Nuclear c-Abl Is a COOH-Terminal Repeated Domain (CTD)-Tyrosine Kinase-specific for the Mammalian RNA Polymerase II: Possible Role in Transcription Elongation

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Abstract
The c-Abl tyrosine kinase has been shown to interact with the COOH-terminal repeated domain (CTD) of mammalian RNA polymerase II and can phosphorylate the tyrosine residues in the CTD. Interestingly, the Drosophila or the yeast CTD were not efficiently phosphorylated by the mammalian c-Abl. This species-specificity was found to be determined by the extreme COOH-terminal CTD sequences that are not conserved through evolution. In vitro, COOH-terminal-truncated CTD could neither bind to, nor be phosphorylated by, c-Abl. In vivo, coexpression of a full length CTD prevents c-Abl from inducing the tyrosine phosphorylation of endogenous RNA polymerase II, and such inhibitory effect was not observed with the coexpression of COOH-terminal-truncated CTD. Serine/threonine phosphorylation of the CTD has been linked to the regulation of transcription elongation. Transcription from the human immunodeficiency virus type 1 (HIV-1) promoter requires CTD-phosphorylation, which is stimulated by the viral Tat protein through the recruitment of cellular Ser/Thr CTD kinases. In transient cotransfection experiments, the c-Abl kinase was found to activate the HIV promoter in the absence of Tat. The activation of the HIV promoter required the nuclear localization of c-Abl and could be correlated with increased tyrosine phosphorylation of RNA polymerase II. These observations suggest that tyrosine phosphorylation of the CTD may be functionally equivalent to its serine/threonine phosphorylation in stimulating transcription elongation.

Introduction
The product of c-abl proto-oncogene encodes a ubiquitously expressed tyrosine kinase that is localized in the nucleus and the cytoplasm of proliferating cells. The NH2-terminal half of the c-Abl protein contains the Src-homology domains 3, 2 and the tyrosine kinase domain (1). The COOH-terminal half of the protein contains three NLSs (2), and a nuclear export signal (3). In addition, the COOH-terminal region also contains three HMG-like boxes that bind A-T-rich DNA (4), an F-actin binding domain (5), a G-actin binding domain (6), and a CTD-ID5 that allows c-Abl to phosphorylate the CTD of RNAP II (7, 8). The c-Abl function is required for the normal growth and development because c-abl-deficient mice exhibit the phenotype of embryonic or neonatal lethality (9). Interestingly, neonatal lethality is also observed with mice that express a COOH-terminal-truncated c-Abl protein that has tyrosine kinase activity but lacks the DNA-binding domain, the actin-binding domain, and the CTD-ID (10). Thus, the biological function of c-Abl requires not only the kinase activity but also the other functional domains that direct this tyrosine kinase to interact with specific cellular components in the nucleus and the cytoplasm.

The CTD of RNAP II is a physiological substrate of the c-Abl tyrosine kinase. The CTD is composed of a consensus heptapeptide motif Tyr-Ser-Pro-Thr-Ser-Pro-Ser that is repeated 52 times in the mammalian RNAP II, 42 times in the Drosophila, and 26–27 times in the yeast RNAP II (11, 12). The c-Abl tyrosine kinase phosphorylates the mammalian CTD to a high stoichiometry (13). Besides the tyrosine kinase domain, three other functional domains of c-Abl are required for the high-stoichiometry phosphorylation of the CTD. The c-Abl SH2 domain binds to tyrosine-phosphorylated CTD and contributes to the processive phosphorylation (14). The CTD-ID of c-Abl binds to unphosphorylated CTD and contributes to the high-affinity interaction between c-Abl and the CTD (7). The DNA binding function of c-Abl is also required, which suggests that c-Abl phosphorylates template-bound RNAP II (7). The large subunit of RNAP II, which contains the CTD, is tyrosine-phosphorylated in cells (13). Interestingly,
the phosphorytrosine content of the large subunit of RNAP II is found to be increased on treatment of cells with DNA-damaging agents such as methylmethane sulfonate (15) or IR (16). This methylmethane sulfonate- and IR-induced tyrosine phosphorylation of RNAP II is not observed in cells lacking either c-Abl (15) or ATM, which has been shown to be required for IR to activate the nuclear c-Abl tyrosine kinase (16). Taken together, the evidence supports a DNA damage-activated pathway in which c-Abl activation by ATM leads to the tyrosine phosphorylation of RNAP II.

Phosphorylation of the CTD plays an important role in the regulation of transcription. The CTD of RNAP II is reversibly phosphorylated on Ser, Thr, and Tyr (17–19). Phosphorylation of the CTD occurs after the formation of the initiation complex and before the processive elongation of transcription (19). The CTD is dephosphorylated by specific phosphatases, and it is the dephosphorylated RNAP II that forms the initiation complex (19–21). Regulated phosphorylation of the CTD is, therefore, a potential mechanism for the control of transcription. A major CTD kinase of mammalian cells is a component of the basal transcription factor TFIIH. TFIIH phosphorylates both the tyrosine and threonine residues of the CTD, enhancing the efficiency of transcription elongation (24–26). The CTD is, therefore, a potential mechanism for the control of transcription. To determine whether tyrosine phosphorylation of the CTD may act in a concerted and sequential manner to achieve the hyperphosphorylation of the CTD and the efficient elongation from the HIV promoter (23).

Because hyperphosphorylation of the CTD by two different CTD kinases seems to be required for transcription from the HIV promoter, it suggests that the RNAP II complex assembled at this promoter is highly sensitive to the phosphorylation status of the CTD. To determine whether tyrosine phosphorylation of the CTD can substitute for Ser/Thr phosphorylation, we tested whether the c-Abl tyrosine kinase can phosphorylate the CTD of RNAP II. The results have led to the proposal that TFIIH and P-TEFb may act in a concerted and sequential manner to achieve the hyperphosphorylation of the CTD and the efficient elongation from the HIV promoter (23).

Results
c-Abl Does Not Phosphorylate the Drosophila or the Yeast CTD. The CTD of RNAP II is composed of heptapeptide repeats with the consensus sequence YSPTSPS. The repeats are highly conserved, the extreme COOH terminus of the CTD is not conserved, and the sequences of mouse, fly, and yeast CTD (Fig. 1). We have shown previously (13) that c-Abl can phosphorylate the mammalian CTD to high stoichiometry in vitro. To determine whether c-Abl can phosphorylate the yeast and Drosophila CTD, each of the CTDs was expressed as a GST–fusion protein in Escherichia coli and purified by glutathione-agata-
The three different GST-CTD fusion proteins were then incubated with immunoprecipitated c-Abl tyrosine kinase or, as a control, purified cdc2/cyclin B kinase and radiolabeled ATP (Fig. 1A). Consistent with previous results, the mouse GST-CTD fusion was phosphorylated by c-Abl on tyrosines (Fig. 1A, Lane 4). Under the same conditions, neither the yeast nor the Drosophila GST-CTD fusion were efficiently phosphorylated by the c-Abl kinase (Fig. 1A, Lanes 5 and 6). In contrast, all three of the GST-CTD fusion proteins were phosphorylated by cdc2/cyclin B kinase (Fig. 1A, Lanes 1–3). The presence of equal amounts of each of the GST-CTD proteins in the kinase reactions was verified by amido-black staining (Fig. 1B).

It is possible that the divergent sequence at the COOH terminus of CTD constitutes a landing site for the c-Abl tyrosine kinase, and this explains the inability of c-Abl to recognize the yeast and the Drosophila CTD as substrates. If so, the divergent COOH-terminal sequence would be expected to interfere with the phosphorylation of the mammalian CTD by c-Abl. To test this, a peptide comprising the last 18 amino acids of the mouse CTD was synthesized (Fig. 2A). As a control, another peptide of identical amino acid composition but with a random sequence was also synthesized and tested (Fig. 2A). The synthetic peptides were then incubated with GST-CTD fusion proteins and incubated with GST-CTD in the presence of increasing concentrations of a specific (Lanes 1–4) or nonspecific (Lanes 5–8) peptide. Each of the reactions contained equal amounts of Abl and 0.2 μg of GST-CTD. Lanes 1–4 contained 0, 50, 200, and 500 ng of the specific peptide, and Lanes 5–8 contained the nonspecific peptide at 0, 50, 200, and 500 ng. The reaction was carried for 30 min, and the product was analyzed by resolving on a 8% SDS-PAGE and by autoradiography.

Taken together, results shown in Fig. 1 and Fig. 2 strongly suggest that the divergent amino acid sequence at the extreme COOH terminus of CTD is important for c-Abl-mediated tyrosine phosphorylation.

Overexpression of a GST-CTD Fragment Inhibits Tyrosine Phosphorylation of RNAP II by c-Abl In Vivo. Previously, we showed that the coexpression of c-Abl or Arg with a GST-CTD (1–52) in COS cells led to the tyrosine phosphorylation of enolase by c-Abl (not shown).
sequences of the CTD are required for c-Abl-mediated tyrosine phosphorylation, we performed competition experiments to test whether the coexpression of CTD could interfere with the tyrosine phosphorylation of the endogenous RNAP II (Fig. 7). In these experiments, we used the human osteosarcoma cells Saos-2 because these cells could be easily transfected to a high efficiency. When Saos-2 cells were transiently transfected with a plasmid expressing c-Abl, a slower migrating RNAP II band was detected by antiphosphotyrosine antibody (Fig. 5B, Lane 3) that was not detected in cells transfected with two different control vectors (Fig. 5B, Lanes 1 and 2). This slower migrating band represented a small fraction of the total RNAP II inasmuch as it was not detected by anti-RNAP II immunoblotting (Fig. 5A). The slower migration of this band was likely due to a high stoichiometry of tyrosine phosphorylation catalyzed by the transfected c-Abl tyrosine kinase. When c-Abl was coexpressed with the full-length CTD, expressed from the pEBG vector as a GST-fusion, the appearance of this slower migrating PTyr band was blocked (Fig. 5B, Lane 4). The GST-CTD became tyrosine-phosphorylated instead (not shown; see Ref. 7), consistent with substrate competition between the transfected GST-CTD and the endogenous RNAP II.

Cotransfection with the COOH-terminal-truncated CTD 1–23, however, did not inhibit the tyrosine phosphorylation of

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**Fig. 3.** Phosphorylation of full-length and various deleted forms of CTD by Abl kinase. **A,** schematic representation of full-length and deletion mutants of CTD. Full-length CTD contains 52 repeats (open boxes) and the additional nonconserved COOH-terminal sequence (filled box). CTD 1–47 contains the first 47 repeats. CTD 1–23 contains the first 23 repeats. The internal-truncation mutant CTD Δ23–39 lacks repeat units 23–39. **B,** murine type IV c-Abl was in vitro translated using reticulocyte lysate and immunoprecipitated using anti-Abl antibody (8E9). The immune complexes were incubated with increasing amounts of full-length and mutant CTD expressed and purified from E. coli. The kinase reactions were carried out as described in “Materials and Methods.” The reactions were terminated with the addition of equal amounts of 3× SDS-sample buffer and resolved on a 5–15% SDS-PAGE. The gel was transferred to Immobilon-P and exposed for autoradiography. The amount of CTD present in the kinase reactions was 10 ng (Lanes 1, 4, 7, and 10); 50 ng (Lanes 2, 5, 8, and 11); and 200 ng (Lanes 3, 6, 9, and 12). **C,** expression and purification of wild-type and various deletion-mutants of CTD shown in panel A as GST-fusion proteins.

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**Fig. 4.** Binding of Abl-CTD-ID (GSE) to full-length and deletion mutants of CTD. His-tagged-CTD was in vitro translated in the presence of [35S]methionine. The lysate was incubated with glutathione-agarose beads containing approximately 0.2 mg of GSE [GST-fusion containing the CTD-ID of c-Abl (7)], or 0.2 mg of GXS [GST-fusion containing the DNA binding domain of c-Abl (4)], and the bound fraction was recovered. The input Lanes (1, 4, 7, and 10) contained 10% of the lysate used in the binding reaction. The samples were resolved on a 5–15% SDS-PAGE, transferred to Immobilon-P, and exposed for autoradiography. [35S]methionine-labeled CTD bound to the GXS beads were loaded in Lanes 2, 5, 8, and 11. [35S]methionine-labeled CTD bound to the GSE beads were loaded in Lanes 3, 6, 9, and 12. Full length CTD was used in Lanes 1–3; CTD 1–23 in Lanes 4–6; CTD Δ23–39 in Lanes 7–9; and CTD 1–47 in Lanes 10–12.
RNAP II (Lane 5). The expression of CTD Δ 23–39 also led to the inhibition of RNAP II tyrosine phosphorylation (Lane 6). The specificity of the anti-PTyr antibody was established by competition with phosphotyrosine (Panel C). The overexpression of equal amounts of c-Abl in these transfections is shown in the Abl immunoblot (Panel D). These observations were consistent with the in vitro results and further supported the requirement of the COOH-terminal divergent sequences for c-Abl-mediated tyrosine phosphorylation of RNAP II in vivo.

Previous studies have identified the SH2 domain (14), and the CTD-ID (7), of c-Abl to be required for the tyrosine phosphorylation of the CTD. Results shown here in Fig. 1–5 have identified the extreme COOH-terminal region of the mammalian CTD as a binding site for the CTD-ID of c-Abl. These multiple interactions between c-Abl and the CTD of mammalian RNAP II are summarized in a schematic model (Fig. 6). Because c-Abl contains multiple interaction domains with the CTD and has been linked to the tyrosine phosphorylation of RNAP II in vivo, this CTD-tyrosine kinase is likely to participate in the regulation of transcription.

Activation of HIV Promoter by Ectopically Expressed c-Abl. Previous studies have shown that c-Abl can activate transcription by 2- to 3-fold from several different promoters, i.e., tk, E2, and c-fos (7, 32, 33). Thus, in transient cotransfection assays, c-Abl enhanced transcription without promoter specificity. The 2- to 3-fold activation of transcription by c-Abl required its kinase activity, nuclear localization, the SH2 domain, and the CTD-ID (7, 32, 33; Fig. 6). Because the phosphorylation of CTD has been linked to transcription elongation, we hypothesized that the small and promoter nonspecific effect of c-Abl on transcription might be due to a stimulation of elongation through the direct interaction with the CTD of RNAP II (such interactions are summarized in Fig. 6). To further evaluate this hypothesis, we tested the effect of c-Abl on the HIV promoter because this promoter is strongly regulated by the control of elongation (23, 24). We reasoned that if c-Abl could stimulate transcription elongation, it...
should have a much stronger effect on the HIV promoter in transient cotransfection experiments.

Transcription from the HIV promoter requires the viral Tat protein (23). One of the functions of Tat is to recruit cellular CTD kinases to the HIV promoter complex (23). This finding suggests that the phosphorylation of the CTD is a rate-limiting step in transcription from the HIV promoter. To determine whether c-Abl could also activate this promoter, we performed transient cotransfection assays using a reporter of CAT driven by the HIV promoter (from nucleotide -633 to +185 of HIV-1). The human Jurkat-T cells were cotransfected with the HIV-CAT reporter and plasmid expressing either the HIV Tat protein or the c-Abl tyrosine kinase (Fig. 7). Cotransfection with the HIV Tat expression plasmid led to, on average, a 20-fold increase in CAT activity (Fig. 7A). The treatment of cells with the phorbol ester TPA, which activates the transcription factor NF-kB in Jurkat-T cells, has been shown to synergize with Tat in activating the HIV promoter (24). This synergy between Tat and TPA was also observed in our experiments, resulting in a 50- to 60-fold activation of the HIV promoter (Fig. 7A, +TPA). Consistent with previously reported results (34), treatment of vector-transfected cells with TPA did not activate the HIV promoter, demonstrating an essential role of Tat (Fig. 7A). Interestingly, cotransfection with c-Abl expression plasmid activated the HIV promoter by 6- to 10-fold in the absence of Tat. The activation induced by the ectopic expression of c-Abl was also enhanced by treatment with TPA, leading to a 40- to 60-fold activation of the HIV promoter (Fig. 7A). The combined expression of Tat and c-Abl did not further increase the degree of activation, regardless of the absence or the presence of TPA (Fig. 7A). Because their stimulatory effects were not additive, Tat and c-Abl were likely to have acted through a similar mechanism, i.e., CTD phosphorylation.

A Tat-responsive element (TAR) has been identified in the HIV genome (nucleotide 24 to 39). The Tat protein binds to the TAR RNA and recruits the cellular CTD kinases to phosphorylate RNAP II (23). Deletion of TAR has been shown to compromise, although not eliminate, the effect of Tat on the HIV promoter activity because Tat can also stimulate transcription initiation (24). To examine whether TAR is required for c-Abl to activate this promoter, we used a CAT reporter driven by a TAR-deleted HIV promoter (Fig. 7B). In Jurkat-T cells, the Tat protein activated the TAR-deleted promoter by 10-fold instead of the 20-fold observed with the wild-type HIV promoter (compare Fig. 7A and 7B). The combined effect of Tat and TPA led to a 20-fold increase in promoter activity on average (Fig. 7B). Thus, deletion of the TAR reduced the extent by which Tat activated the HIV promoter. The c-Abl tyrosine kinase activated the TAR-deleted promoter by 10-fold, similar to the activation observed with the wild-type HIV promoter (compare Fig. 1A and 1B). The combined effect of c-Abl and TPA increased the HIV(ΔTAR) promoter activity by 30- to 40-fold, which was within the range of activation observed with the wild-type HIV promoter (compare Fig. 7A and 7B). Overall, Tat activated the wild-type HIV promoter better than c-Abl (Fig. 7A), whereas c-Abl activated the HIV (ΔTAR) promoter better than Tat (Fig. 7B). Taken together, these results showed that c-Abl can activate the HIV promoter in the absence of Tat. The c-Abl-mediated activation does not seem to be dependent on the TAR element. However, c-Abl and Tat are likely to act through a similar mechanism because their effects are not additive.

Activation of HIV Promoter Requires the Nuclear Localization of c-Abl Kinase. The c-Abl tyrosine kinase is localized to the cytoplasm and the nucleus (3). If c-Abl activates the HIV promoter through the tyrosine phosphorylation of the CTD, then the cytoplasmic c-Abl should not have this activity. We have recently shown (3) that c-Abl shuttles between the nuclear and the cytoplasmic compartments because it contains a NES. Whereas the wild-type c-Abl is distributed in both compartments, the NES-defective c-Abl is found to be exclusively nuclear (3). In addition, Wen et al. (2) have identified three NLS sequences in c-Abl and have mutated all three of the NLS to create a c-Abl mutant that is exclusively cytoplasmic. To distinguish between the nuclear and cytoplasmic c-Abl, we coexpressed the NES- and the NLS-defective c-Abl with the HIV-CAT reporter (Fig. 8). A
kinase-defective c-Abl was also included in the experiment. The NES-defective c-Abl activated the HIV-CAT by 5-fold (Fig. 8A). This activity was lower than the wild-type c-Abl, possibly because the NES-defective c-Abl accumulated to a lower level in the transfected cells (Fig. 8B; compare Lanes 2 and 4). In contrast, the NLS-defective c-Abl, which cannot be translocated into the nucleus, was unable to activate the HIV-CAT (Fig. 8A), despite a level of expression comparable to the wild-type c-Abl (Fig. 8B; compare Lanes 2 and 5). The combined expression of both the NES and the NLS mutants, restoring c-Abl in both subcellular compartments, gave a level of activation similar to that observed with the NES-mutant alone (Fig. 8A). Thus, the cytoplasmic pool of c-Abl did not seem to make any contribution to the observed activation of the HIV promoter. As expected, the kinase-defective c-Abl did not stimulate HIV-CAT activity (Fig. 8A). These results demonstrate that the nuclear localization of an active c-Abl tyrosine kinase is required for the activation of the HIV promoter. The cytoplasmic pool of c-Abl does not seem to make any contribution to the regulation of the HIV promoter in these Jurkat cells.

Overexpression of CTD Fragment Inhibits the Activation of HIV Promoter by c-Abl. In other experiments, we found that mutation of the SH2 domain or the CTD-ID of c-Abl also abolished the trans-activating function of c-Abl (not shown). To further determine whether tyrosine phosphorylation of RNAP II was involved in the activation of HIV promoter, we tested whether the CTD fragments (Fig. 3A and Fig. 5B) could interfere with the c-Abl-mediated activation of HIV-CAT (Fig. 9). In Saos-2 cells, transfection of HIV-CAT with c-Abl led to a 3- to 5-fold activation of the HIV-promoter activity (Fig. 9, Vector and c-Abl). Cotransfection with the full-length CTD fragment (1–52) abolished this activation (Fig. 9, CTD 1–52 + c-Abl). Cotransfection with the internal-deletion mutant CTD Δ23–39 also blocked the activation; however, cotransfection with the two COOH-terminal-truncation mutants (CTD 1–23 and CTD 1–47) did not affect the trans-activating function of c-Abl. These results correlated well with those shown in Fig. 5 and suggested that the increased tyrosine phosphorylation of the endogenous RNAP II was important for the observed activation of the HIV promoter in these transient cotransfection experiments.

Discussion

Multiple Interactions between c-Abl and the Mammalian CTD. Previously, we reported that c-Abl can phosphorylate and associate with the CTD of RNAP II (7). A CTD-ID has
Tat-independent Activation of HIV Promoter by c-Abl

Fig. 9. Overexpression of CTD fragments prevents c-Abl from activating the HIV-1-LTR-CAT. Saos-2 cells were cotransfected with 0.5 μg of pBK-CMV expression c-Abl, 0.1 μg of the HIV-LTR reporter construct, and 2.5 μg each of the pEBG plasmids expressing either full-length or deleted CTD (CTD 1–23, CTD Δ23–39, and CTD 1–47). At 48 h after transfection, cells were harvested and CAT assays were performed as described previously (41). Values represent the percent acetylation of [14C]chloramphenicol, normalized to β-galactosidase activity. The means and SDs of results from three independent experiments are shown.

been mapped in c-Abl, and this region has been shown to be conserved in the Abl-related tyrosine kinase Arg (8). The binding site for the CTD-ID, based on the results here, involves the extreme COOH-terminal sequences of the CTD. We found that a full-length CTD fragment (with 52 repeats) and an internal-truncation mutant of CTD (Δ23–39) were phosphorylated by c-Abl in vitro and could inhibit the tyrosine phosphorylation of RNAP II by c-Abl in transfected cells. On the other hand, two COOH-terminal-truncation mutants (CTD 1–23 and CTD 1–47) were not phosphorylated by c-Abl in vitro and had no inhibitory effects on the tyrosine phosphorylation of RNAP II in transfected cells. That the extreme COOH-terminal sequences of the mammalian CTD are important in the c-Abl-catalyzed tyrosine phosphorylation is also supported by two other lines of evidence: (a) c-Abl does not phosphorylate the Drosophila or the yeast CTDs, which are divergent from the mouse CTD at the extreme COOH terminus; and (b) a peptide corresponding to the divergent COOH-terminal sequence of the mammalian CTD inhibits the c-Abl-mediated tyrosine phosphorylation of the CTD in vitro. Finally, the full-length CTD could bind to a c-Abl domain that contains the previously defined CTD-ID, but the COOH-terminal-truncated CTD did not bind to the CTD-ID under identical experimental conditions.

Taken together, three different interactions between c-Abl and the CTD have been identified (summarized in Fig. 6):

(a) there is the kinase-substrate interaction at the catalytic center. The close proximity of proline to tyrosine in the CTD is likely to be important for catalysis because the c-Abl kinase domain has been shown to select tyrosines in a consensus sequence of YXXP (35);

(b) there is the SH2-Ptyr interaction that allows c-Abl to associate with tyrosine-phosphorylated CTD (14). Previous study (14) has demonstrated the importance of this interaction in the processive phosphorylation of CTD to a high stoichiometry; and

(c) there is the interaction between the CTD-ID of Abl with the extreme COOH terminus of the CTD. The interaction through the Abl CTD-ID has previously been shown (7) to account for the 1 μM $K_m$ of the mammalian CTD for the c-Abl kinase. This interaction can also account for the inefficient phosphorylation of the Drosophila and the yeast CTDs by c-Abl.

The direct interaction between c-Abl and the CTD may explain the nonspecific activation of several promoters by c-Abl in transient transfection assays. Physiologically, regulated increase in the tyrosine phosphorylation of RNAP II by DNA-damaging agents has been shown to require the activation of nuclear c-Abl tyrosine kinase (15, 16).

Tyrosine Phosphorylation of the CTD and Transcription Regulation. Phosphorylation of the CTD by Ser/Thr kinases has been correlated with promoter clearance and transcription elongation (19). In addition to the basal transcription factor TFIIH and the elongation factor P-TEFb, a yeast CTD kinase I, has also been shown to stimulate transcription by modulating the efficiency of transcription elongation (36). The observation that c-Abl can activate the HIV promoter in the absence of Tat supports the notion that tyrosine phosphorylation of CTD may also regulate promoter clearance and transcription elongation. Although these results provided a strong correlation, they did not prove that CTD-tyrosine phosphorylation was directly involved in the activation of the HIV promoter. For example, the ectopically produced c-Abl may activate another factor to release the paused polymerase complex. The direct demonstration that c-Abl can phosphorylate the CTD of RNAP II at the HIV promoter will await further experimentation with in vitro transcription systems and purified proteins.

Although c-Abl can activate the HIV promoter in transient cotransfection experiments, there is no evidence that c-Abl has a physiological role in the regulation of HIV gene expression. The observed stimulatory effect on the HIV promoter was likely due to the overproduction of c-Abl, which may be recruited to the HIV promoter through its interaction with the CTD. At its physiological levels, the nuclear c-Abl tyrosine kinase is not likely to be recruited to the HIV promoter. However, c-Abl has been found to associate with sequence-specific DNA binding complexes, including RB-E2F (33) and RFX1 (37). It is conceivable that the endogenous c-Abl may phosphorylate the CTD of RNAP II at specific cellular promoters to regulate transcription elongation.

A number of protein kinases have been found to phosphorylate the CTD. The most notable among them is the cyclin-dependent kinase CDK7 present in the basal transcription factor TFIIH, which has been shown to function as a major CTD kinase in vivo (22). With the HIV promoter, at least one other CTD kinase (in P-TEFb) recruited by the Tat protein is additionally required for transcription (23). Given this precedent, CTD kinases such as c-Abl may have the potential to phosphorylate the CTD in a promoter-specific manner. The nuclear c-Abl tyrosine kinase is activated at the G1-S transition and further activated after DNA damage (16, 32, 38).
Increase in the tyrosine phosphorylation of the RNAP II largest subunit has been observed after the exposure of cells to DNA-damage agents, and this increase was dependent on the activation of c-Abl tyrosine kinase (15, 16). Thus, c-Abl tyrosine kinase may act as a transducer of either S-phase or DNA-damage signals to regulate transcription elongation of specific genes through the tyrosine phosphorylation of the CTD.

Materials and Methods

Cell Culture and Transfections. Saos-2 cells were cultured in DMEM containing 10% supplemented calf serum (HyClone). Jurkat-T cells were cultured in RPMI 1640 with 10% fetal bovine serum (Intergen). Cells were grown at 37°C in 5% CO₂ and in the presence of the following supplements: glutamine (2 mM), penicillin G (100 units/ml), streptomycin (100 μg/ml), and streptomycin (100 μg/ml). Transfections were carried out using lipofectamine reagents as per manufacturer’s protocol (Life Technologies, Inc.). For RNAP II phosphotyrosine content analysis, cells were cotransfected with a ouabain-resistant plasmid, and the transfected cells were selected by a 24-h incubation with 1 μM ouabain before harvesting (7).

Plasmids. Full-length murine type IV Abl was expressed from a CMV-based promoter construct, pB-KCMV (32). The coding sequence of a murine CTD in pGEX-2T (39) was transferred to the pRSET vector (Invitrogen) for the expression of GST-CTD protein. The pEBG-Drosophila CTD 1–23 were constructed by digestion with the indicated restriction enzymes and the rejoinder of the fragments. The CTDs of Drosophila and yeast RNAP II were subcloned into pGEX-2T (Invitrogen) vector to express GST-CTD protein. The pEBG-GST-CTD plasmid that expresses the wild-type-CTD as GST-fusion proteins into pGEX-2T (InVitrogen) vector to express GST-CTD protein. The pEBG-Drosophila and yeast RNAP II were subcloned into pGEX-2T (Invitrogen) vector to express GST-CTD protein. The pEBG-GST-CTD plasmid that expresses the wild-type-CTD as GST-fusion proteins were obtained by subcloning the 1.1-kb BamHI-EcoRI fragment encoding the CTD from pGEX-2T-CTD into pEBG (7). Mutants of CTD were constructed by digestion at the indicated restriction enzymes and the rejoinder of the fragments. Thus, the truncation mutants CTD 1–47 and CTD 1–23 were constructed by digestion with EcoRI plus either Ddel or Spel. CTD Δ23–39 was obtained by digestion with Spel and rejoinder of the fragments. The CTDs of Drosophila and yeast RNAP II were subcloned into pGEX-2T (Invitrogen) vector to express GST-CTD protein. The pEBG-GST-CTD plasmid that expresses the wild-type-CTD as GST-fusion proteins were obtained by subcloning the 1.1-kb BamHI-EcoRI fragment encoding the CTD from pGEX-2T-CTD into pEBG (7). The mutant pEBG-GST-CTD (CTD 1–23, CTD 1–47, and CTD Δ23–39) constructs were made by subcloning various CTD fragments from pGEX-CTD constructs into pEBG.

Antibodies. Polyclonal antibody against the RNAP largest subunit was kindly provided by Dr. Mike Dahnus (University of California, Davis, CA). Monoclonal anti-PTyr (PY20) was provided by Dr. Bart Sefton (Salk Institute, La Jolla, CA). Monoclonal anti-Abl antibody (E99) was purchased from PharMingen. Horseradish peroxidase (HRP)-conjugated secondary antibody against mouse IgG or rabbit antibody was purchased from Life Technologies, Inc.

Immunoprecipitation and Kinase Reaction. Full-length Abl protein was obtained in vitro translation with TNT reticulocyte lysate and T3 RNA polymerase (Promega). Abl immunoprecipitation and CTD kinase reaction were carried out as described previously (13). The amounts of CTD used in the kinase reactions are given in the figure legends. For cdc2/cyclin B kinase assays, mitotic HeLa cells obtained by nocodazole treatment and mitotic shake-off, were lysed in 1x radioimmunoprecipitation assay buffer containing [25 μg Tris-HCl (pH 7.4), 50 μg NaCl, 0.5% sodium deoxycholate buffer, 0.1% SDS, 0.2% NP40, and 0.05 mg/ml aprotinin and leupeptin], and cdc2 kinase was immunoprecipitated using anti-cdc2 antibody (40). The beads were washed five times in radioimmunoprecipitation assay buffer and twice with kinase buffer containing 50 μg Tris-HCl (pH 7.4), 10 μg MgCl₂, and 1 mM DTT. The kinase reaction was initiated by the addition of the CTD substrate and 20 μCi of [γ-32P]ATP at 700 Ci/mmol of ATP in a final volume of 20 μl. The reaction was terminated by the addition of an equal volume of SDS-sample buffer. The samples were resolved on a 5% SDS-PAGE transferred to Immobilon-P and exposed for autoradiography.

CAT Assays. At 48 h after transfection, the cells were harvested, and extracts were prepared as described (41). CAT assays were performed as described previously (41). Transfection efficiencies were normalized by cotransfection of β-galactosidase and measurement of that enzyme activity.

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