Binding of HTm4 to Cyclin-dependent Kinase (Cdk)-associated Phosphatase (KAP)–Cdk2-Cyclin A Complex Enhances the Phosphatase Activity of KAP, Dissociates Cyclin A, and Facilitates KAP Dephosphorylation of Cdk2*

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Cyclin-dependent kinase 2 (cdk2) activation requires phosphorylation of Thr160 and dissociation from cyclin A. The T-loop of cdk2 contains a regulatory phosphorylation site at Thr160. An interaction between cdc-associated phosphatase (KAP) and cdk2 compromises the interaction between cdk2 and cyclin A, which permits access of KAP, a Thr160-directed phosphatase, to its substrate, cdk2. We have reported that KAP is bound and activated by a nuclear membrane protein, HTm4. Here, we present in vitro data showing the direct interaction between the HTm4 C terminus and KAP Tyr141. We show that this interaction not only facilitates access of KAP to Thr160 and accelerates KAP kinetics, but also forces exclusion of cyclin A from the KAP-cdk2 complex.

HTm4 (MS4A3) is the third member of subfamily A in an extensive membrane-spanning four-domain gene family. These genes are only loosely related at the sequence level, but their encoded proteins share a common four-transmembrane topology, including CD20 (MS4A1) and FcεRI (MS4A2). To date, few functions for the MS4 family of proteins have been ascribed. However, a diverse functionality is beginning to emerge. These functions include roles such as cell surface signaling receptors and intracellular adapter proteins (1–5, 22).

Cell cycle progression is regulated by the sequential activation, and inactivation, of the cyclin-dependent kinases (cdks).1 Cdk2, themselves, are substrates for regulatory phosphorylation and dephosphorylation. The phosphorylation status of cdks is controlled by a discrete class of regulatory kinases and phosphatases (6). Cdk activity is controlled by activatory and inhibitory proteins, the latter exemplified by p21 and p27. Binding of inhibitors such as p21 and p27 to different cyclin-cdk complexes is sufficient to arrest the cell cycle (7–12). Conversely, inactive, monomeric cdk can be activated via association with a specific cyclin and the concurrent phosphorylation of a conserved and essential threonine residue, such as threonine 160 (Thr160) in cdk2, which is located within the activation segment (T-loop) of the kinase.

In addition to cyclin association, full activation of cdk2 requires phosphorylation of Thr160 and dephosphorylation of Thr14 and Tyr15. Thr160 of cdk2 is phosphorylated by CAK (cdk-associated kinase), whereas its dephosphorylation is critical for inactivation. This dephosphorylation is executed by a serine/threonine-directed phosphatase, KAP. It has been shown that exogenous expression of KAP slows the G1 phase cell cycle progression in HeLa cells and that aberrant KAP transcripts are detected in some hepatocellular carcinomas (13, 14). These observations suggest that KAP has the same biological effects as cdk inhibitors, although their modes of actions are different. KAP can bind to cdk2 either in the presence or absence of cyclins (15–17). However, KAP can only dephosphorylate cdk2 when cyclin A is degraded or disassociated, in a mechanism that may control access of KAP to its substrate Thr160 (16). Cyclin binding may therefore control access of KAP to its substrate phosphothreonine 160. We reported previously that HTm4 interacts directly with the KAP via its C terminus (18, 19) and that exogenous expression of HTm4 leads to dephosphorylation of cdk2 and cell cycle arrest at the G2/M phase. Here, we show that the presence of HTm4 in the KAP-cdk2-cyclin A complex controls cdk2 activity in a dual fashion. First, HTm4 binding causes exclusion of cyclin A from its interaction with cdk2. Second, HTm4 binding potentiates KAP enzymatic activity and causes conformational changes that regulate access to Thr160.

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**RESULTS**

**Direct Binding of KAP and HTm4 in Vitro**—We have shown previously that HTm4 and KAP phosphatase may be coimmunoprecipitated from hematopoietic cells (18). Because these data form the basis for a novel model where HTm4 is a component of the cell cycle machinery, it is important that we determine whether the interaction is direct or if it involves an intermediary protein. We have since established a purified protein-protein interaction system that allows us to examine whether HTm4 and KAP interact directly.

His-tagged HTm4 was produced in *E. coli* and purified by fast protein liquid chromatography (Fig. 1a). The eluted peak corresponding to HTm4 was visualized by Coomassie Blue staining (Fig. 1b) and then analyzed by Western blot. Both anti-HTm4 and anti-His antibodies detected a 27-kDa band corresponding to HTm4 (Fig. 1, c and d). Surface plasmon resonance was used to detect direct binding of purified HTm4 to KAP. Full-length KAP protein was coupled to a sensor chip CM-5 shell (Amersham Biosciences). Compared with either uncoupled or nonspecifically coupled chips (coupled to anti-β2m antibody BMB.1, an immediate increase in mass was observed when either full-length HTm4 (Fig. 2a) or C-terminal peptide from HTm4 (Fig. 2b) was passed over KAP-coupled sensor chips. Moreover, premixing HTm4 with KAP abolished the KAP/HTm4 selective binding (data not shown). These in vitro data confirm that HTm4 and KAP interact directly and that the HTm4 C terminus is necessary and sufficient to mediate binding to KAP.

**KAP Phosphatase Activity Is Enhanced in the Presence of HTm4**—HTm4 is a direct binding partner for KAP, as shown in our previous yeast two-hybrid data. We hypothesize that HTm4 may regulate the enzymic activity of the KAP phosphatase. In the current experiment, KAP phosphatase activity was measured using pNPP as a generic substrate for KAP. In the presence of HTm4 and the cdk2-cyclin A-binding complex, a biphasic
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**FIG. 1.** Purification and characterization of HTm4. *a*, elution of His-HTm4 from a nickel column. His-tagged HTm4 was expressed in E. coli, refolded with the column methods described, and eluted with a gradient concentration of 0.5 M imidazole. *b*, the eluted peak corresponding to HTm4 was visualized by Coomassie Blue staining. *c*, Western blot analysis of HTm4 fraction of E. coli lysate. The HTm4-containing fraction was confirmed by Western blot using an anti-HTm4 antibody. *d*, Western analysis of HTm4 fraction of E. coli lysate. The HTm4-containing fraction was confirmed by Western blot with anti-His tag antibody.

**FIG. 2.** KAP and HTm4 interaction by BioSensor analysis. *a*, binding of full-length HTm4 protein to KAP. The interactions of HTm4 with immobilized proteins were analyzed on a sensor chip (CM-5) cell. KAP was coupled to the surface of the sensor chip at 2,800 RU; nonspecific human immunoglobulin (NS) was coupled to the surface of the sensor chip (anti-β2m antibody BBM.1) at 2,900 RU; UC, uncoupled sensor chip. Binding of 20 μM full-length HTm4 protein in 50 mM HEPES containing 1 mM EDTA, 1 mM DTT, 5% glycerol, and 0.1% Tween 20 was examined at a flow rate of 10 μl/min. *b*, binding of HTm4 C-terminal to KAP. The interactions of HTm4 with immobilized proteins were analyzed on a sensor chip (CM-5) cell. KAP was coupled to the surface of the sensor chip at 2,800 RU; nonspecific human immunoglobulin was coupled to the surface of the sensor chip (anti-β2m antibody BBM.1) at 2,900 RU. Binding of 1 mM HTm4 C-terminal peptide in 50 mM HEPES containing 1 mM EDTA, 1 mM DTT, 5% glycerol, and 0.1% Tween 20 was examined at a flow rate of 10 μl/min. Responses from uncoupled cells were subtracted.

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sic kinetic for KAP activity was observed. As shown in Fig. 3a, after the addition of pNPP substrate, an exponential phase (in the first 20 min) was followed by a plateau phase (after 30 min). The presence of HTm4 linearly increased the phosphatase activity of KAP (y = 1.45x + 0.01; r² = 0.98) (Fig. 3b). Without HTm4, the Vmax and Km for KAP phosphatase activity were 1.30 nm/min and 2.68 μM, respectively (Fig. 3, c and d). However, when HTm4 was present, the Vmax of KAP phosphatase activity increased dramatically to 17.5 nm/min. A parallel increase in Km (to 8.33 μM) was also observed. Taken together, these data suggest that the presence of HTm4 directly affects the activity of the KAP phosphatase.

**KAP Dephosphorylation of Peptide or Protein Substrates—**KAP dephosphorylation of phosphorylated proteins or peptide substrates was examined using the Ser/Thr Phosphatase Assay Kit 1 according to the manufacturer’s instructions. Phosphorylated Thr160 of cdk2 (p-cdk2) and K-R-pT-I-R-R peptides were used as substrates for the assay. Concentrations of KAP and HTm4 were both 20 μM; concentrations of p-cdk2 and peptide substrates (K-R-pT-I-R-R) were 10 and 25 μM, respectively. Reactions were performed in pNPP Ser/Thr Assay Buffer, containing 50 mM Tris-HCl, pH 7.0, and 100 μM CaCl2. To examine the necessity of the HTm4 C-terminal region and to confirm the KAP phosphatase activity enhancement effect of HTm4, a peptide containing the last 22 amino acids of the HTm4 C terminus (CNANCNSSREEISSPPNSV) was synthesized. The phosphatase activity of the HTm4 C-terminal region was examined using the assay protocol described above. All assays were performed in triplicate, with the average values and standard deviations displayed on the plot.

We further evaluated the major factors affecting KAP phosphatase activity. As we have shown, KAP phosphatase activity is significantly enhanced in the presence of HTm4. When an inactive KAP mutant (C140S) is substituted for the wild-type KAP, the phosphatase activity is almost completely abolished. The inactive KAP mutant thus serves as a negative control (15, 20). We found that when HTm4 was absent or was replaced with a nonrelated peptide, KAP phosphatase activity was almost completely inhibited compared with control reactions containing wild-type KAP, HTm4, and cdk2-cyclin A complex (Fig. 3e). It is interesting to note that a cdk2-cyclin A complex, rather than either a monomeric cdk2 or cyclin A, was required for a stronger KAP phosphatase activity induced by HTm4. Additionally, our data suggest that KAP activity is optimal in the context of a multicomponent protein complex that contains its substrate, cdk2, and the HTm4 protein. Our data demonstrate that wild-type KAP and HTm4 are essential for KAP phosphatase activity.

Based on our previous yeast two-hybrid and immunoprecipitation data, we aimed to confirm whether the C-terminal region of HTm4, alone, was sufficient to mediate the HTm4-KAP interaction and to induce the KAP phosphatase activity. To this end, we utilized the same synthesized peptide containing the hypothesized KAP binding domain (CNANCNSSREEISSPPNSV), as described above. When introduced into our in vitro
KAP activity assay, the HTm4 C-peptide showed a comparable enhancement effect on KAP phosphatase activity compared with HTm4 wild-type protein (Fig. 3e). These data both confirm our previous observation that the C terminus of HTm4 mediates direct binding of HTm4 to KAP and show that the C terminus is the functional domain of HTm4 which regulates KAP activity.

**HTm4 Controls the Phosphatase Activity of KAP toward Its Physiological Substrates**—The data presented above demonstrate that HTm4 enhances the activity of the KAP phosphatase toward a generic substrate compound, pNPP. We asked whether HTm4 could also modify the activity of KAP toward its physiological substrates. We evaluated the ability of KAP to dephosphorylate two of its substrates (Fig. 3f). First, we examined cdk2 with a phosphorylated Thr\(^{160}\) (p-cdk2), which acts as a natural substrate for KAP. We then used a synthesized threonine phosphopeptide, K-R-pT-I-R-R (Upstate). We found that HTm4 increases the dephosphorylating activity of KAP toward phosphorylated cdk2 by more than 6-fold, when cdk2 is presented in the context of the cdk2\(^{\text{cyclin A}}\) complex. We also demonstrated that HTm4 potentiates dephosphorylation of the synthesized phosphopeptide K-R-pT-I-R-R. However, no KAP dephosphorylation activity could be detected if HTm4 was not presented. Interestingly, no dephosphorylation effect was observed when phosphorylated cdk2 was presented in the cdk2\(^{\text{cyclin A}}\)-binding complex. These data imply that the presence of HTm4 confers a substrate-specific enhancement of KAP phosphatase activity.

**Structural Change of KAP by Adding C-peptide of HTm4**—Based on our observation that C-peptide of HTm4 protein enhances the activity of the KAP phosphatase, we hypothesized that the binding of HTm4 C-peptide to the KAP molecule induced KAP conformational changes.
FIG. 5. Effects of the cdk2-cyclin A complex in the presence or absence of HTm4. a, cyclin A, in the absence of HTm4, 10 μM of 10 μM cdk2-cyclin A and 10 μM His-tagged KAP were mixed, and the same amount of 10 μM HTm4 (left), 1 μM HTm4 (middle), or its buffer (right) was added to the mixture. After that, 5 μl of nickel magnet beads was added. Bound fractions were electrophoresed by SDS-PAGE and then detected by immunoblotting with anti-cyclin A antibody as the primary antibody, and alkaline phosphatase-conjugated anti-rabbit IgG was used as the secondary antibody. The band of cyclin A is indicated with an arrow. b, HTm4 released cyclin A. 10 μL of 10 μM cdk2-cyclin A and 10 μL of 10 μM His-tagged KAP were mixed in 20 mM Tris-HCl, pH 7.5, buffer containing 0.15 M NaCl, 1 mM 2-mercaptoethanol, and 0.1% Tween 20. After incubation at room temperature for 5 min, 10 μL of 10 μM HTm4 (without His tag) or its buffer was added to the mixture and allowed to incubate at room temperature for 5 min. 5 μl of nickel magnet beads was added to each mixture. The bound fractions through His-tagged KAP were washed with the Tris buffer and boiled for SDS-PAGE. The gel was then silver stained. Bands of cyclin A, cdk2, and KAP are indicated with arrows.

change. To confirm this hypothesis, we examined conformational changes of KAP in the presence or absence of the C-peptide by using CD spectroscopy. The CD spectrum of a protein in the “near-ultraviolet” spectral region (250–350 nm) can be sensitive to certain aspects of tertiary structure. At these wavelengths the chromophores are the aromatic amino acids and disulfide bonds, and the CD signals they produce are sensitive to the overall tertiary structure of the protein. Signals in the region from 250 to 270 nm are attributable to phenylalanine residues, signals from 270 to 290 nm are attributable to tyrosine, and those from 280 to 300 nm are attributable to tryptophan. Disulfide bonds give rise to broad weak signals throughout the near-ultraviolet spectrum. As shown in Fig. 4, we found a spectral change at 270–280 nm in the difference spectrum of KAP sample A (with HTm4 C-peptide) and sample B (without C-peptide). The 270–280 nm difference spectrum demonstrated a conformational change in tyrosine when HTm4 C-peptide was added into KAP solution. Because HTm4 C-peptide has no tyrosines residues, this major change in the spectrum must be caused by conformational changes in the KAP tyrosine residues. Hence, this result demonstrated that HTm4 could cause direct conformational/tertiary structural change in KAP after its binding to KAP by the C terminus.

HTm4 Dissociates Inhibitory Cyclin A from the cdk2-cyclin A-binding Complex—The activity of cdk2 is controlled by its protein-protein interactions with KAP and cyclin A. The binding of KAP phosphatase to the cdk2-cyclin A complex dephosphorylates, and hence inactivates, cdk2. However, the binding of cyclin A inhibits the ability of KAP to dephosphorylate cdk2. Dissociation of cyclin A by HTm4 facilitates cdk2 dephosphorylation by KAP. Interestingly, HTm4 enhances the KAP dephosphorylation effect even on phosphorylated cdk2 that is presented in a complex with cyclin A. This result suggests that HTm4 may facilitate cdk2 inactivation by alleviating the inhibitory effect of cyclin A. Our model suggests that HTm4 may regulate cdk2 activity, via KAP, and by physically excluding cyclin A from its inhibitory interaction with cdk2.

To verify our hypothesis, we used an affinity purification method to evaluate the cdk2-cyclin A interaction both with and without HTm4. We isolated His-tagged KAP protein using a Ni²⁺ binding column. Without HTm4, cyclin A remained in the His-purified KAP-cdk2-cyclin A-binding complex (Fig. 5a).
However, when purified HTm4 (after His tag cleavage) was added to the KAP-cdk2-cyclin A reaction mixture, cyclin A dissociated from the complex in a dose-dependent manner, and the remaining protein complex was found to contain only KAP and HTm4 (Fig. 5b). These observations demonstrate that HTm4 inactivates cdk2 by activating KAP and by causing concomitant dissociation of cyclin A from cdk2. Together with the observation that HTm4 enhances KAP phosphatase activity, we suggest that HTm4 is positioned as a key regulator in the activation of KAP and for cdk2 activity.

**DISCUSSION**

**Mechanism for HTm4-induced Dissociation of Cyclin A from Cdk2-Cyclin A**—*In silico* modeling was used to probe the potential nature of the HTm4 regulatory interaction that promotes KAP phosphatase activity and potentially leads to the exclusion of cyclin A. Based on our findings, we show that KAP undergoes conformational changes following allosteric binding to the HTm4 C-terminal domain and that these conformational changes may subsequently facilitate the interaction between the KAP substrate and its active site. To examine this hypothetical model, we produced a steric structure model using the coordinates of cdk2-cyclin A (1JST) (21) and cdk2-KAP (1FQ1) (20) from the Protein Data Bank (PDB). These structures were overlapped utilizing Homology in Insight II software (MSI). The resulting model complex is shown in Fig. 6a. Both cdk structures significantly overlap, and the overall RMS deviation is 0.92 Å, with the exception of the T-loop region. On the reverse side of the interface, phosphothreonine 160 of cdk2 can be inserted into the entrance of the active site of cdk2, which is consistent with the observed conformational changes in the T-loop region.

**FIG. 7. Van der Waals interaction in KAP, cdk2, and cyclin A.** *a,* Van der Waals interaction between the T-loop of cdk2 (red) and cyclin A (green) in the cdk2-cyclin A complex. *b,* the second interaction (between Phe152 and Ile182) is completely abolished by moving the T-loop.

**FIG. 8. KAP, cdk2, and cyclin A complex models.** *a,* existence of ionic interaction between Arg157 of the T-loop of cdk2 and cyclin A Glu268 in the cdk2-cyclin A complex but not in the cdk2-KAP complex. The calculated distances are based on coordinates of cdk2-cyclin A in PDB and our model in cdk2-KAP. *b,* amino acid multialignment for cdks. The numbers on the top of our alignment are from the amino acid sequence of cdk2. *c,* stereo view of the T-loop side in cdk2-cyclin A (1JST). Pro155 of cdk2 (carbon, green; oxygen, red; nitrogen, dark blue) is seen in the deep concave. The Pro residue seems not to be accessible by an isomerase. Right, cyan is cdk2; left, white molecule is cyclin A. *d,* this view is a reverse side of Fig. 6b (KAP, cdk2 active site) and is also shown by CPK model. Thr160 of cdk2 (yellow) and cyclin A (pink) are seen on the back side of the cdk2-KAP complex (KAP, blue; cdk2, white). We can stereographically see a broad concave that HTm4 C-terminal domain would bind. Tyrosines residues 87 (dark blue) and 141 (green) of KAP represent the candidate conformational change sites after the HTm4-C-terminal binding shown by CD difference spectrum (Fig. 4).
HTm4 Affects KAP-Cdk2-Cyclin A Complex

Table 1

| PDB code | ω (Degree) | TL* | Cyclin A/KAP |
|----------|------------|-----|--------------|
| 1JSS     | -13.76     | cis | E            |
| 1JST     | 5.87       | cis | E            |
| 1H9H     | 0.06       | cis | E            |
| 1H2S     | 6.61       | cis | E            |
| 1QMZ     | 36.92      | cis | E            |
| 1FQ1     | 175.31     | trans | S        |
| 1HCK     | 170.27     | trans | S        |
| 1PF8     | 166.29     | trans | S        |
| 1PW2     | 177.23     | trans | S        |

* TL: T-loop; E, extended; S, shortened.

Site of KAP in the absence of cyclin A, whereas a small molecule of substrate such as pNPP may still be able to enter the entrance even in the presence of cyclin A. This finding may explain why KAP activity toward pNPP is enhanced at moderate levels in the presence of the cdk2-cyclin A complex.

Mode of Structural Change of the Cdk2 T-loop in the Absence and Presence of HTm4—The T-loop is a conserved segment in cdks, which plays an important role in cdk activation. In the cdk2-cyclin A complex, phosphorylation is associated with the mobility of the T-loop (12). However, viral cyclins (K-, H-cyclins) can still activate cdk6 without Thr177 phosphorylation, which corresponds to Thr160 phosphorylation in cdk2. The degree of interaction between the T-loop of cdk6 and V-cyclin is shown to be critical because the interaction area between cdk6 and V-cyclin was 20% larger than that of cdk2 and cyclin A (15). This increased interaction is contributed mainly by the T-loop of cdk6. Hence, the movement of the T-loop is critical for cdk activation.

In the cdk2-cyclin A activation model, van der Waals interactions exist between Ala151, Phe152, and Tyr159 of the cdk2 T-loop and Phe267, Ile182, and Ile270 of cyclin A (Fig. 7a) (6). However, in our KAP-cdk2-cyclin A complex model, the second cdk2-cyclin A interaction (Phe152-Ile182) is completely abolished if the T-loop is moved because of the interaction of KAP (Fig. 7b). This result demonstrates that binding of KAP to cdk2 weakens the interactions between cdk2 and cyclin A. Moreover, it is reported that interactions between the T-loop of cdk2 and KAP comprise van der Waals interactions between residues Tyr141 and Phe267 of KAP with residue His161 of cdk2 (20).

Besides the van der Waals interaction between the T-loop of cdk2 and cyclin A, a specific ionic interaction also exists between the -NH at Arg157 of cdk2 and the -OH of Glu268 of cyclin A in cdk2-cyclin A complex. This interaction disappears when the cyclin A molecule is dissociated, as seen in the cdk2-KAP complex (Fig. 8a). Thus, the extension of the T-loop in cdk2-cyclin A (blue) is inhibited by this ionic interaction.

In PDB models, more than 20 sets of coordinates are deposited for cdk2, both with and without cyclin A or its inhibitors. We investigated each structure of the T-loop and measured dihedral angles between Val154 and Pro155, which are conserved in several cdks. Fig. 8b shows amino acid multialignments for several cdks. When cdk2 is associated with cyclin A, the T-loop is shortened with cis-Pro at position 155. Conversely, without cyclin A, the loop is extended with trans-Pro (Table 1). When bound to KAP (without cyclin A), the T-loop of cdk2 shows extension in the trans form. According to the predicted crystal structure of cdk2-cyclin A, this cis-trans conversion at Pro155 is thought to occur automatically because the residue Pro155 would no longer be accessible to an isomerase (Fig. 8c).

When cyclin A dissociates from cdk2, the T-loop will extend through cis-trans conversion at the Pro155 residue, and phosphothreonine 160 can then be inserted into the active site of KAP. Thus, the susceptibility of phosphothreonine 160 to KAP phosphatase occurs in response to HTm4 binding to KAP. Pro155 commonly exists in cdk3, cdk5, but not cdk4, cdk5 as shown in Fig. 8b, whereas its counterpart residue Glu258 is conserved in cyclins A, B, E, and C but not in cyclin D. This disparity suggests that mechanisms for cdk inactivation by KAP may differ between cdk species and hence between different phases of the cell cycle.

Binding Site of HTm4—In Fig. 8d, we show the close allosteric position between Thr160 of cdk2 and Tyr84, Tyr141 of KAP, which suggests that the concave face of KAP accepts the HTm4 C terminus via the Tyr141 residue of KAP. Immediately following HTm4 binding, the conformational change of the Tyr141 residue of (Fig. 4) will weaken the van der Waals interaction between cdk2 and cyclin A and then affect continuously along-side T-loop (from residues 142 to 165) involving Ala151, Phe152, and Tyr159. This HTm4 binding eventually dissociates cyclin A from cdk2, thereby inducing a structural change of cdk2. At the reverse side of the interface, phosphothreonine 160 of cdk2 is inserted into the entrance of the active site of KAP, after cyclin A is released.

Model of Action Mechanism for HTm4—HTm4 has four transmembrane domains (22). Topologically, this structure provides two tails that are available for protein-protein interactions (2). HTm4 localizes to the nuclear membrane. We hypothesize that the tails of HTm4 extend into the nuclear lumen and tether the KAP-cdk2-cyclin A complex (Fig. 9). The interaction between HTm4 and the KAP-cdk2-cyclin A complex is likely reversible and may occur only at certain stages in the cell cycle. HTm4 significantly affects the activity of KAP, cdk2, and cyclin A. HTm4 also regulates cdk2 activity in a dual fashion, by concurrently activating KAP activity and facilitating the accessibility of Thr160 to KAP by causing dissociation of inhibitory cyclin A (Fig. 9). In conclusion, we show that HTm4 regulates the cell cycle in hematopoietic cells through its ability to control cdk2 status.

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