIP2 associates with the β -myosin heavy chain promoter to repress its activity. Thereby, CTIP2 might contribute to the regulation of the size of heart sarcomeres in physiological or pathological conditions.

Results

CTIP2 Is Associated with the Inactive P-TEFb Complex. First, we investigated, whether or not CTIP2 associates with one of the previously described P-TEFb complexes. We performed immunoprecipitation experiments, revealing that CTIP2 coimmunoprecipitates with the CyclinT1 and Cdk9 components of the P-TEFb complex (Fig. 1A). To further define the CTIP2-containing complexes, we separated the previously described P-TEFb complexes by gel filtration chromatography (Fig. 1B). As shown in Fig. 1B, Cdk9 was detected in the low molecular weight (LMW) complex ("free" P-TEFb complex) and in the high molecular weight (HMW) complex that coeluted with HEXIM1 and the 7SK snRNA. Interestingly, we found CTIP2 in those latter fractions containing the HMW P-TEFb complex (Fig. 1B). These observations suggested that CTIP2 may be part of an inactive HEXIM1/7SK-including P-TEFb complex. To confirm this hypothesis, we performed additional coimmunoprecipitation experiments targeting the active and the inactive form of P-TEFb. We found that CTIP2 copurified with CyclinT1, Cdk9, HEXIM1, and 7SK but not with Brd4, making CTIP2 a previously undescribed component of the inactive P-TEFb complex. Confocal observations further confirmed the colocalization of CTIP2, P-TEFb, and HEXIM1 in the previously described CTIP2-induced nuclear structures (22) (Fig. S1). As suggested by the gel filtration elution profile, CTIP2 was not found in the active Brd4/P-TEFb complexes. The 7SK snRNA functions as a scaffold RNA facilitating the interaction between HEXIM1 and P-TEFb. To investigate how CTIP2 associates with the inactive P-TEFb complex, we performed coimmunoprecipitation experiments following RNase treatment

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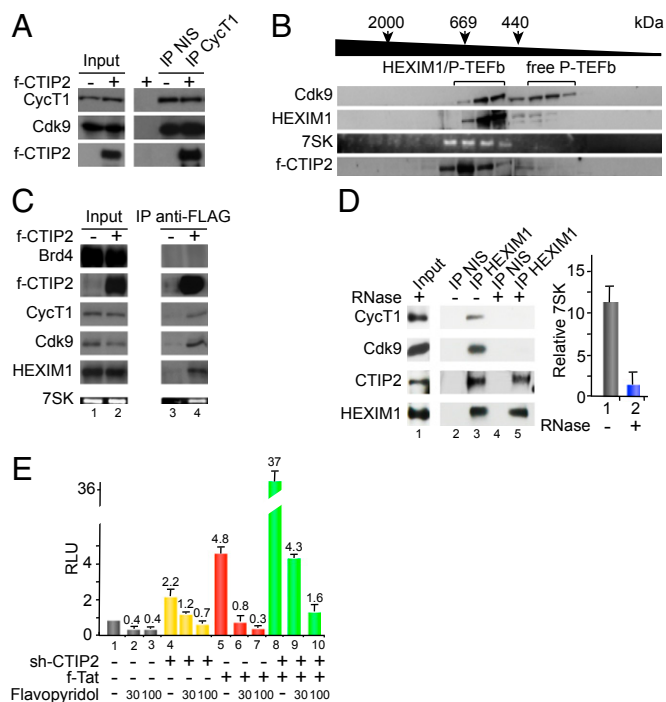


Fig. 1. CTIP2 is associated with the inactive P-TEFb complex. (A) HEK293T cells expressing FLAG-CTIP2 were lysed and immunoprecipitated with anti-CyclinT1 antibody or with nonimmune serum IgG (NIS). Input and immunoprecipitated proteins were probed with anti-FLAG (f-CTIP2), anti-CyclinT1, and anti-Cdk9 antibodies. (B) Protein complexes from microglial cell nuclear extracts were separated in 29 fractions by HPLC gel filtration. Each collected fraction was subjected to Western blot analysis with the indicated antibodies and processed for 7SK detection by RT-PCR. Before all separations, the column was calibrated with protein standards (dextran blue, 2,000 kDa, fraction 9; thyroglobulin, 669 kDa, fraction 14; ferritin, 440 kDa, fraction 17). (C) HEK293T cells expressing FLAG-CTIP2 were lysed and immunoprecipitated with anti-FLAG antibody. Input (lines 1 and 2) and immunoprecipitated proteins (lines 3 and 4) were probed with anti-FLAG, anti-Brd4, anti-CyclinT1, anti-Cdk9, and anti-HEXIM1 antibodies. The presence of the 7SK snRNA was assessed by RT-PCR amplification. (D) Cellular extracts were treated (+) or not (-) with RNase before being subjected to immunoprecipitation with anti-Hexim1 antibody or nonimmune serum (NIS) as indicated. Inputs (line 1) and immunoprecipitated proteins (lines 2, 3, 4, and 5) were probed with anti-CTIP2, anti-CyclinT1, anti-Cdk9, and anti-HEXIM1 antibodies. 7SK snRNA degradation was controlled by RT-PCR. (E) Cells were transfected with the LTR-LUC (HIV-1 promoter) epizomal reporter construct in the presence of Tat and/or sh-CTIP2 plasmids or the respective control constructs. Cells were treated with 30 or 100 nM flavopyridol 24 h posttransfection and subjected to luciferase assays 48 h posttransfection. Efficiencies of knockdown and overexpression were controlled by Western blot as in Fig. 2 F and G. Results are representative for at least three independent experiments performed in triplicates.

to degrade the 7SK snRNA (Fig. 1D). As previously described, degradation of the 7SK snRNA (Fig. 1D, Right) induced the dissociation of P-TEFb and HEXIM1 (Fig. 1D, Left, column 5 vs. 3). However, CTIP2 remained associated with HEXIM1 upon RNase treatment, suggesting that CTIP2 directly interacts with HEXIM1 and that the 7SK RNA is required to associate with P-TEFb. These biochemical observations were supplemented by functional experiments. Because the HIV-1 LTR is a bona fide P-TEFb-sensitive promoter, we quantified its activity upon CTIP2 knockdown, Tat transactivation, and P-TEFb inhibition. We observed that knocking down CTIP2 increased the Tat-dependent transcriptional activity of the HIV-1 promoter, suggesting that endogenous CTIP2 might contribute to the repression of Tat by repressing P-TEFb function (Fig. 1E). In support of this hypothesis, treatments with Flavopyridol, a potent P-TEFb inhibitor,

abrogated this synergistic transcriptional activation. Therefore, we conclude that CTIP2 may contribute to P-TEFb repression in the inactive form of the P-TEFb complex. Along this line, we further investigated CTIP2 function in this P-TEFb-, HEXIM1-, and 7SK snRNA-including nucleoprotein complex (Fig. 2).

CTIP2 Interacts with the 7SK snRNA. First, gel shift experiments were performed to define whether CTIP2 interacts with the 7SK snRNA in vitro. As shown in Fig. S2, increasing amounts of purified GST-CTIP2 protein shifted increasing amounts of 32 P-labeled 7SK snRNA, indicating that CTIP2 interacts with the 7SK in vitro. As a control, GST-CTIP2 did not bind to TAR RNA in vitro. To define whether this interaction also exists in vivo, we performed immunoprecipitations of CTIP2-associated complexes and probed for the presence of 7SK RNA by RT-PCR. We found that the 7SK snRNA copurified with CTIP2, suggesting that this association exists in vivo (Fig. 2A). Micro scale thermophoresis experiments confirmed this result and allowed us to precise the domain of 7SK involved in this interaction. CTIP2 associates with the Loop 2 domain of the 7SK snRNA, but not with a point-mutated Loop 2 (Fig. 2B and C). We next investigated which domain of CTIP2 is involved in the interaction with 7SK and P-TEFb. To do so, we performed immunoprecipitation experiments with truncated forms of CTIP2 (Fig. 2D). This mapping confirmed the need of the 7SK snRNA for the interaction with P-TEFb but not with HEXIM1 (Fig. 2D and Fig. S3). Indeed, a CTIP2 mutant lacking amino acids 355–813 was unable to associate with 7SK snRNA and P-TEFb, but still associated with HEXIM1 (Fig. 2D, column 3). Moreover, we identified the domain from 349 to 475 aa of CTIP2 as the potential interface for P-TEFb binding and thereby for the 7SK snRNA interaction (Fig. S4). Surprisingly, the deletion of the 717 first amino acids (out of 813) of CTIP2 was needed to release the interaction with HEXIM1 (Fig. 2D, column 1). Next, we tested whether or not CTIP2 regulates P-TEFb activity.

CTIP2 Inhibits Cdk9-Mediated Phosphorylation. To explain CTIP2-mediated repression of the P-TEFb function, we first hypothesized that CTIP2 may favor P-TEFb recruitment into the inactive complex. CyclinT1 immunoprecipitation experiments were performed to confirm this hypothesis. Surprisingly, CTIP2 overexpression did not favor recruitment of P-TEFb into the inactive 7SK snRNA-containing complex (Fig. 2E). Because the amount of P-TEFb in the inactive complex was not impacted by CTIP2, we next tested the inhibition of the P-TEFb kinase activity. We first quantified the Cdk9 kinase activity of the CyclinT1-immunoprecipitated complex in the presence or absence of CTIP2 overexpression. As shown in Fig. 2F, CTIP2 overexpression significantly inhibits Cdk9 activity (Fig. 2F and Fig. S5). To confirm that this repression also occurs in physiological conditions, we analyzed the global level of RNA Pol II serine 2 phosphorylation in CTIP2 knockdown cells. Accordingly, higher levels of RNA Pol II serine 2 phosphorylation were observed in CTIP2-depleted cells (Fig. 2G). These observations suggest that CTIP2 represses P-TEFb function by inhibiting Cdk9 activity.

CTIP2-Including Complexes Containing P-TEFb- and Chromatin-Modifying Enzymes Are Mutually Exclusive. We have reported that CTIP2 silences HIV-1 gene expression by the recruitment of HDAC1, HDAC2, and SUV39H1 to the viral promoter (19). To examine the existence of multiple CTIP2 complexes, we performed sequential immunoprecipitation experiments. For this purpose, CTIP2 associated complexes (IP1) were further immunoprecipitated with anti-HEXIM1 or anti-HDAC2 antibodies to discriminate between potential P-TEFb- and chromatin-modifying enzyme-containing complexes, respectively (IP2). As shown in Fig. 3, HDAC2 and Suv39H1 were excluded from the P-TEFb complex whereas neither Cdk9, CyclinT1, nor HEXIM1 was found in the complex containing

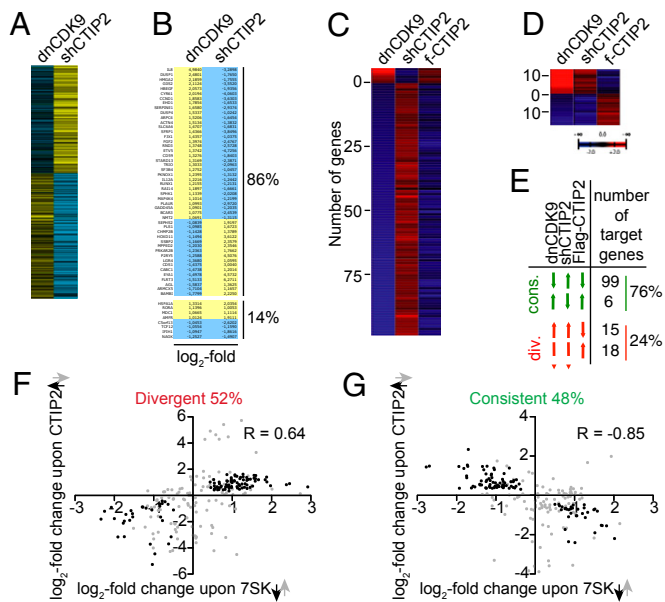


Fig. 4. CTIP2 regulates P-TEFb-sensitive genes. (A) Genes modulated by the knockdown of CTIP2 in microglial cells were compared with the genes modulated by an inhibition of Cdk9 activity (expression of the dnCdk9 construct) (4). Shades of yellow and blue represent relative activation and repression, respectively. (B) Genes highly sensitive to both dnCdk9 expression and CTIP2 knockdown (modulation average: $\text{Log}_2 > 1$) were clustered and identified. (C) Transcriptome heatmap of genes significantly ($P \ll 0.05$) oppositely regulated by Cdk9 and CTIP2: comparison of the expression of dnCdk9 with CTIP2 overexpression and knockdown in HEK293 cells. (D) As in C, but for the statistically significantly coregulated genes. (E) Table showing the number of target genes differentially expressed in each of the comparisons from C and D. (F) Scatter plot of LogQ values for the 112 genes significantly ($P < 0.05$) coregulated by CTIP2 overexpression (black arrow up) and 7SK knockdown (black arrow down). The LogQ values of the same set of genes upon CTIP2 knockdown (gray arrow down) and 7SK overexpression (gray arrow up) are illustrated in gray. (G) Same as in F, but for the 105 genes antiregulated in these conditions.

best-characterized P-TEFb-associated pathologies: hypertrophic cardiomyopathy (HCM). HCM was triggered by phenylephrin (PE) administration to adult mice for 15 d. PE is an alpha-adrenergic receptor agonist known to trigger cardiac hypertrophy in mice. Gene expression profiles from HCM mice were compared with those obtained upon Cdk9 overexpression and CTIP2 knockdown (Fig. 5). Cdk9 overexpression regulated half of the HCM-modulated genes, confirming the influence of P-TEFb on this specific pathological gene expression pattern (Fig. 5A and Dataset S6). We observed a significant correlation between the gene expression levels from both conditions (Fig. 5B). By comparing this cluster of genes with CTIP2-sensitive genes, we observed that 50% of the Cdk9-sensitive HCM genes were consistently regulated by CTIP2 knockdown (Fig. 5C and Dataset S7). Interestingly, key HCM pathways are enriched in the CTIP2/Cdk9 cluster of modulated genes (Fig. 5E). We found that the gene of the sarcomeric β -myosin heavy chain (MYH7) was regulated by CTIP2 consistently with a repression of Cdk9 function (Fig. 5F). Moreover, a network analysis of the HCM Cdk9- and CTIP2-sensitive genes highlights the major impact of CTIP2 on the regulation of cardiac hypertrophy (Fig. 5G). Indeed, key HCM pathways, such as the MAPK, the Ca^{2+} /Calmodulin, the NF- κ B/NFAT, and the PI3K/Akt pathways, are regulated by CTIP2 (Fig. 5G). We found endogenous CTIP2, CyclinT1, Cdk9, and HEXIM1 bound to the MYH7 proximal promoter but not to $-1,000$ bp and $-2,000$ bp upstream

or $+2,000$ bp downstream of the transcription start site (Fig. 6B). Accordingly, with our model, the recruitment of Cdk9 (Fig. 6, column 3), CyclinT1 (Fig. 6, column 4), and HEXIM1 (Fig. 6, column 5) is favored by the presence of CTIP2. These observations argue for a CTIP2-mediated anchorage of an inactive P-TEFb complex at the MYH7 promoter and suggest a major influence of CTIP2 in the control of the size of heart sarcomeres. The proposed mechanistic model is presented in Fig. 7.

Discussion

The cellular factor CTIP2 is a key transcriptional repressor involved in development (14–17, 23), T lineage commitment (15, 24), cancer (18, 25), and HIV-1 gene silencing (19). In association with chromatin modifying enzymes, CTIP2 promotes the establishment of a local heterochromatin environment at target promoters such as the cellular p21 gene promoter (26) and the viral HIV-1 gene promoter (19, 22, 27). Interestingly, both genes have been reported to be highly sensitive to the P-TEFb elongation complex (28–30). To secure the cell integrity, P-TEFb is

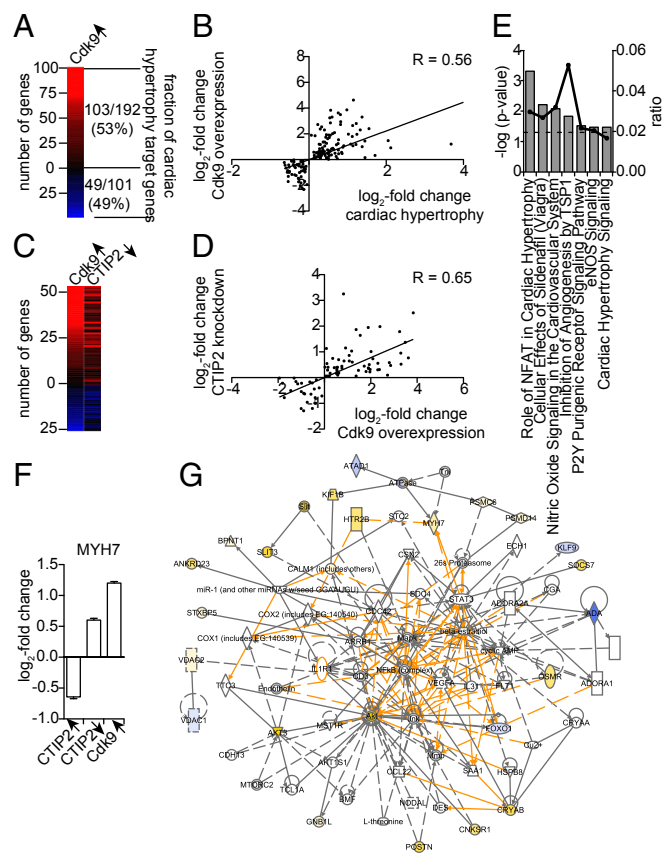


Fig. 5. CTIP2 regulates P-TEFb-sensitive genes in hearts of hypertrophic cardiomyopathic mice. (A) Heatmap of genes significantly ($P < 0.01$) differentially expressed in hearts of hypertrophic cardiomyopathic compared to healthy mice, whose expression is affected in the same manner upon overexpression of Cdk9 in HEK293 cells. The fraction of up- and down-regulated cardiac hypertrophy target genes in the Cdk9 condition is indicated. (B) Scatter plot of the genes shown in A. The Pearson correlation coefficient (R) is indicated. (C) Heatmap of the fraction of cardiac hypertrophy target genes affected by Cdk9 overexpression and shRNA-mediated knockdown of CTIP2. (D) Scatter plot of the genes shown in C. (E) Canonical cardiovascular pathway enrichment among the genes shown in C. The P value threshold ($P < 0.05$) is indicated as a dotted line. (F) Expression changes of the sarcomeric protein MYH7 upon CTIP2 overexpression, knockdown, and overexpression of Cdk9. (G) Gene network of the genes shown in C. Up-regulated genes are shown in yellow, and down-regulated genes are shown in blue.

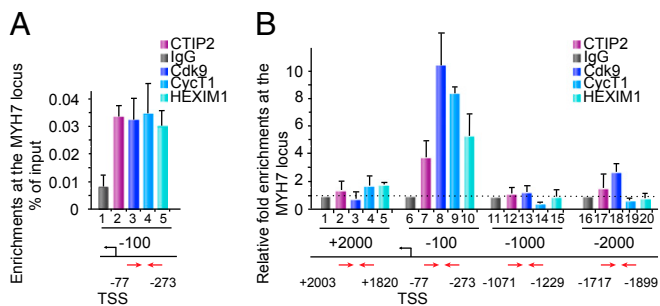


Fig. 6. CTIP2 binds to the MYH7 gene promoter region. (A) Cells were subjected to chromatin immunoprecipitation experiments with the indicated antibodies. The associated promoter region of the MYH7 gene was quantified by Q-PCR. Enrichments are presented as % of input. (B) Cells transfected with the control FLAG-pcDNA3 or the FLAG-CTIP2 plasmids were subjected to chromatin immunoprecipitation experiments with the indicated antibodies 48 h posttransfection. The associated promoter, the $-1,000$ bp, the $-2,000$ bp, and the $+2,000$ bp regions of the MYH7 gene were quantified by Q-PCR. Fold enrichments are presented relative to the enrichments observed in the control condition. Results are representative of at least three independent experiments.

dynamically regulated at physiological conditions. 7SK snRNA and HEXIM1 inhibit Cdk9 activity in an inactive HEXIM1/7SK/P-TEFb complex (7–10). We show here that CTIP2 associates with the inactive HEXIM1/7SK/P-TEFb complex, which is distinct from the previously described CTIP2-associated complex containing chromatin-modifying enzymes (19). Our genome-wide comparison of Cdk9- and CTIP2-sensitive cellular genes is consistent with CTIP2 being a P-TEFb-repressive component. Mechanistically, we observed that RNA Pol II Serine 2 phosphorylation levels increase in CTIP2-knockdown cells. In addition, *in vitro* kinase assays identified CTIP2 as a direct inhibitor of Cdk9 enzymatic activity. Based on these observations, we propose that, when associated with HEXIM1/7SK/P-TEFb, CTIP2 significantly represses Cdk9 kinase activity and thus contributes to the inhibition of P-TEFb function. Interestingly, the presence of the 7SK snRNA is crucial for recruiting CTIP2 to P-TEFb. CTIP2 is able to bind the 7SK snRNA *in vitro* and *in vivo*, suggesting a direct interaction between both components. The 7SK snRNA has been shown to have at least two major functions in gene expression regulation: it negatively regulates P-TEFb activity and it acts as a negative regulator of the architectural transcription factor HMGA1 (31–34). However, whether CTIP2 impacts other 7SK-including complexes such as the 7SK/hnRNP (K, A1/A2, or Q1/R) complexes (35) remains under question. Recently, multifunctional HIV-1 Tat-associated P-TEFb complexes were identified. Tatcom2 includes the 7SK snRNA, LARP7 but not HEXIM1, and Tatcom1 includes MLL-fusion partners and the PAF1 complex (12). Preliminary results suggest that CTIP2 may associate with PAF1 and LARP7 (Fig. S7). However, deciphering the influence of Tat expression on the CTIP2/7SK/HEXIM1 complex and defining the impact of CTIP2 on the Tat-associated complexes will need more investigations. Interestingly, CTIP2 and HMGA1 both associate with the loop 2 region of 7SK RNA. Moreover, preliminary data suggest that CTIP2 collaborates with HMGA1 to inhibit HIV-1 gene transcription and the viral replication. Because we have also observed that endogenous CTIP2 represses the Tat/P-TEFb-mediated transactivation of the HIV-1 promoter (Fig. 1E), the repressive activity of CTIP2 and HMGA1 on this P-TEFb-sensitive promoter is now attested. In addition, this control of P-TEFb might contribute to the persistence of the latently infected HIV-1 reservoirs by counteracting the major viral reactivation events. Although $\sim 50\%$ of the cellular genes were similarly modified upon CTIP2 overexpression or 7SK knockdown, the remaining half showed an inverse correlation.

This subset of genes was identified as activated by CTIP2, but repressed by 7SK RNA. These genes are likely regulated by CTIP2 in a Cdk9-independent manner. D'Orso and Frankel showed the recruitment of the inactive P-TEFb complex to the Sp1 binding sites of the HIV-1 promoter (13). However, no mechanistic evidence was provided on the way of this recruitment. Because CTIP2 is anchored to the Sp1 region via an association with Sp1 and LSD1 proteins (27, 36), CTIP2 may constitute a platform for the recruitment of inactive P-TEFb to the HIV-1 promoter. Together with AIDS, hypertrophic cardiomyopathy is a well-described P-TEFb-dependent pathology (for review, see refs. 20 and 21). Upon HCM, the overexpression of the β -myosin heavy chain induces an increase of the size of the sarcomeres and a pathological thickening of the heart muscle. This final target gene expression is controlled by multiple pathways, all contributing to HCM (20). Our results suggest that CTIP2 contributes to the control of the MAPK, the Ca^{2+} /Calmodulin, the NF- κ B/NFAT, and the PI3K/Akt pathways. In addition, we show that CTIP2 inhibits the expression of the β -myosin heavy chain (MYH7) consistently with the repression of Cdk9 activity. Moreover, the CTIP2-mediated anchorage of an inactive P-TEFb complex at the MYH7 promoter further argues for the presence of the inactive P-TEFb complex at promoters of P-TEFb sensitive genes. In fact, a recent study by Ji et al. has demonstrated the assembly of 7SK RNA-containing complexes at gene promoters (37). Taken together, our results suggest that CTIP2 is a potent inhibitor of P-TEFb function in two of the best characterized P-TEFb sensitive pathologies: HCM and AIDS. As a part of the 7SK/HEXIM1/P-TEFb complex, CTIP2 controls P-TEFb-sensitive gene expression and thereby may constitute a pharmaceutical target for fighting P-TEFb-dependent pathologies.

Materials and Methods

Cell Culture. The human microglial cell line (provided by M. Tardieu, Paris, France) (38) and HEK293T cell lines were maintained in Dulbecco's modified Eagle medium (DMEM) containing 10% (vol/vol) FCS and 100 U/mL penicillin/streptomycin.

Coimmunoprecipitation Assays and Antibodies. Two days posttransfection, immunoprecipitations were performed using the standard technique with M2 anti-FLAG (Sigma), anti-Hexim1 (Abcam), or anti-CyclinT1 (Santa Cruz) antibodies overnight at 4 °C with or without RNase or HMBA treatments. Finally, the immunoprecipitated complexes were processed for SDS/PAGE and Western blot analysis, real time quantitative PCR (qRT-PCR), or RT-PCR assay. Proteins were detected in Western blot analyses using antibodies directed against the FLAG epitope (M2 mouse monoclonal from Sigma), CyclinT1 and Cdk9 (Santa Cruz), and CTIP2, Brd4, and HEXIM1 proteins (Abcam).

Gel Filtration Experiments. The 3 mg of microglial cellular nuclear extracts were concentrated by using a Microcon YM-10 Centrifugal Filter Unit (3,000 Nominal Molecular Weight Limit) and separated by gel filtration on a

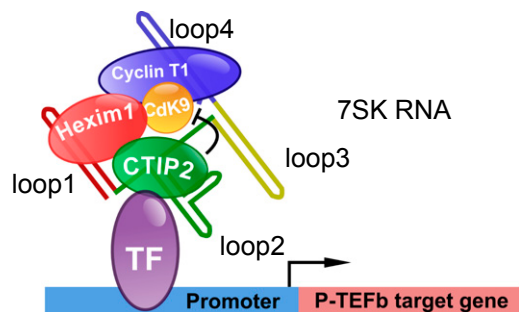


Fig. 7. Model for CTIP2-mediated repression of P-TEFb target genes. Bound to target promoters, CTIP2 interacts with HEXIM1 and the 7SK snRNA to repress P-TEFb kinase activity and thus the expression of P-TEFb-sensitive genes.

Superose 6 PC 3.2/30 column (Amersham Biosciences) as previously described (39). The 29 fractions collected were accessed by SDS/PAGE and Western blot analysis. Upon RT-PCR amplification, the presence of the 75K was visualized by agarose gel electrophoresis.

Micro Scale Thermophoresis. Micro Scale Thermophoresis (MST) experiments were conducted with Fam6-labeled 75K L2 RNA or 75K L2 m137 RNA and increasing concentrations of FLAG-CTIP2 using a Monolith NT.115 (Nanotemper Technologies) as described previously (40) (Laser-power 20%, Laser-on time 60s, LED-power 30%).

Transcriptome Analyses. Transcriptome analyses were performed using either Agilent Human whole-genome array (G2534-60011), Applied Biosystems human arrays (Product nos. 4339628 and 4336875), or an Affymetrix GeneChip MOE 430 2.0 array according to the manufacturer's instructions. Data quality was determined using a QC procedure (41). Data were normalized using NeONORM with $k = 0.02$ (42–44). Subtraction profiling was performed as in refs. 31 and 32 using the CDS test (45).

Generation of Cardiac Hypertrophic Mice. Micropumps (Alzet 2002 Osmotic Pumps) were set up to deliver (R)-(-) Phenylephrine hydrochloride (Sigma P6126-5G) at $80 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ or vehicle buffer only (PBS, 0.002% ascorbic

acid) for 15 d. The micropumps were implanted under the back skin of five C57BL/6N mice for each group under 2% Isoflurane (Abbott), 2% in oxygen. Cardiac hypertrophy was validated by the increase of heart weight to body weight ratio in treated animals. Protocols are in accordance with the ethic recommendations of the Université Pierre et Marie Curie, Paris 6 University.

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