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CONTROL OF RNA POLYMERASE II ACTIVITY BY DEDICATED CTD KINASES AND PHOSPHATASES

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1. ABSTRACT

The elongation phase of eukaryotic transcription by RNA polymerase II (RNAPII) is an important target for regulation of gene expression. An interplay of positive and negative elongation factors determines the elongation activity of RNAPII in different promoters. The phosphorylation status of the carboxyl-terminal-domain (CTD) of the larger subunit of RNAPII appears to be the regulatory focus of different factors regulating mRNA processivity. The emerging model of the transcription cycle proposes that the phosphorylation state of the CTD is dynamic during elongation with different forms predominating at different stages of transcription. Shortly after initiation RNA polymerase II comes under the control of negative elongation factors and enters abortive elongation. Escape from the action of these negative controls requires the action of at least one positive elongation factor identified in the P-TEFb complex composed of the Cyclin-Dependent Kinase CDK9 and its regulatory subunit cyclin T. Finally, the requirement of CTD phosphatase activity, identified in the FCP1 protein, has been invoked as necessary to recycle the hypophosphorylated form of the RNA polymerase II competent to reinitiate the transcription cycle.

2. INTRODUCTION

The elongation stage of eukaryotic mRNA transcription represents an important regulatory step in the control of gene expression (1-2). Accumulation of promoter-paused RNA polymerase II (RNAPII) downstream the transcription start site has been documented for many genes, including hsp70 (3), c-myc (4), c-myb (5), c-fms (6) c-fos (7) and HIV-1 (8). It has been proposed that escape of the paused RNAPII is a major rate-limiting step in the transcription control of these genes (9). Accumulating evidence has strongly indicated that the carboxyl-terminal repeat domain (CTD) of the large subunit of RNAPII participates at several levels in the regulation of gene expression (10). The mammalian CTD consists of a series of 52 heptapeptide repeats (Tyr-Ser-Pro-Thr-Ser-Pro-Ser) that can be extensively phosphorylated at multiple sites, and its phosphorylation status plays a dominant role in transcription regulation and posttranscriptional RNA processing. The hyperphosphorylated form CTD (RNAPIIo) is associated with transcription elongation complexes, while only the hypophosphorylated form (RNAPIIa) can assemble into preinitiation complexes (11,12). The transition from initiation to elongation is invariable, characterized by conversion of RNAPIIa to the RNAPIIo form. Recently, a more complex

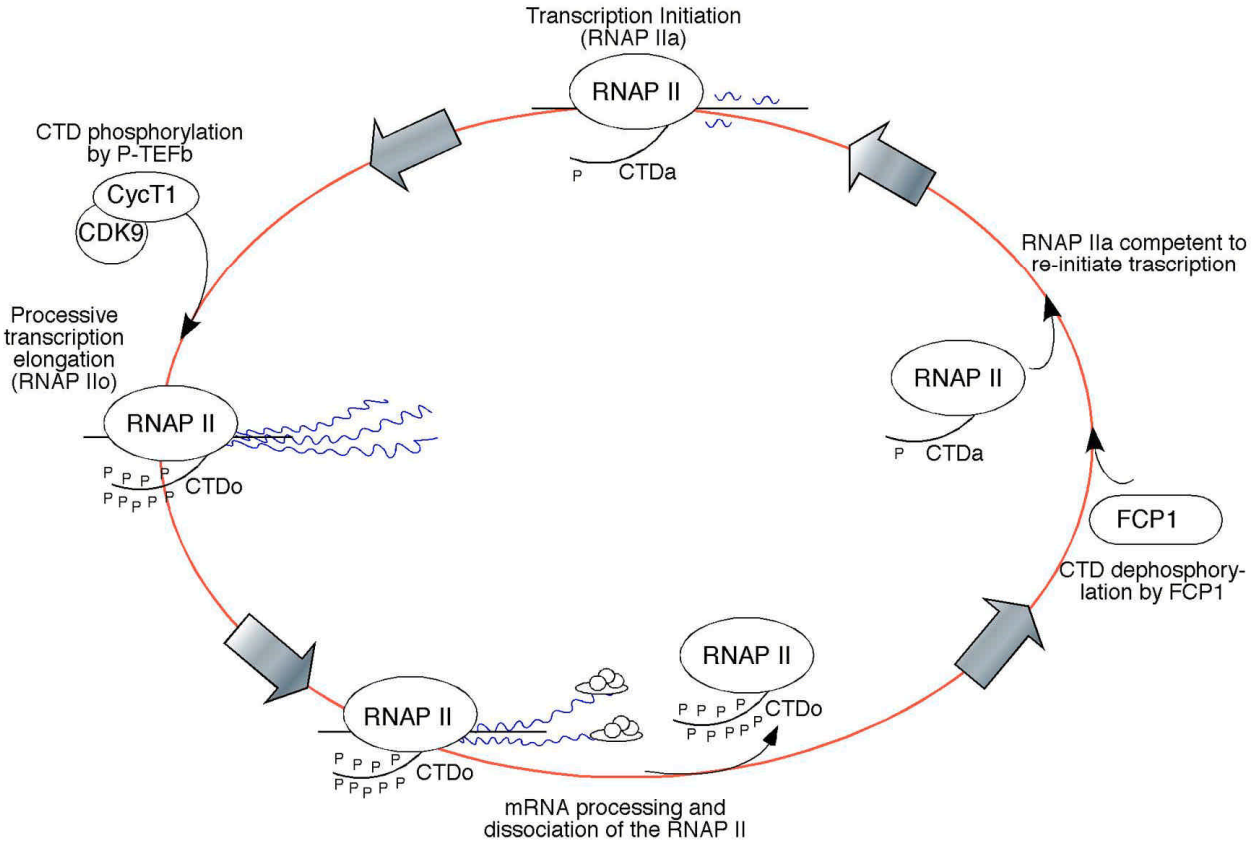

Figure 1. Model illustrating RNAPII phosphorylation status during the transcription cycle. The suggested involvement of the CTD kinase complex P-TEFb and the dedicated phosphatase FCP1 at different steps during the process is indicated.

CTD cycle in which different modified forms, phosphorylated at different Ser residues, predominate at different stages of transcription has been suggested (13). The emerging overall picture of the transcription cycle proposes that the phosphorylation state of the CTD and association of RNA processing factors are dynamic during elongation. Consequently, the presence of dedicated CTD kinases and phosphatases is required for the dynamic activity of RNAPII (figure 1).

2.1. Scope of the review

We will focus this review on the P-TEFb complex, which is thus far the only CTD kinase able to modify the functional properties of RNA polymerase II. P-TEFb has been shown to phosphorylate the CTD of RNAPII at the time it is known to functionally modify the elongation properties of the polymerase. (14,15). Moreover, recent results led to the hypothesis that RNAPII cycles during the transcription cycle and invoke the requirement for a CTD phosphatase. (16) While several kinases are putative candidates of RNAPII phosphorylation changes during the transcription cycle (2,9,12,13), FCP1 phosphatase protein is the only CTD phosphatase identified thus far (17,18). Recent evidence demonstrating that the FCP1 phosphatase is involved in RNAPII transcription regulation will be discussed.

2.2. Cyclin-dependent kinases and RNAPII CTD phosphorylation

At least 10 cellular kinases that can phosphorylate the CTD *in vitro* are known, and 3 of them are members of the cyclin-dependent kinase family (19). In the human genome, the CDK/cyclin family contains five CDKs (Cdk1, 2, 3, 4 and 6) and four cyclin classes (cyclin A, B, D and E) that regulate cell cycle progression, and three CDKs (Cdk7, 8 and 9) and three cyclin classes (cyclin C, H and T) that regulate transcription. Other CDK and cyclin classes with less defined roles also exist in the human genome (20). To date, it is likely that the three CDKs 7, 8 and 9 are the only CDKs involved in transcription. CDK7 is found in association with cyclin H, which is a subunit of transcription factor TFIIH (21). CDK8/cyclin C is a component of the RNA polymerase II holoenzyme (22) and the CDK9/cyclin T complex is present in the positive transcription elongation factor P-TEFb (23, 24, 25, 26,27,28). Indeed, all three CDKs have been reported as being capable of utilizing the CTD as a substrate leading to the hyperphosphorylation of RNAPII.

3. THE P-TEFb COMPLEX

The nucleoside analogue 5,6-dichloro-1-b-D-ribofuranosyl-benzimidazole (DRB) is an inhibitor of transcription elongation in *in vivo* and *in vitro* nuclear transcription extracts (29,30). The use of DRB has been instrumental for the isolation of DRB-sensitive transcription elongation factors. Price and colleagues isolated a DRB-sensitive factor P-TEFb, as an elongation factor that stimulates a shift from production of short to long transcripts *in vitro* (31). Subsequently, CDK9 was

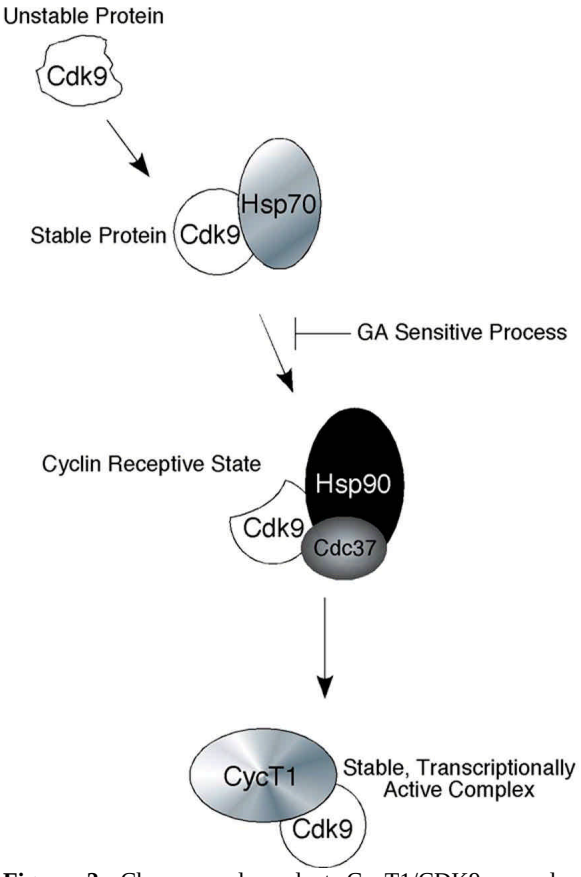

Figure 2. Chaperone-dependent CycT1/CDK9 complex assembly pathway. The general chaperone Hsp70 stabilizes the CDK9 protein. The kinase-specific chaperone complex Hsp90/Cdc37 leads to the formation of the CycT1/CDK9 complex. The GA-sensitive step is indicated.

identified as a component of the P-TEFb multi-protein complex involved in transcription elongation in Drosophila and mammals (26). Drosophila CDK9 cDNA was first isolated and sequence database searches revealed that the identified cDNA was the Drosophila homologue of a human gene named PITALRE. PITALRE was already known as a human gene sharing sequence similarity with other cyclin-dependent kinases (32). Contemporarily, other groups identified PITALRE as a component of the Tat-associated Kinase (TAK) (33). The involvement of PITALRE in Tat transactivation was further supported by the results of a screening for drugs that inhibit the process. All compounds that were found to block Tat transactivation also inhibited the kinase activity (34). Albeit PITALRE is required for Tat transcription activation *in vitro*, it was shown that Tat does not associate directly with PITALRE. Similar to other CDKs, a cyclin partner of PITALRE called cyclin T1 was isolated and it was found to directly interact with Tat. Subsequently, the kinase subunit has been renamed CDK9 (25,35,36,37,38,39).

3.1. The CDK9 partners

It has been shown that CDK9 associates specifically with multiple cyclin subunits. Thus far two cyclin T genes, T1 and T2 (23) and a separate and unique cyclin K gene product (40), all of which are related to cyclin C, have been found in association with CDK9. Furthermore, alternative splicing of the cyclin T2 gene locus yields two T2 transcripts, T2A and T2B. *In vitro* studies have shown that both cyclin T1/CDK9 and cyclin T2/CDK9 complexes function in transcription. In addition, the purified CDK9-cyclin K heterodimer is able to substitute for immunodepleted P-TEFb during in vitro transcription assays (40). However, *in vitro* binding data and the CTD phosphorylation activity (40,41), suggest that CDK9 binds less efficiently to cyclin K and this complex has a reduced CTD phosphorylation activity *in vitro* and *in vivo*. It remains to be demonstrated whether this difference in activity might reflect a lower specific activity of the CDK9-cyclin K complex or might indicate that the CTD is not the preferred substrate of this complex. Clearly the presence of diverse cyclins capable of forming stable complexes with CDK9 opens the possibility that different regulatory cyclins might determine specific functions of CDK9 activity.

3.2. Chaperone-assembly of P-TEFb complex

In addition to cyclin T (T1 and to a lesser extent T2), CDK9 has also been shown to interact with several other proteins of unknown identity. Consequently, the exact subunit composition of P-TEFb has not been determined. Recently, the identity of CDK9-associated proteins has been explored by affinity purification. It has been reported that in addition to forming a heterodimer with cyclin T1, CDK9 interacts with the molecular chaperone Hsp70 and with the kinase-specific chaperone complex, Hsp90/Cdc37, to form two separate chaperone-CDK9 complexes (42). The three CDK9 containing complexes seem to have different roles in the cell and only the cyclin T/CDK9 complex shares all the known activities of the P-TEFb complex. Association of CDK9 with Hsp70 or Hsp90/Cdc37 does not confer to the formed complexes any ability in the activation of gene expression. The existence of a concerted chaperone-dependent pathway has been suggested for the stabilization and folding of CDK9 leading to the assembly of the functional cyclin T/CDK9 complex (figure 2). The general chaperone protein Hsp70 seems to be responsible for the folding of the CDK9 kinase and its targeting to the kinase-specific chaperone complex Hsp90/Cdc37. Only after the binding of CDK9 to Hsp90/Cdc37, the kinase can associate with its regulatory partner cyclin T1 to form a functional transcription competent complex. Moreover, it has been shown that cyclin T/CDK9 is a very stable complex, with a half-life of about 36 hours, while free and unprotected CDK9 appears to be degraded rapidly (with a half-life of about 6 hours). The stability of the CDK9 protein is increased only when it is bound to cyclin T1. The presence of Geldanamycin (GA), a drug that inhibits Hsp90 from assembling with the target protein, prevents the association between CDK9 and cyclin T1 and causes the accumulation of the CDK9/Hsp70 complex in the cell. Consequently, the assembly of the P-TEFb complex is a chaperone-regulated process in which Hsp70 leads to the folding of the kinase, and the Hsp90/Cdc37 complex renders the kinase competent for the association with cyclin T and allows it to function as P-TEFb complex. P-TEFb represents the mature and

functional form of the complex and it is the only CDK9-containing complex capable of supporting Tat transcription and phosphorylating the CTD of RNAPII (42).

3.3. The P-TEFb substrates

Several pieces of evidence suggest that the CTD domain of the large subunit of RNA polymerase II is an important physiological target of P-TEFb *in vitro* and *in vivo* (41,43). The CTD is phosphorylated during the transcription cycle at the time P-TEFb is known to act and the CTD is strictly required for nascent RNA transcript elongation. Nonetheless, although the CTD is the generally proposed physiological target, it has recently been found that other molecules involved in RNAPII-dependent transcription are substrates of P-TEFb phosphorylation at least *in vitro*. This is the case of TFIIF and the SPT5 subunit of DSIF, which both can be phosphorylated by P-TEFb (43,44). Particularly, it has been demonstrated that P-TEFb preferentially phosphorylates the repetitive carboxyl domain of hSPT5 as compared with the CTD, at least *in vitro* (45). In addition, CDK9 protein itself can be a substrate of CDK9 autophosphorylation (46,47). Interestingly, CDK9 autophosphorylation has been demonstrated to be required for high affinity binding of the HIV-1 Tat-P-TEFb complex to TAR RNA.

P-TEFb shares responsiveness to DRB with DSIF (DRB sensitivity-inducing factor) and NELF. DSIF forms a protein complex with the negative elongation factor NELF to inhibit promoter proximal elongation by RNAPII (48,49). Release from this inhibition is mediated by P-TEFb specifically through phosphorylation (50). DSIF is a heterodimer comprising the mammalian homologues of yeast Spt4 and Spt5 and interacts biochemically and genetically with RNAPII through Spt5. This interaction is believed to restrict RNAPII to the synthesis of short transcripts. P-TEFb has been demonstrated to be able to phosphorylate the C-terminal region (CTRI) of Spt5 at least *in vitro* (51). This region shares sequence homology with the C-terminal region of RNAPII (CTD), suggesting that P-TEFb-mediated phosphorylation of either the CTD or the Spt5 CTR1 domain or both leads to removal of DSIF inhibition. Although Spt4 and Spt5 are required for DRB-mediated inhibition of transcription elongation, it has been demonstrated that these proteins can also stimulate transcription elongation in *in vitro* transcription assay, suggesting that they can regulate transcription elongation in both a positive and negative manner (52).

DSIF is also important for Tat activation. It has been shown that nuclear extracts depleted of hSPT5 do not support Tat-dependent elongation *in vitro* and *in vivo* overexpression of hSPT5 stimulates Tat activation (48,53). Additional evidence of the role of Spt5 and Spt6 in transcription elongation comes from recent studies in Drosophila. It has been demonstrated that Drosophila Spt5 and Spt6 colocalize at a large number of transcriptional active chromosomal sites on polytene chromosomes. Their localization is highly coinciding with localization of elongating phosphorylated PolII, suggesting that Spt5 and Spt6 are present at regions of active transcription. In addition, it has been found that heat shock triggers the rapid recruitment of Spt5, Spt6, along with P-TEFb, to heat shock loci (54,55,56). The global distribution of Spt5, Spt6 and P-TEFb on polytene chromosomes and their local occupancy on heat shock genes strongly suggest a concerted function of these factors to increase the rate of escape of the DSIF-paused RNAPII in productive elongation.

3.4. P-TEFb recruitment activates transcription

While the involvement of P-TEFb in Tat transactivation is now clear, the role and the mechanism of action of cyclin T/CDK9 complex in uninfected cells is at the moment unknown. P-TEFb function requires its kinase activity, but how this activity is normally directed toward the elongation complex is not understood. Genetic studies have shown that chimeric cyclin T1 and CDK9 proteins can activate transcription if tethered directly to either RNA or DNA elements, indicating that the primary role of Tat and TAR is to recruit cyclin T1-CDK9 to RNA. A hybrid Rev-CDK9 fusion protein, in the absence of Tat, activates transcription from HIV-1 LTR where TAR has been replaced by a Rev-responsive-element RRE (57,58), and both the kinase activity and cyclin T1 are required for these effects. Results from our and other laboratories have demonstrated that the recruitment of Cyclin T1/CDK9 kinase complex to the promoter template, through fusion to a DNA-binding domain of P-TEFb, stimulates transcription of RNAPII-dependent promoters bearing the cognate DNA-binding sites *in vivo*. The artificially DNA-bound CDK9 kinase functions in transcription at a step subsequent to TFIID recruitment by enhancing transcription elongation (59). The ability of the Cyclin T1/CDK9 kinase to function in a catalytic-dependent manner when bound to promoter upstream sequences raises the possibility that promoter-specific activation of transcription elongation might occur through interaction between CyclinT/CDK9 complex and a dedicated DNA-bound activator. The first example of natural recruitment of P-TEFb to an activator comes from the finding that CIITA functionally recruits P-TEFb containing cyclin T1 to major histocompatibility complex Class II promoters (60). Furthermore, it has been reported that upon heat shock, P-TEFb is rapidly recruited to the heat shock loci in a manner consistent with models in which P-TEFb acts to stimulate promoter-paused RNAPII to enter into productive elongation (54). Finally, it has been recently shown that the androgen receptor interacts with P-TEFb and enhances the elongation stage of transcription (61).

4. P-TEFb AND TAT FUNCTION

Activation of human immunodeficiency virus type 1 (HIV-1) transcription by the virus-encoded transcription factor, Tat, provides an important paradigm for understanding the mechanism by which the P-TEFb complex is involved in regulation of transcription elongation by RNA polymerase II. HIV-1 encodes a transactivator protein, Tat, which stimulates transcription elongation through interaction with the transactivation response (TAR) RNA element located at the 5' end of the nascent transcript (62). Two cyclin-dependent kinase (CDK)-cyclin pairs have been implicated as Tat co-factors,

which could phosphorylate the CTD: the TFIIH, whose kinase activity resides in the CDK7 subunit, and the TAK (Tat-associated Kinase) complex. CDK7, together with cyclin H and Mat 1, forms the CDK-activating kinase CAK complex that phosphorylates CDKs involved in the regulation of the cell cycle. The second CTD kinase, TAK, was found to be equivalent to P-TEFb (13,33,34,63,64). The interaction between Tat and P-TEFb is mediated through human cyclin T1. It has been found that only human cyclin T1 specifically binds the Tat activation domain both as a subunit of the cyclin T/CDK9 complex and on its own (25,65,66). Our recent data show that distinct regions of cyclin T1 are required for binding to CDK9 and for recruitment to the HIV-1 Tat/Tar complex (67). Most importantly, cyclin T1 increases the affinity of Tat for TAR (25). In addition, we and others have demonstrated that Tat function can be rescued in rodent cells only by human cyclin T1, and that enforced expression of human cyclin T2 inhibits this process in rodent cells, and represses Tat transactivation in human cells (66,68,69). The inhibitory function of cyclin T2 is likely due to the lack of binding to Tat, and it is conceivable that over-expressed cyclinT2 would squelch cyclin T1 for binding to CDK9.

It has been found that most of the P-TEFb isolated from human cells appears to be inactive in forming stable P-TEFb-Tat-TAR complexes, at least *in vitro*. Recently presented data have proposed a mechanistic view of how a high affinity P-TEFb-Tat-TAR complex is assembled *in vitro* (70,71). Assembly of this complex seems to be a regulated process involving the relief of two autoinhibitory mechanisms in P-TEFb (46,47). P-TEFb undergoes conformational changes in at least two controlled steps .The first autoinhibition arises from the requirement of CDK9-dependent phosphorylation. High affinity binding of the Tat–P-TEFb complex to TAR requires prior phosphorylation of P-TEFb, which may occur by autophosphorylation or through the action of other cellular kinases. Enhanced binding to TAR requires catalytically active CDK9 and a hydrolysable ATP substrate and is accompanied by phophorylation of CDK9 and cyclin T1 (46). Multiple Ser or Thr phosphorylation sites have been mapped at the c-terminus tail of CDK9, and it has been demonstrated that truncation of this region of CDK9 destroys autophosphorylation without affecting kinase activity (47). Autophosphorylation of CDK9 seems to overcome inhibition by inducing conformational changes in P-TEFb exposing a region in cyclin T1 involved in physical interaction with TAR. The second autoinhibitory mechanism relies on the capability of an intramolecular interaction between the N- and C-terminal regions of cyclin T1 that sterically block the P-TEFb-TAR interaction *in vitro*. The intramolecular interaction of the cyclin T1 C-terminal region with the N-terminal half have been proposed to create a steric hindrance that blocks the access to TRM by TAR. It has been suggested that disruption of this intramolecular interaction requires the transcription elongation factor Tat-SF1 which interacts with the C-terminal region of cyclin T1 and increases the binding of the Tat-SF1-PTEFb-Tat complex to TAR.

5. P-TEFb AND CELL DIFFERENTIATION

In most eukaryotic cells examined, the level of CDK9 and cyclin T proteins does not vary during the cell cycle. However, an increase in P-TEFb kinase activity has been found upon activation of peripheral blood lymphocytes and differentiation of promonocytic cell lines (72,73), and up-regulation of P-TEFb activity has been correlated with an increase of cyclinT1 (72). Moreover, it has been shown that the levels of cyclin T1 are up-regulated by two independent signaling pathways triggered by phorbol myristate acetate or phytohemagglutinin (72). Moreover, following activation, CDK9/cyclin T1-mediated CTD kinase activity is greatly increased. Most notably, it has been recently shown that enforced expression of a catalytic inactive mutant of CDK9 (CDK9dn) in Jurkat T cells and U937 promonocytic cells sensitized cells to apoptosis, particularly after PMA-induced differentiation to macrophage-like cells (74). Because P-TEFb activity is induced in PMA-treated cells, it has been suggested that P-TEFb activity may play an antiapoptotic role during monocyte differentiation.

Accumulating evidence suggests a role of P-TEFb in mouse cell differentiation. Murine CDK9, which is 98% identical to the human protein, is expressed at high levels in brain and kidney tissues. Furthermore, the kinase activity and protein expression has been found highest in terminally differentiated tissues such as the muscle and brain (75).

Another example of the putative role of P-TEFb in cell differentiation comes from a study of *in vitro* induced differentiation of mouse myoblasts. While asynchronously growing C2C12 myoblasts containing the CDK9 protein localize only in the nucleus, *in vitro* differentiation induces a shift of the kinase into the cytoplasm (76). One possible explanation for the heterogeneous subcellular localization is that CDK9 is a protein that can shuttle between the nucleus and the cytoplasm. It has been recently reported that cyclin T1 and CDK9 co-localize throughout the non-nucleolar nucleoplasm, with an increased signal present at numerous nuclear foci (77). However, our unpublished data indicate that CDK9 can be found in both nuclear and cytoplasmic subcellular compartments, and it can shuttle between the nucleus and cytoplasm, and that Leptomycin B, a specific inhibitor of nuclear export, inhibits this process (G. Napolitano and B. Majello, *unpublished data*). It remains to be demonstrated whether the change of subcellular localization of CDK9 reflects altered P-TEFb activity.

6. THE FCP1 CTD-PHOSPHATASE

During the transcription cycle concomitant with or following the termination of transcription, dephosphorylation of the CTD must occur in order to regenerate the nonphosphorylated form of the enzyme that appears to be recruited to promoters. While several RNAPolII CTD kinases have been described, only a single CTD phosphatase named FCP1 has been isolated (figure 3). CTD phosphatase was originally purified from HeLa

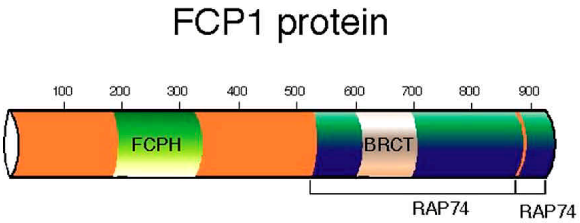

Figure 3. Schematic representation of the FCP1 protein phosphatase. The FCPH (FCP1 Homology domain), BRCT and the two RAP74 domains are indicated.

cells and subsequently from S. Cerevisiae (78,79,80). The activity of this CTD phosphatase was stimulated by the RAP74 subunit of the general transcription factor TFIIF. Using a two-hybrid screen with RAP74 as bait, the human FCP1 gene was isolated (18). Two forms of FCP1 were described, FCP1a, which encodes an 842-amino acid polypeptide, and a shorter splicing variant denoted as FCP1b. A third form of human FCP1 was subsequently isolated and denoted as FCP1 (16). Sequence comparison between FCP1 and FCP1a revealed that FCP1a denotes a truncated form of FCP1 lacking 120 aa at the N-terminus of the protein, while FCP1b lacks the last 139 amino acids of FCP1a. The homologous yeast gene, Fcp1, also interacts directly with RAP74. The primary sequences of both human and yeast FCP1 genes contain a motif common to a family of phosphatases that act on phosphate esters. This phosphatase motif is present within residues that are conserved among different putative CTD phosphatases and other database proteins of unknown function, and it has been designated as the FCP homology (FCPH) domain. The FCP1 protein has been classified as a type–2C-protein phosphatase (resistant to okadaic acid and requiring Mg2+ for activity). Another characteristic feature of FCP1 is the presence of a BRCT domain in the C-terminus of the protein partially overlapping with most of the 5' RAP74 binding region. The BRCT domain (BRCA1 C-terminus), first identified in the breast cancer suppression protein BRCA1, is an evolutionary conserved domain found in more than 50 proteins, many of which have defined roles in the cellular response to DNA damage (81). Although a common molecular function for this domain has not been uncovered, the BRCT sequences of some proteins serve as sites of protein-protein interaction (82).

6.1. Role of FCP1 phosphatase in transcription

It has been demonstrated that human FCP1 is able to dephosphorylate the CTD of RNAPII *in vitro*, and that the phosphatase activity resides in the N-terminus of the protein since deletion of the amino-terminal 119 amino acids results in a compromised phosphatase activity (16). Recent studies performed with yeast Fcp1 have demonstrated the importance of two Aspartates, D180 and D182, within the conserved phosphatase motif shared by FCP1 proteins and a small family of phosphotransferases and phosphohydrolases. It has been shown that the wild type Fcp1, but none of Fcp1 mutations in the two aspartate residues, is able to complement a chromosomal deletion of Fcp1, suggesting that Fcp1 functions as a phosphatase *in vivo* (83). However, it is still controversial whether the human FCP1 protein is the catalytic or the regulatory subunit of the CTD phosphatase. It has been found that the phosphatase activity of Fcp1 requires the presence of additional factors. Similarly, the human FCP1 phosphatase activity seems to require RNAPII and could not be reconstituted with the isolated CTD. However, recent results (84) demonstrate that a highly purified recombinant yeast Fcp1 copurifies precisely with an activity that dephosphorylates an *in vitro* labeled RNAPII CTD.

The effect of the FCP1 CTD-phosphatase on transcription has been studied using a highly purified reconstituted transcription system. These studies have demonstrated that the phosphorylated form of RNAPII is compromised in its ability to direct transcription because of a poor incorporation into the transcription initiation complexes. This phosphorylated form of RNAPII can be reactivated *in vitro* by addition of a catalytic active FCP1 protein, demonstrating the ability of this protein in recycling the RNA polymerase and allowing efficient incorporation of RNAPII into the transcription initiation complex (16). The overall effect of RNAPII dephosphorylation mediated by the FCP1 protein results in a global stimulation of elongation of nascent transcripts. Interestingly, *in vitro* stimulation of transcription elongation by FCP1 is independent of its catalytic activity. These findings suggest that FCP1 may remain associated with RNAPII during elongation.

FCP1 phosphatase activity has been also demonstrated to be required for a different step in the transcription cycle: the dissociation of capping enzymes from the elongation complex. Recent data have demonstrated that reduced CTD dephosphorylation during elongation in a Fcp1 ts-mutant prevents the dissociation of capping enzymes, implying that CTD dephosphorylation mediated by FCP1 is at least part of the signals for release of capping enzymes (85). Finally, genome-wide expression studies have shown that transcription by RNAPII in S. Cerevisiae generally requires Fcp1 activity. The experiments performed with a Fcp1 ts mutant in yeast revealed a large decline in the total amount of poly (A)+ mRNA after a shift to the non-permissive temperature (84). These results are sufficient to explain why FCP1 is an essential gene in yeast.

6.2. Role of FCP1 *in vivo*

The FCP1 protein was originally identified as a RAP74 interaction partner in a yeast two-hybrid screening. Interaction between RAP74 and Fcp1 likely involves amino acids conserved in yeast and human proteins, since yeast Fcp1 can bind human RAP74 and human FCP1a can bind yeast RAP74. Recently, a direct interaction between yeast Fcp1 and the general transcription factor TFIIB has been demonstrated. The binding sites for TFIIB have been shown to be very similar to the previously identified binding sites for RAP74. Accurate analysis of the protein sequences of RAP74 and TFIIB revealed a short amino acid sequence of high similarity between the two proteins, suggesting a common biological function (83). However, there is no evidence thus far of a physiological role of the FCP1-TFIIB interaction.

The finding that FCP1 is essential for the growth of S.Cerevisiae (17,84) highlights the importance of FCP1 *in vivo*. Moreover, this finding strongly suggests that dephosphorylation of the RNAPII CTD is likely an essential function in yeast. Genetic experiments showed that the BRCT domain is essential for viability in yeast. In fact, the integrity of this domain has been recently demonstrated to be essential for Fcp1 to function in S. Cerevisiae (83). Deletion mutants in the BRCT region and a specific Fcp1 ts mutant bearing a point mutation in the BRCT domain accumulate an excess of the hyperphosphorylated form of RNAPII, resulting in loss of viability (83). These results demonstrate that the integrity of the BRCT domain of Fcp1 is essential for cell viability and for CTD dephosphorylation activity *in vivo*.

6.3. FCP1 recruitment activates transcription

FCP1 has been found to be a component of the RNAPII holoenzyme complex as well as the general transcription factors RAP74 and TFIIB. A number of components of the RNAPII holoenzyme complex have been shown to be able to activate transcription when artificially tethered to a promoter via fusion to a heterologous DNA-binding domain (86,87,88). Recent data demonstrate that a LexA-Fcp1p fusion is able to activate a LacZ reporter gene bearing LexA binding sites. A deleted version of LexA-Fcp1p lacking the domain involved in binding to both RAP74 and TFIIB is impaired in its ability to activate transcription (83). Similar results have been obtained by our group for the human FCP1 protein in transient transfection in mammalian cells. We found that artificial recruitment of a fusion FCP1protein bearing the sequence-specific DNA binding domain GAL4, strongly activates transcription of promoters containing appropriate GAL4 DNA-binding sites. Similar to the results obtained for yeast Fcp1, we found that a deleted version unable to bind RAP74 is severely impaired in activating transcription (89). Collectively, these results reveal a good correlation between the ability of FCP1 to activate transcription *in vivo* when brought into the vicinity of a promoter and its ability to bind RAP74.

6.4. Tat and FCP1

As discussed in the above sections, cyclin T1, a subunit of the P-TEFb complex, is a dedicated human-specific cofactor for Tat, causing hyperphosphorylation of RNAPII CTD to produce full-length viral mRNAs. Because an increased level of CTD phosphorylation can also result from inhibition of CTD phosphatase this class of nuclear phosphatases might represent potential Tat cellular cofactors. It has been shown that Tat can down-regulate FCP1-mediated dephosphorylation of CTD *in vitro* (90). Furthermore, we and others have provided evidence that FCP1 binds directly to Tat (90,91). Thus, Tat has the ability to interact with proteins that can either phosphorylate or dephosphorylate the CTD. Moreover, we have recently found that over-expression of FCP1 suppresses Tat-activated expression of the HIV-1 LTR *in vivo*. We further show that FCP1 does not affect either HIV-1 basal activity or P-TEFb-mediated transcription, suggesting that FCP1 selectively inhibits Tat function. These findings suggest that over-expression of FCP1 might inhibit Tat function by inducing ectopic dephosphorylation of the CTD.

7. PERSPECTIVE

Elongation control of nascent transcripts seems to be have a central role in the regulation of expression of most cellular genes. The conserved CTD of the RNA polymerase II large subunit has been found to be a landing pad for positive and negative elongation factors. A determining role in processive elongation has been attributed to the positive transcription elongation complex P-TEFb. It has been proposed that P-TEFb determines the fate of RNAPII at the early phase of elongation causing RNAPII to become competent to produce full-length transcripts. Phosphorylation and dephosphorylation of the CTD conserved domain of RNAPII seem to be the key regulatory steps controlling the entry of the RNA polymerase II into processive elongation. Nonetheless, recent evidence suggests that phosphorylation of the CTD is invoked also in the regulation of different steps of the transcription cycle. Recent findings suggest that RNAPII phosphorylation allows the recruitment of processing factors, including capping, polyadenylation and splicing factors. On the other hand, phosphatase activity has been postulated to be necessary to regenerate the bulk of free unphosphorylated RNA polymerase II. Thus far, FCP1 is the only specific CTD phosphatase identified in both mammals and yeast. FCP1-mediated CTD dephosporylation has been shown to be required not only to recycle free RNA Polymerase II competent to re-enter the transcription cycle but also mediate dissociation of capping enzymes from the elongation complexes. Consequently, CTD phosphorylation and dephosphorylation seem to control different steps during the RNA polymerase II transcription cycle. A likely model would predict that negative and positive factors cause a dynamic and reversible form of RNAPII-CTD, which in turn will allow the assembly and replacement of factors required for the elongation and processing steps of the transcription cycle.

8. ACKNOWLEDGMENTS

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