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Corepressor MMTR/DMAP1 is an intrinsic negative regulator of CAK kinase to regulate cell cycle progression

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ABSTRACT

We have previously reported that MMTR (MAT1-mediated transcriptional repressor) is a co-repressor that inhibits TFIIH-mediated transcriptional activity via interaction with MAT1 (Kang et al., 2007). Since MAT1 is a member of the CAK kinase complex that is crucial for cell cycle progression and that regulates CDK phosphorylation as well as the general transcription factor TFIIH, we investigated MMTR function in cell cycle progression. We found that MMTR over-expression delayed G1/S and G2/M transitions, whereas co-expression of MAT1 and MMTR rescued the cell growth and proliferation rate. Moreover, MMTR was required for inhibition of CAK kinase-mediated CDK1 phosphorylation. We also showed that the expression level of MMTR was modulated during cell cycle progression. Our data support the notion that MMTR is an intrinsic negative cell cycle regulator that modulates the CAK kinase activity via interaction with MAT1.

1. Introduction

Dnmt1-associated protein 1 (DMAP1) was originally identified a DNMT1-interacting molecule and is implicated in gene regulation through modification of chromatin [1]. In addition, the DMAP1–DNMT1 complex was reported to interact with the p33ING1–Sin3–HDAC complex and to localize at pericentric heterochromatin to maintain the heterochromatin structure and histone modifications in the late S phase [2]. DMAP1 was demonstrated to co-localize with DNMT1 throughout the S phase in order to mediate transcriptional repression activity or with HDAC2 and DNMT1 at DNA replication foci during the late S phase in order to form transcriptionally repressive chromatin. Recent studies have also revealed that DMAP1 is a core component of the NuA4/Tip60 histone acetyltransferase (HAT) complex and the ATP-dependent chromatin-remodeling complex Swr1/SRCAP, and that it plays primary roles in transcription, cellular response to DNA damage, and cell cycle control [3,4]. However, the role of DMAP1 in cellular functions remains largely unknown.

We independently isolated and characterized the MAT1-mediated transcriptional repressor (MMTR) from mouse embryonic stem cells as a novel clone and found it to be identical to DMAP1 [5]. MMTR is a key component of RNA Pol II-mediated gene expression that interacts with HDAC1 and modulates of TFIIH kinase activity via MAT1 [6]. We showed that the coiled-coil domain in the middle of MAT1 interacts with the C-terminal half of MMTR and that MMTR-mediated transcriptional repression activity was completely restored by over-expressed MAT1 in the presence of TSA. MMTR inhibited *in vitro* phosphorylation of the TFIIH kinase substrate, the C-terminal domain of the RNA Pol II largest subunit, which is important for efficient promoter escape via suppression of early Pol II elongation intermediates [7]. We hypothesized that MMTR also regulates cell cycle progression since MAT1 is also an assembly/targeting factor for cyclin-dependent kinase (Cdk)-activating kinase (CAK), a sub-complex of TFIIH [8]. Moreover, the free CAK complex composed of the catalytic subunit cdk7, the regulatory subunit cyclin H, and MAT1 preferentially phosphorylates Cdks to induce G1/S and G2/M phase transitions. Here, we show that MMTR is, indeed, involved in the regulation of cell cycle progression as an intrinsic negative regulator of CAK that regulates mitotic cell cycle progression.

2. Materials and methods

2.1. Cell culture and transfection

293T human embryonic fibroblasts and HeLa cells were grown in Dulbecco's modified eagle medium (DMEM) and Dulbecco's modified Eagle's medium nutrient mixture F-12 HAM (DMEM/F12;

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Sigma) supplemented with 10% fetal bovine serum (FBS, Gibco-BRL) at 37 °C in 5% CO_2. For synchronization of cells at the G1/S boundary phase, we used the double thymidine block method. Briefly, cells were exposed to 2 mM thymidine with two 16-h periods separated by 10-h incubation without thymidine for 293T cells or a single 20-h period of exposure to 1 mM thymidine for HeLa cells.

2.2. Plasmid construction

To generate pLV-TH-GFP-MMTRi, an EcoRI/ClaI fragment from pSUPER-retro-MMTRi was sub-cloned into the EcoRI/ClaI site of pLV-TH-GFP (a kind gift from Dr. Didier Trono, University of Geneva, Switzerland). pSUPER-retro-MMTRi was constructed by sub-cloning PCR products using the following primers: primer 1; 5′- GAT CCC CCT TCG TAG TTA TTC ACG ATT TCA AGA GAA TCG TGA ATA ACT ACG AAG TTT TTG GAA A-3′, primer 2; 5′-AGC TTT TCC AAA AAC TTC GTA GTT ATT CAC GAT TCT CTT GAA ATC GTG AAT AAC TAC GAA GGG G-3′. The GST-CDK1 expression vector was provided by Dr. Philipp Kaldis (Yale University, CT, USA). Other expression vectors used in this study have been previously described [6].

2.3. FACS analysis

Cells were grown in six-well tissue culture plates to 70% confluency and then trypsinized, pelleted, washed with chilled phosphate-buffered saline, and fixed with 100 μl citrate buffer (250 mM sucrose, 40 mM trisodium citrate, 5% DMSO, pH 7.6). Cells synchronized at the G1–S boundary by the double thymidine block were trypsinized, centrifuged, and fixed with 70% cold ethanol for 30 min. After washing with PBS, pelleted cells were sequentially resuspended in solutions containing RNase A for 5 min and propidium iodide for 5 min. The cells were subjected to FACS analysis using a FACS Vantage flow cytometer (Becton Dickinson, Franklin Lakes, NJ).

2.4. TUNEL assay

For in situ detection of apoptotic cells, the TUNEL assay was performed using the horseradish peroxidase (POD) in situ cell death detection kit (Roche Molecular Biochemicals). The fixed cells were incubated with TUNEL reaction mixture for 60 min at 37 °C in a humidified atmosphere, and then converter-POD was added for 30 min at 37 °C. DNA fragments were stained using DAB-substrate solution.

2.5. Kinase assay

Cell extracts of pCMV-HA-MMTR which over-expressed 293T cells and the mock-transfected 293T cells were prepared in a lysis buffer [20 mM Tris–HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, 1% NP-40, 0.1% SDS, 1 mM PMSF, and protease inhibitor cocktail (Roche)]. Recombinant GST-CDK2 (2 μg) in *in vitro* kinase assay buffer [20 mM Hepes (pH 7.4), 10 mM $MgCl_2$, 1 mM DTT, 0.5 mM ATP, and 2 μCi [γ-^{32}P]ATP (Amersharm Biosciences)] was added to 3 μg protein extract, and the reaction mixtures were incubated at 30 °C for 60 min and then quenched with SDS–PAGE loading buffer. Western blotting was performed to confirm that an equal amount of CAK complex was used in each reaction. ^{32}P-labeled protein was detected using autoradiography.

3. Results

3.1. MMTR over-expression reduces the cell proliferation rate by prolonging the G2/M phase

Since MAT1, a component of TFIIH, is also a subunit of the CAK complex [8], we investigated whether it affects cell cycle progression. CAK regulates cell cycle progression by inducing phosphorylations of CDK1, CDK2, CDK4 and CDK6. We transiently transfected the MMTR expression vector (pCMV-HA-MMTR) into 293T cells and monitored the cell growth and proliferation rate for up to 48 h before the cells reached confluence. Growth of the MMTR-transfected cells inhibited at least 30% compared with that of cells transfected with vector control (Fig. 1A, left panel). The transfection efficiency was routinely greater than 70% (data not shown), and over-expression of MMTR was confirmed via Western blot (data not shown). To more accurately assess the MMTR-mediated inhibition of cell proliferation, we co-transfected the MMTR expression vector with pEGFP-C1 and scored the number of EGFP-expressing cells 36 h after transfection. The number of EGFP-positive cells in the MMTR and EGFP co-over-expressing culture was ~60% of that of cells expressing EGFP alone (Fig. 1A, right panel). The number of EGFP-positive cells in a culture that co-expressed the C-terminal half of MMTR (MMTR-C) and EGFP was ~50% of that of cells expressing EGFP alone, while the number of cells co-expressing the N-terminal half of MMTR (MMTR-N) and EGFP was similar to that of cells transfected with EGFP alone (Fig. 1A, right panel). Thus, our data indicate that MMTR inhibits cell proliferation and that MMTR-C containing the MAT-1 interaction domain is sufficient to repress cell proliferation. Furthermore, since intact MMTR is required to repress HDAC1 and TFIIH-mediated transcriptional repression [6], our results also suggest that the mechanism by which MMTR inhibits cell proliferation is distinct from that of transcriptional repression.

The reduction in cell proliferation might be caused by cell death or by cell cycle arrest. To test whether the MMTR effect on cell proliferation is caused by the induction of cell death, we performed TUNEL staining analyses using DNase I-induced DNA fragmentation as a positive control. MMTR-transfected or control mock-transfected 293T cells did not show any DNA fragmentation, whereas cells treated with DNase I clearly showed DNA fragmentation, as revealed by both AP staining and fluorescence microscopy (Fig. 1B). This result suggests that the reduced cell proliferation caused by over-expression of MMTR is not due to apoptosis. Next, to test the effect of MMTR on cell cycle progression, pEGFP-MMTR was transiently transfected into 293T cells, and the distribution of EGFP-positive cells was analyzed using flow cytometry 36 h after transfection. EGFP-MMTR-transfected cells showed an increase in the proportion of cells in the G2/M phase (from 16% to 32%) at the expense of the G0/G1 population, which decreased from 69% to 47%, compared with those of control cells expressing EGFP alone (Fig. 1C, left panel). Interestingly, cells with >90% knock-down of MMTR by transient transfection of the pLV-TH-GFP-MMTR RNAi vector showed a similar cell cycle distribution profile to that of the control (Fig. 1C, right panel). Taken together, these data indicate that over-expression, but not down-regulation, of MMTR reduces the cell proliferation rate by prolonging the G2/M phase. We also confirmed the MMTR-mediated G2/M transition delay in HeLa cells. pEGFP-MMTR vector or control pEGFP vector was transiently transfected into HeLa cells, and the thymidine block method was used to arrest cells at the G1/S boundary. When cell cycle progression of EGFP-positive cells in the pEGFP-MMTR-transfected group was analyzed, the G2/M period was increased (Fig. 1D, middle panel), whereas the cell cycle progression profile of EGFP-negative cells in the pEGFP-MMTR-transfected group was not different from that of EGFP-positive or EGFP-negative cells in the pEGFP-transfected group (data not shown). These data indicate that the MMTR-mediated G2/M transition delay is not a cell type-specific phenomenon.

To more precisely dissect the MMTR-mediated cell cycle delay, we sub-gated the pEGFP-MMTR-transfected population into 3 groups; EGFP-negative cells (red region in dot plots in the left panel of Fig. 1D), EGFP^{low} cells (blue region) and EGFP ^{high} cells

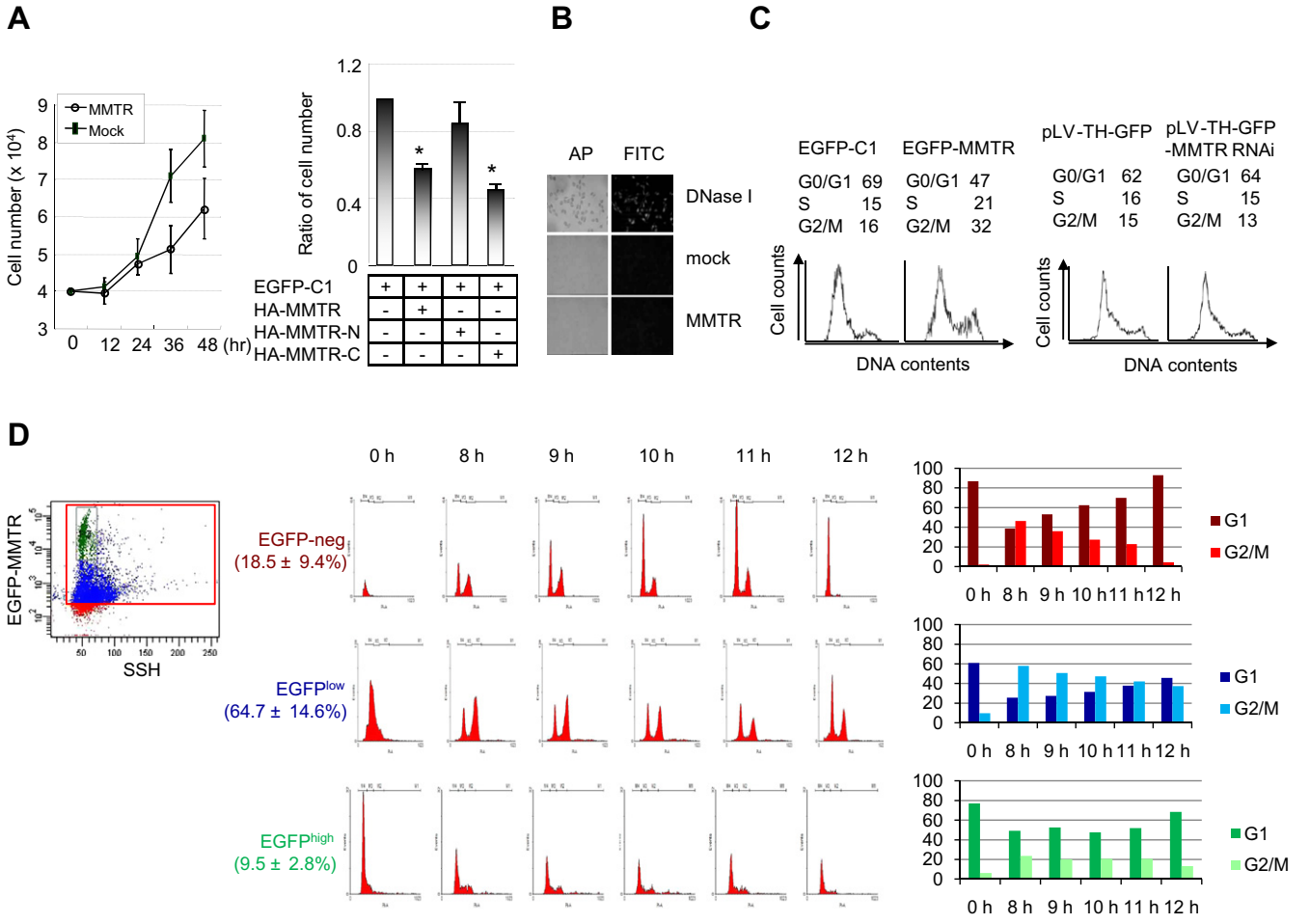

Fig. 1. MMTR over-expression reduced the cell proliferation rate by prolonging theG2/M phase. (A) Inhibition of cell growth rate by MMTR over-expression. Growth curves were determined by counting cell numbers every 12 h after transfection of pCMV-HA-MMTR or mock vector into 293T cells (left panel). The ratios of EGFP-expressing cells relative to the total cell numbers following transfection of pCMV-HA-MMTR, pCMV-HA-MMTR-N or pCMV-HA-MMTR-C in combination with pEGFP-C1 into 293T cells (right panel). EGFP-expressing cells were scored under fluorescence microscopy 36 h post-transfection. Error bars indicate standard deviation from triplicate wells. * p-value < 0.05. (B) Analysis of apoptosis. AP; alkaline phosphatase staining, FITC; fluorescence microscopy for FITC. (C) Cell cycle analyses of pEGFP-C1 and pEGFP-MMTR-transfected 293T cells (left panel) and pLV-TH-GFP and pLV-TH-MMTR RNAi transfected 293T cells (right panel) using FACS at 36 h after transfection. (D) Cell cycle analyses of pEGFP-MMTR-transfected HeLa cells. A representative example of the cell FACS profile (left panel) and cell cycle profile at each time point after release of pEGFP-MMTR transfected HeLa cells from the thymidine block are shown (middle panel).

(green region). We also indexed the pEGFP-MMTR-transfected cells in specific stages of the cell cycle at different time points after release from the thymidine block (0–12 h) (Fig. 1D, middle panel). As expected, EGFP^{low} cells (64.7% of the total cells) showed an MMTR-mediated G2/M transition delay, whereas the EGFP-negative cells (9.5% of the total cells) showed normal cell cycle progression (compare Fig. 1D, top and middle rows of the middle panel). Interestingly, some of the EGFP-MMTR cell population that highly expressed of EGFP (EGFP ^{high}) (and thus highly expressed MMTR) showed obvious G1 arrest (compare Fig. 1D, top and bottom rows of the middle panel). These data indicate that higher levels of MMTR expression lead to G1 arrest, whereas moderate MMTR over-expression causes only a G2/M transition delay.

3.2. MMTR-mediated cell cycle arrest is rescued by over-expression of MAT1

To determine whether MMTR-mediated cell cycle retardation is rescued by over-expression of MAT1, we transiently co-transfected the MAT1 expression vector with pEGFP-C1 and pHA-MMTR into 293T cells, individually or in combination, and monitored the growth rate by counting the EGFP-expressing cells 36 h post-transfection. The number of MMTR-expressing cells in the MMTR-transfected group was reduced by ~30% compared with that of the control, and this inhibition of cell proliferation was rescued to the level of the control by over-expression of MAT1 (Fig. 2A, compare lanes 1 though 4). Interestingly, the rescue effect of MAT1 was recapitulated in cells containing only the C-terminal region of MMTR (MMTR-C) (Fig. 2A, lane 8). We also confirmed the rescue effect of MAT1 on cell cycle progression using FACS analyses (Fig. 2B); i.e., the cell cycle progression profile of the EGFP-MMTR/MAT1 cells, where pEGFP-MMTR and pFlag-MAT1 were co-transfected, was not different from that of the control EGFP cells. Our data suggest that MAT1 suppresses over-expressed MMTR or MMTR-C to restore the normal cell proliferation rate and further indicates that the action mechanism of MMTR-mediated cell division control is distinct from that of MMTR-mediated transcriptional repression, which requires intact MMTR to repress HDAC1 and TFIIH-mediated transcriptional repression [6].

3.3. MMTR inhibits the CAK kinase activity against CDK1

Cell division is controlled by cyclin-dependent kinases (CDKs). In metazoans, the S phase onset coincides with activation of Cdk2, whereas Cdk1 triggers mitosis onset [8]. Both Cdk1 and Cdk2 require cyclin binding and T loop phosphorylation for full

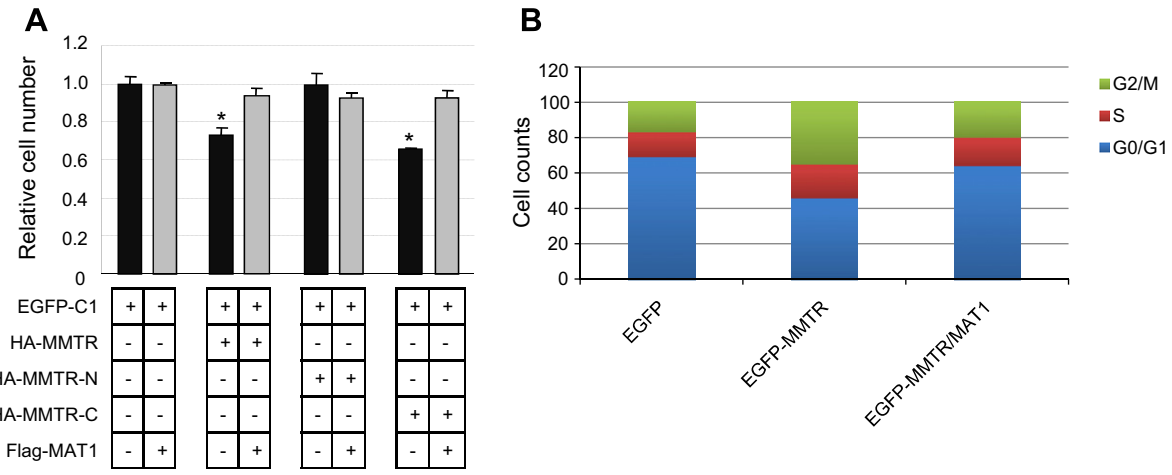

Fig. 2. MMTR-mediated cell cycle arrest is rescued by over-expression of MAT1. (A) Growth rates of EGFP-positive 293T cells 36 h after co-transfection of pCMV-HA-MMTR, pCMV-HA-MMTR-N, or pCMV-HA-MMTR-C with pEGFP-C1 in the presence or absence of pcDNA3-Flag-MAT1. Error bars indicate standard deviation from triplicate wells. * p-value < 0.05. (B) FACS analyses of DNA content of 293T cells transfected with pEGFP-C1, pEGFP-MMTR, and pEGFP-MMTR/pcDNA3-Flag-MAT1 expression vectors 36 h after transfection.

activity. Since MMTR over-expression predominantly induced cell cycle arrest at the G2/M phase (see Fig. 1D), we selected CDK1 as a potential target of the CAK complex and tested whether MMTR could inhibit the kinase activity of CAK against the substrate CDK1.

To examine the effect of MMTR on the phosphorylation status of CDK1, *in vitro* kinase assays were performed using purified GST-CDK1 as a substrate. GST-CDK1 phosphorylation was observed when GST-CDK1 was incubated with 293T cell extracts (Fig. 3A, lane 3 of the left panel). In contrast, GST-CDK1 phosphorylation was barely detected in the reaction with cell extracts containing over-expressed MMTR (Fig. 3A, lane 4 of the left panel). Similar results were also observed in the reaction containing 293T cell extracts and recombinant GST-MMTR compared to those containing over-expressed MMTR (Fig. 3A, middle panel). Furthermore, GST-MMTR inhibited GST-CDK1 phosphorylation in a dose-dependent manner (Fig. 3A, middle panel), suggesting that MMTR directly inhibits the kinase activity for CDK1 phosphorylation in the cells. To more precisely examine the effect of MMTR on the inhibition of CAK-mediated CDK1 phosphorylation, we used affinity-purified CAK subunits in the *in vitro* kinase reactions. Expression vectors encoding MAT1, Cyclin H, CDK7 (pcDNA3-Flag-MAT1, -cyclin H and -Cdk7) and MMTR (pcDNA3-HA-MMTR) were co-transfected in combination into 293T cells, and the CAK complex was immunoprecipitated with anti-Flag antibody. Kinase assays were performed on reaction mixtures containing the immunoprecipitates and GST-CDK1. We assumed that MMTR would be co-immunoprecipitated with the CAK complex due to protein–protein interaction between MMTR and MAT1 when MMTR was coexpressed with Flag-tagged MAT1, Cyclin H, and CDK7. As expected, the kinase activity toward GST-CDK1 was much stronger in the reaction containing the tripartite CAK complex than in a bipartite CAK complex with CDK7/MAT1 or CDK7/cyclin H (Fig. 3A, compare lanes 1 though 4 in the right panel). Co-expression of MMTR reduced the level of phosphorylation of GST-CDK1 to that of the bipartite complex (Fig. 3A, lane 5 in the right panel), indicating that MMTR directly affects the activity of CAK kinase for the phosphorylation of GST-CDK1. Therefore, we concluded that MMTR directly interacts with MAT1 and inhibits CAK kinase activity.

3.4. MMTR over-expression inhibits CAK kinase activity and perturbs cell cycle progression in both the G1 and G2/M phases

To test whether MMTR-mediated inhibition of the cell cycle progression is really due to inhibition of CAK kinase, we monitored the phosphorylation status of CDK1 during cell cycle progression of mock and MMTR-transfected 293T cells. Cell cycle synchronization was induced by the thymidine double block, and the cell cycle stage and expression levels of cell cycle markers at each time point after releasing from the block were analyzed using FACS and Western blotting, respectively. As expected, after releasing from block, the majority of the control mock cells were in the G0/G1 stage (Fig. 3B, top of the left panel). Starting from 2 h after release, the percentage of cells in G2/M gradually increased at the expense of S phase cells and persisted for 10 h, with the maximum percentage observed after 6 h. Accordingly, expressions of Cyclin A and Cyclin B started to increase in parallel with the entrance of cells into the S phase and persisted until cells entered the G2/M phase. CDK1 phosphorylation also appeared to be correlated with the G2/M transition (Fig. 3B, bottom of left panel). In contrast, in MMTR- over-expressing cells, the cycle progression was delayed with prolonged G1 and G2/M phases (Fig. 3B, top of right panel), and the expression pattern of phosphorylated CDK1 was greatly perturbed (Fig. 3B, bottom of right panel). These results indicate that MMTR over-expression perturbs cell cycle progression at both G1/S and G2/M transitions where CAK kinase mediates the phosphorylations of CDK2 and CDK1, respectively.

3.5. MMTR expression is modulated during cell cycle progression

Since the results described above were obtained under conditions in which MMTR was ectopically over-expressed, we examined the expression profiles of endogenous proteins involved in cell cycle progression, including MMTR, in 293T cells. Interestingly, while MAT1 expression was consistent during cell cycle progression, MMTR expression was down-regulated from 2 h after release from the thymidine double block, showed a minimum expression level at 6 h and slowly increased thereafter (Fig. 4). Thus, MMTR expression was at a maximum in the G1 phase and at a minimum in the G2/M transition. Down-regulation of MMTR during cell cycle progression occurred concurrently with down-regulation of Cyclin E, which is expressed in G1 and associates with CDK2 to form an active kinase [8]. Changes in the MMTR expression level inversely correlated with those of Cyclin A/Cyclin B and p21. These data are consistent with those data shown in Fig. 1D, where higher levels of MMTR expression led to G1 arrest, whereas moderate MMTR over-expression resulted in only G2/M transition delay. Thus, our data suggest that less than 25% down-regulation of MMTR expression at the G1 phase is sufficient to allow cells to enter into the S phase,

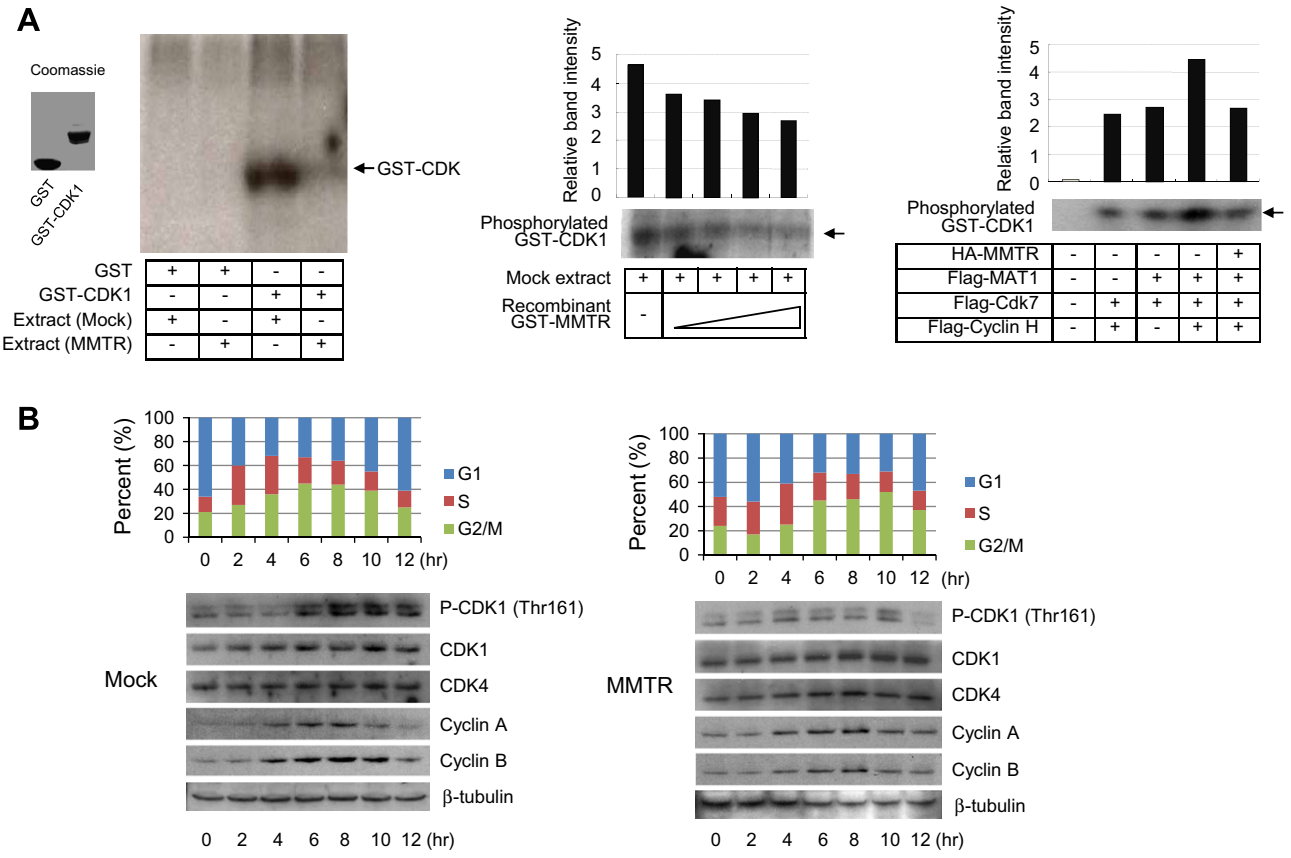

Fig. 3. MMTR over-expression perturbs cell cycle progression by inhibiting CAK kinase activity in the G1 and G2/M phases. (A) MMTR inhibits CAK kinase activity. Kinase assays with mock- or MMTR-transfected 293T cell nuclear extracts (left panel), mock 293T extracts and increasing amounts of recombinant GST-MMTR(middle panel), and Flag-antibody immunoprecipitated proteins from extracts of 293T cells following co-transfection with pCMV-HA-MMTR, pcDNA3-Flag-MAT1, pcDNA3-Flag-CDK7 and pcDNA3-Flag-cyclin H in the combinations as indicated (right). GST-CDK1 was used as a kinase substrate and [^{32}p] ATP was included in the reaction. The insert in left panel shows Coomassie-stained purified GST fusion proteins. (B) MMTR over-expression inhibits CAK kinase activity and perturbs cell cycle progression in both the G1 and G2/M phases. Cell cycle indexing (top panel) and phosphorylation status of CDK1 (bottom panel) were monitored after release from the double thymidine block in the mock- (left panel) and MMTR-over-expressing (right panel) 293T cells.

whereas > 50% down-regulation is required for the G2/M transition. Our data also suggest that down-regulation of MMTR expression is important for CAK kinase function at both G1/S and G2/M phase transitions.

4. Discussion

MMTR is known to be a co-repressor that inhibits TFIIH-mediated transcriptional activity through interaction with MAT1 [6]. Here, we report that MMTR is also involved in the regulation of cell cycle progression. We found that MMTR inhibited cell proliferation due to delays of G1/S and G2/M transitions, such that higher levels of MMTR expression led to G1 arrest, whereas moderate MMTR over-expression caused only G2/M transition delay (Fig. 1). Co-expression of MAT1 and MMTR rescued both cell growth and proliferation rate (Fig. 2). Furthermore, we demonstrated that MMTR blocked the CAK kinase-mediated phosphorylation of CDK1 that is important for normal G2/M transition both *in vitro* and *in vivo* (Fig. 3). Finally, we revealed that the expression level of MMTR was modulated during cell cycle progression, such that down-regulation of MMTR expression was correlated with G1/S and G2/M transitions (Fig. 4B). Thus, our data strongly support the notion that MMTR is an intrinsic negative cell cycle regulator that modulates CAK kinase activity via interaction with MAT1. Furthermore, our findings suggest that the mechanism of action of MMTR-mediated cell division control is distinct from that of MMTR-mediated transcriptional repression since only the C-terminal half of MMTR containing the MAT1 binding domain is sufficient to inhibit cell cycle progression via CAK-mediated Cdk1 phosphorylation (Figs. 2 and 3), whereas intact MMTR is required to repress HDAC1 and TFIIH-mediated transcriptional repression [6].

We showed that two distinct execution points, G1/S and G2/M transitions in the cell cycle, are regulated differently by MMTR. A higher level of MMTR expression was required for G1 arrest, whereas moderate MMTR over-expression caused a G2/M transition delay (Fig. 1D). Furthermore, during normal cell cycle progression the G1/S transition correlated with a moderate decrease in the MMTR protein level, whereas the G2/M transition occurred when MMTR reached its lowest level (Fig. 4). These data suggest that MMTR-mediated cell cycle arrest is dependent on different threshold levels of MMTR that might modulate the stage-specific CAK kinase activities. Our findings are consistent with recent reports that G1/S and G2/M transitions are regulated by different modes of CDK activation by the CAK complex. For example, selective inhibition of Cdk7 in G1 prevents activation (but not formation) of Cdk2/cyclin E complexes and delays the S phase, whereas inhibition of Cdk7 in G2 blocks entry into mitosis and disrupts Cdk1/cyclin B complex assembly [9]. In addition, it was reported that Cdk1 and Cdk2 are activated by kinetically distinct mechanisms, even though they share the same CDK-activating kinase, Cdk7, such that Cyclin A assembles with Cdk1 only after complex formation with Cdk2 reaches a plateau during the late S and G2 phases [10].

MAT1 constitutes the first example of an assembly factor that appears to be essential for the formation of an active CDK-cyclin

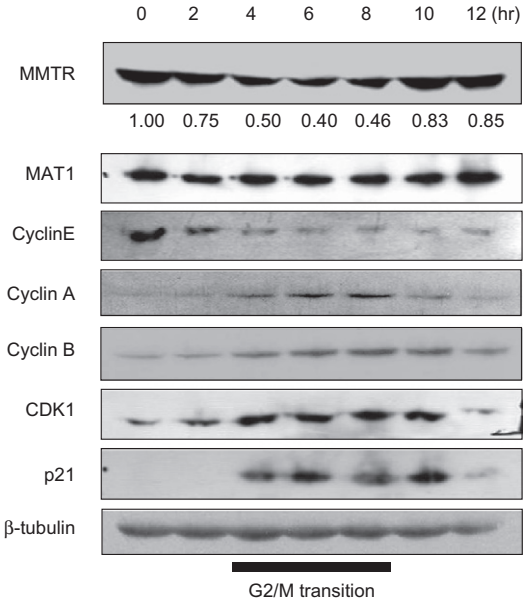

Fig. 4. The MMTR expression level is modulated during cell cycle progression. Expression levels of MMTR, MAT1 and additional cell cycle markers in the mock-transfected 293T cells shown in Fig. 3B were analyzed using Western blotting. Quantified MMTR signals were normalized to that of MMTR protein at time 0, with the highest value set to 1.00. Western blots for Cyclin A and Cyclin B are duplicates of panel a in Fig. 3B.

complex [8]. It has been reported that deregulation of CAK function via abrogation of MAT1 induces G1 phase arrest, and interaction of MAT1 with pRb stimulates the phosphorylation of pRb by the CAK complex and E2F-mediated trans-activation of genes that trigger the G1/S transition [8]. However, the association of the three proteins in the CAK complex is near stoichiometric and invariant throughout the cell cycle and, likewise, phosphorylation of the CDK7 T-loop does not fluctuate during the cell cycle [11]. In this study, we showed that the MMTR protein level is modulated during cell cycle progression, consistent with the idea that the G1/S and G2/M phase transitions require CAK kinase function for CDK2 and CDK1 phosphorylations (Fig. 4). Thus, MMTR might be the crucial regulator of the CDKs that control cell division. Taken together, we conclude that the cell cycle regulation properties of MMTR are strictly related to its expression level, and that the MMTR protein has a dose-dependent anti-proliferative effect on somatic cells. These findings should facilitate the understanding of MMTR/DMAP1 function in mammalian cells and elucidate the molecular links between cell cycle progression and regulation of transcriptional activity.

Acknowledgments

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