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Mitotic Control of RUNX2 Phosphorylation by Both CDK1/Cyclin B Kinase and PP1/PP2A Phosphatase in Osteoblastic Cells

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Abstract Skeletal development and osteoblast maturation require the phenotype promoting activity of the transcription factor RUNX2, which controls both cell growth and differentiation in osteoblasts. We have recently shown that in actively proliferating cells RUNX2 regulates the expression of specific target genes as cells enter and exit mitosis. In this study, we addressed whether post-translational modifications of RUNX2 control its activity during mitotic exit. Western blot analysis of proteins from osteoblastic Saos-2 cells released from mitotic inhibition into early G_1 show a phosphatase-sensitive shift in the mobility of RUNX2 in SDS gels. The slowly migrating hyper-phosphorylated form of RUNX2 is immunoreactive with a CDK related phospho-antibody (MPM2) only in mitotic cells and is converted into a faster migrating hypo-phosphorylated RUNX2 when cells complete mitosis. This conversion is inhibited by okadaic acid, an inhibitor of protein phosphatases 1 and 2 (PP1 and PP2A), but not by deltamethrin which blocks PP2B phosphatase. Mitotic phosphorylation of RUNX2 is sensitive to the CDK inhibitors roscovitine and olomoucine. Furthermore, RUNX2 can directly interact with CDK1 and is phosphorylated in vitro by the CDK1/cyclin B kinase complex. Hence, RUNX2 is hyper-phosphorylated by CDK1/cyclin B during mitosis, and dynamically converted into a hypo-phosphorylated form by PP1/PP2A-dependent dephosphorylation after mitosis to support the post-mitotic regulation of RUNX2 target genes. J. Cell. Biochem. 100: 1509–1517, 2007.

Key words: mitosis; RUNX2; cyclin B; CDK1; osteoblast; osteosarcoma; okadaic acid; roscovitine

The osteogenic transcription factor RUNX2 is a critical regulator of bone formation and controls the expression of both bone phenotypic and cell growth related genes [Stein et al., 2004]. Mutations in the RUNX2 locus in mouse or human cause major skeletal defects [Komori et al., 1997; Mundlos et al., 1997; Otto et al., 1997; Choi et al., 2001] that are directly attributable to the ability of RUNX2 to control the normal growth and differentiation of osteoblasts. We recently observed that RUNX2 deficiency increases the proliferative potential of calvarial mesenchymal cells and that forced expression of RUNX2 impedes cell-cycle progression [Pratap et al., 2003]. One mechanism for cell-cycle inhibition may involve the RUNX2-dependent induction of p21 and p27 [Westendorf et al., 2002; Thomas et al., 2004], but additional cell growth regulatory pathways are likely to be operative. RUNX2 proteins are cell-cycle regulated in normal osteoblastic cells and present at low levels during mitosis [Galindo et al., 2005]. RUNX2 interactions with its target genes during mitosis may be a key component of an epigenetic mechanism that sustains osteogenic cell identity and maturation-stage specific phenotypes [Zaidi et al., 2003; Young et al., 2004, 2005].

The mitotic function of RUNX2 is reflected by its association with chromosomes during mitosis. Upon mitotic exit, RUNX2 becomes

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punctately distributed during interphase at subnuclear sites to organize the dynamic nucleation of multimeric protein-protein complexes that activate or repress genes through chromatin modifications [Zaidi et al., 2003]. The scaffolding function of RUNX2 can be regulated by interactions with a number of co-regulatory proteins including its heteromeric partner core binding factor-β (CBF-β) through the N-terminal Runt homology DNA binding domain, as well as the Yes associated protein (YAP), SMADs, transducin-like enhancer of split (TLE), and histone deacetylases (HDACs) which each interact with RUNX2 through its C-terminal domain [Hanai et al., 1999; Javed et al., 2000; Kundu et al., 2002; Westendorf et al., 2002; Zaidi et al., 2004].

RUNX2 is regulated by phosphorylation events that modify its activity and/or the potential for protein–protein interactions [Lian et al., 2004]. Phosphorylation of RUNX2 is mediated in part by mitogen activated protein kinase (MAPK) related signaling pathways, including extracellular signal-regulated kinase (ERK) [Xiao et al., 2000, 2002]. Moreover RUNX2 is a substrate for protein kinase A (PKA) [Selvamurugan et al., 2000] and protein kinase C [Kim et al., 2006]. One key question is how the subcellular re-distribution of RUNX2 and the dynamic association with transcriptional co-regulators is controlled when cells initiate mitosis and subsequently exit into the next interphase. Phosphorylation of RUNX proteins may also signal the onset and progression of distinct cell-cycle sub-stages. One of the main findings of the present study is that RUNX2 is reversibly phosphorylated at mitosis by cyclin-dependent kinase 1 (CDK1/CDC2) in complex with cyclin B.

MATERIALS AND METHODS

Cell Culture and Mitotic Block

Experiments were performed with human osteosarcoma (Saos-2) cells that were maintained in McCoy's 5A modified medium (Invitrogen, Carlsbad, CA) supplemented with 15% FBS. For mitotic synchrony, Saos-2 cells were grown in 150 mm plates to 70% confluency and nocodazole (100 ng/ml; Sigma, St. Louis, MO) was added for 19 h. Next day mitotic cells were shaken off, washed twice in PBS and processed for analysis. Okadaic acid and Deltamethrin (Calbiochem, EMD Biosciences, La Jolla, CA) were added for the last 4 h of the nocodazole treatment.

SDS–PAGE and Western Blotting

Samples were separated in 8% or 12% SDS-polyacrylamide gels (SDS–PAGE), and then transferred to nylon membrane. The blots were incubated in blocking buffer (5% non-fat dry milk, 0.1% Tween 20 in PBS) for at least 60 min and then incubated with primary antibody for 1 h at room temperature in 1% non-fat milk and PBST (i.e, phosphate buffer saline plus 0.1% Tween 20). The blots were washed three times with 10 ml PBST and incubated for 1 h more at room temperature with the appropriate horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA) in 1% non-fat milk and PBST. Finally, the blots were washed three more times with 10 ml PBST and visualized via enzyme-linked chemiluminescence (ECL) using the ECL kit (Amersham/GE Healthcare, Piscataway, NJ). The primary RUNX2 antibody is a mouse monoclonal antibody from R + D systems (Minneapolis, MN).

Kinase Assay

Full-length recombinant RUNX2 protein was expressed as a glutathione-*S*-transferase (GST) fusion protein (GST-RUNX2) using the pGEX-2TK vector (Amersham/GE Healthcare). The fusion protein was expressed and purified according to the manufacturer's protocol. In vitro phosphorylation experiments were carried out at 30°C in a final volume of 25 μl with 8 mM MOPS, 0.8 mM EDTA, and 200 μM ATP. In this buffer, purified GST RUNX2 (5 μg) was incubated with purified recombinant human CDK1/cyclin B (Upstate Biotechnology, Lake Placid, NY) (10 ng). Reactions were initiated via the addition of [γ^{32}P] ATP (1 μci/μl) and were allowed to proceed for 30 min at room temperature before being stopped with SDS–PAGE sample buffer.

Immunoprecipitation

Saos-2 cells were harvested and lysed in 500 μl of ice-cold lysis buffer (50 mM Tris HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% NP-40, Phosphatase inhibitor cocktails I & II, 25 μM MG132, and 1 Complete^{TM} protease inhibitor cocktail) by sonication. Cell lysate was clarified via centrifugation at 12,000 rpm for 10 min. A small fraction of the supernatant was removed at this point and incubated with

SDS–PAGE sample buffer in order to examine expression of proteins in the whole cell extract. The remaining supernatant was incubated with 1.5 μg of RUNX2 rabbit polyclonal (Santa Cruz) or anti-MPM2 (Upstate Biotechnology) for 2 h with end-over-end rotation at 4°C before being incubated with Protein A/G agarose beads (Sigma) for 1 h. Immunoprecipitated proteins were eluted from the beads with sample buffer, resolved by SDS–PAGE, and subjected to western blot analysis with mouse monoclonal anti-RUNX2 antibody.

DNA Constructs, Site Directed Mutagenesis, and Transfection

Deletion mutants for RUNX2 were previously reported (i.e., RUNX2 1–406 [Zaidi et al., 2001]; RUNX2 1–376 [Afzal et al., 2005]). Expression vectors for mouse RUNX2 (P1/MASNS) were cloned in pcDNA 3.1(+). Serine mutants of mouse RUNX2 were generated using the Quick change site directed mutagenesis kit (Stratagene, La Jolla, CA) according to the manufacturer's protocol. U2OS cells were transfected with different RUNX2 constructs using FuGENE 6 (Roche Applied Sciences, Indianapolis, IN). The transfection reagent was diluted in serum-free medium and the appropriate RUNX2 constructs were added. After incubation for 20 min, the DNA complex was added dropwise to the cells.

RESULTS

RUNX2 Is Phosphorylated During Mitosis

We have previously demonstrated that RUNX2 binds to mitotic chromosomes [Zaidi et al., 2003; Young et al., 2004, 2005]. Here we have examined the post-translational status of RUNX2 during mitosis and subsequent entry into G_1. In cultures of Saos-2 cells that are arrested in mitosis using colcemid or nocodazole, we find that RUNX2 exhibits reduced mobility in SDS–PAGE as compared to RUNX2 isolated from asynchronous, nearly confluent cultures which are almost devoid of mitotic cells (Fig. 1A). Reduced mobility in SDS–PAGE is typical for proteins that are (hyper-)phosphorylated, and mobility differences can be exacerbated by the presence of proline residues. Indeed, the C-terminal half of RUNX2 was originally defined as a PST domain as it contains a series of proline, serine, and threonine residues [Ogawa et al., 1993].

To examine whether the reduced mobility of RUNX2 is due to phosphorylation, we treated mitotic cell lysates with recombinant λ phosphatase and assessed whether treatment results in differences of mobility. As shown Figure 1B, λ phosphatase activity increases the mobility of RUNX2 from mitotic cells, while incubation of RUNX2 in the absence of λ phosphatase does not. Thus, RUNX2 is subject to mitotic phosphorylation.

Dynamic Dephosphorylation of RUNX2 by an Okadaic Acid-Sensitive Phosphatase Upon Exit From Mitosis

The phosphorylation status of RUNX2 reflects the balance between kinase activity and enzymatic removal of the phosphate moiety by phosphatases. To understand the dynamics of this post-translational modification, we next examined the phosphorylation status of RUNX2 as Saos-2 cells exit mitosis into G_1. Cells were synchronized in mitosis using nocodazole for 19 h and then replated after removal of nocodazole to induce cell-cycle progression from M to G_1. The phosphorylation-dependent mobility of RUNX2 was examined by western

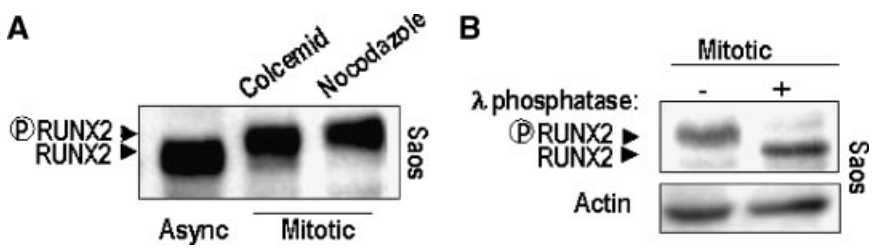

Fig. 1. Mitotic mobility shift of RUNX2 is due to phosphorylation. **A**: Asynchronous cells and Saos-2 cells were arrested in mitosis (using nocodazole and colcemide) and lysed by sonication. Following centrifugation to remove cellular debris, the supernatant was electrophoresed in an 8% gel, transferred to a membrane and probed with a RUNX2 antibody. The arrowheads indicate the two forms of RUNX2 that exhibit differences in electrophoretic mobility. **B**: Lysates from asynchronous and mitotic Saos-2 cells were treated with 600 U of λ phosphatase for 30 min at 30°C. The reaction was terminated by the addition of 6× protein loading dye. Samples were separated by 8% SDS/PAGE followed by western blotting using a RUNX2 antibody.

blot analysis. The results clearly show that RUNX2 exhibits dramatically faster migration within 2 h of release from mitotic block (Fig. 2A) suggesting that RUNX2 is actively dephosphorylated as cells progress into G_1.

We then directly examined whether the mobility of RUNX2 changes due to active dephosphorylation immediately after mitosis. As above, Saos-2 cells were treated with nocodazole to achieve a mitotic arrest and released in the presence or absence of phosphatase inhibitors. We observed that the PP1/PP2A inhibitor okadaic acid can quantitatively prevent the decreased mobility and hence dephosphorylation of RUNX2 (Fig. 2B). In contrast, the PP2B selective inhibitor deltamethrin did not affect the mobility of RUNX2 (Fig. 2C). These data indicate that the slower migrating hyper-phosphorylated form of RUNX2 is dephosphorylated at the completion of mitosis by a PP1/PP2A related phosphatase.

Purified CDK1/Cyclin B Complexes Phosphorylate RUNX2

The cyclin B-dependent kinase CDK1 (CDC2) is one of the major kinases in mitotic cells and RUNX2 contains at least one CDK consensus motif [(S/T)P X (R/K)]. We therefore postulated that CDK1/cyclin B may be responsible for altering the electrophoretic migration and thus phosphorylation of RUNX2. We examined whether CDK1/cyclin B can phosphorylate either recombinant RUNX2 or endogenous RUNX2 in Saos-2 cells (Fig. 3). GST-RUNX2 fusion protein (Fig. 3A) or endogenous RUNX2 isolated by specific immunoprecipitation (Fig. 3B) are both radio-labeled by ^{32}P-γ-ATP following incubation with CDK1/cyclin B kinase. Phosphorylation is increased by increasing the amount of RUNX2 in the reaction indicating that phosphorylation is substrate dependent (Fig. 3B). Taken together, the results show that CDK1 is capable of phosphorylating RUNX2 in vitro.

RUNX2 Phosphorylation Is Sensitive to the CDK Inhibitor Roscovitine and Occurs at a CDK1 Specific Phospho-Epitope

To examine whether CDK1 can phosphorylate RUNX2 in live cells, we assessed whether RUNX2 phosphorylation is sensitive to CDK selective inhibitors. Mitotically arrested cells were treated with the cyclin-dependent kinase inhibitor roscovitine in the last 4 h of nocodazole treatment. The results show that roscovitine does not affect RUNX2 phosphorylation in

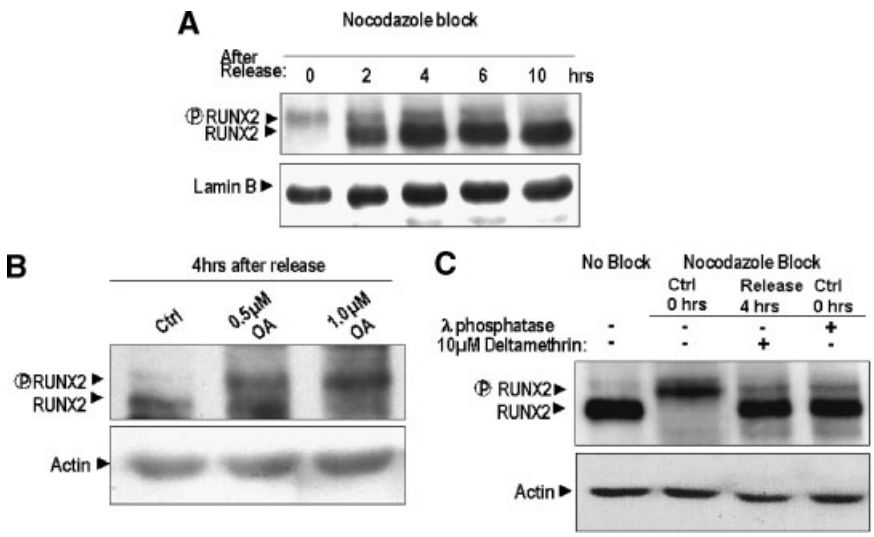

Fig. 2. RUNX2 is dephosphorylated during mitotic exit by an okadaic acid-sensitive phosphatase. **A**: Saos-2 cells were blocked at mitosis with nocodazole (100 ng/ml) for 19 h, and released by administering fresh medium without the inhibitor. Cells were harvested at the indicated times following release. Samples were analyzed by Western blotting using antibodies against RUNX2 (**upper panel**) and lamin B loading control (**lower panel**). **B**: Mitotic Saos-2 cells were blocked as above by nocodazole, and replated in the absence (**left lane**) or presence of 0.5 μM (**middle lane**) or 1 μM (right lane) of okadaic acid (i.e., a PP1/PP2A specific inhibitor) for 4 h. RUNX2 levels were examined by Western blotting as described above, and Actin was used as loading control (**lower panel**). **C**: The panel shows RUNX2 in asynchronously growing cells (**left lane**), nocodazole blocked cells (**lane 2**), cells blocked and then released for 4 h in the presence of deltamethrin (**3rd lane**), and nocodazole blocked cells treated with phosphatase (**4th lane**).

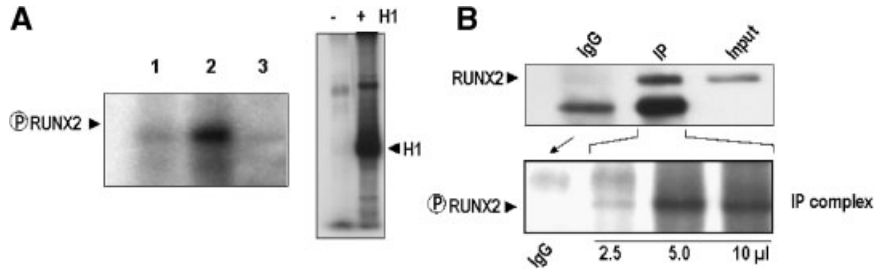

Fig. 3. CDK1/Cyclin B phosphorylates RUNX2. **A**: Recombinant GST/RUNX2 fusion protein is a substrate for CDK1/Cyclin B. The **left panel** shows in vitro kinase reactions that were carried out with purified GST-RUNX2 or GST alone with CDK1/cyclin B kinase (10 ng) for 30 min at 30°C. An aliquot (10 µl) of the total reaction (30 µl) was separated by 8% SDS/PAGE. At the completion of electrophoresis, the gel was dried and exposed to autoradiographic film. The in vitro kinase reactions contained, respectively, 0.2 µg of GST-RUNX2 (**Lane 1**), 1 µg of GST-RUNX2 (**Lane 2**), 1 µg of GST (**Lane 3**). The right panel shows a positive control for the in vitro kinase reaction. Histone H1 (10 ng) was omitted from (−) or added to (+) a kinase reaction mixture containing purified CDK1/cyclinB kinase (10 ng) for 30 min at 30°C. An aliquot (5 µl) of the reaction was analyzed by 10% SDS/PAGE. The gel was dried and subjected to autoradiography. **B**: Immunoprecipitated RUNX2 from asynchronously growing cells is phosphorylated by recombinant CDK1/cyclin B. The **upper panel** shows a western blot of input lysate and immunoprecipitates obtained with either RUNX2 antibody or non-specific IgG-complex. The lower panel shows phosphorylation of increasing amounts (2.5, 5, and 10 µl) of immunoprecipitated RUNX2 complexes that were subjected to in vitro kinase reactions (30 µl) with purified CDK1/cyclin B kinase (10 ng). Samples were analyzed by 8% SDS/PAGE followed by autoradiography of the dried gel. The first lane shows a reaction performed with non-specific IgG (2.5 µl).

asynchronous cells, but inhibits mitotic RUNX2 phosphorylation (Fig. 4A). This finding suggests that RUNX2 phosphorylation in vivo is mediated by the CDK1/cyclin B complex.

Several monoclonal antibodies have been developed in different laboratories against mitotic cell lysates. These antibodies react with subsets of proteins that are phosphorylated upon entry into mitosis. One of the best characterized antibodies is MPM-2 which recognizes a CDK-related epitope containing phosphorylated serine or threonine followed by proline [phospho (S/T)P]. We performed immunoprecipitation experiments with both asynchronous and mitotic cell lysates using both RUNX2 and MPM-2 antibody and analyzed the immunoprecipitates by western blot with RUNX2 antibody. As expected, the RUNX2 antibody precipitates RUNX2 from both mitotic and asynchronous cell lysates (Fig. 4B). More importantly, the MPM-2 antibody is capable of complexing only with mitotic RUNX2. This result demonstrates that RUNX2 is phosphorylated in vivo at a CDK site during mitosis.

Formation of a Stable RUNX2/CDK1/Cyclin B Complex at Mitosis

Having established that RUNX2 can be phosphorylated in vitro and in vivo by CDK1/cyclin B or a closely related kinase, we investigated whether endogenous CDK1/cyclin B can form a stable complex with RUNX2 in Saos-2 cells. We find that CDK1/cyclin B co-immunoprecipitates with mitotic RUNX2 but this association cannot be detected in asynchronous cells (Fig. 5). Hence, CDK1/cyclin B complexes

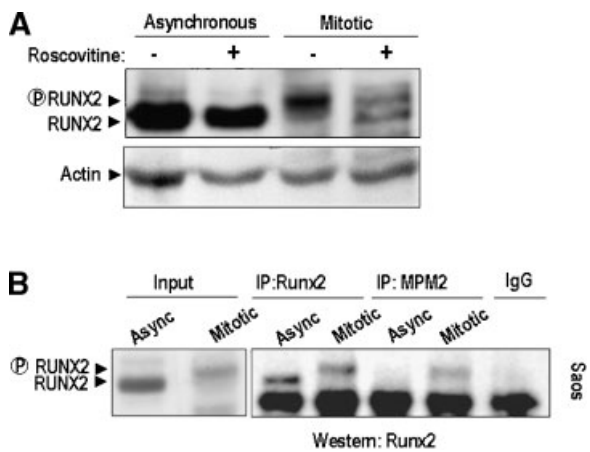

Fig. 4. CDK phosphorylation of endogenous RUNX2 at mitosis. **A**: Roscovitine inhibits mitotic Runx2 phosphorylation. Saos-2 cells were arrested in mitosis with nocodazole (100 ng/ml) for 19 h in the presence or absence of the CDK inhibitor Roscovitine (25 µM) for the last 5 h of the nocodazole treatment. Asynchronous control cells and mitotic cells were harvested and analyzed by western blotting using antibodies for RUNX2 (**upper panel**) or actin (**lower panel**). **B**: RUNX2 mitotic phosphorylation occurs at a CDK2 related phospho-epitope. Lysates from asynchronous and mitotic Saos-2 cells were subjected to immunoprecipitation using antibodies for RUNX2 or the CDK phospho-epitope MPM2, or non-specific IgG. The immunoprecipitated complexes were resolved by 8% SDS/PAGE and analyzed by western blotting using a RUNX2 antibody. The two electrophoretic forms of RUNX2 are indicated in **Panels A** and **B** by the two arrowheads.

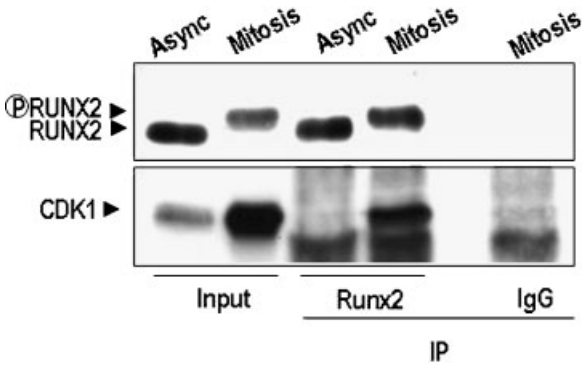

Fig. 5. CDK1 associates with mitotic RUNX2. The **upper panel** shows a RUNX2 western blot of immunoprecipitates obtained using a mouse monoclonal RUNX2 antibody (**lanes 3** and **4**) or non-specific rabbit IgG (**lanes 5** and **6**) and lysates from asynchronous or mitotic cells (**lanes 1** and **2**). Immunocomplexes were resolved by 8% SDS/PAGE prior to membrane transfer. The **lower panel** shows the same immunoprecipitates from both asynchronous and mitotic cells that were separated by 12% SDS/PAGE and probed with a CDK1 antibody.

physically associate with RUNX2 in vivo. Taken together, our results strongly suggest that RUNX2 interacts stably with CDK1/cyclin B complexes and is a CDK substrate during mitosis.

RUNX2 Mutant S472A Is Phosphorylated at Mitosis

Bioinformatics analysis reveals that there are 12 putative (S/T)P phosphorylation sites which are conserved in both human and mouse RUNX2. However, only one of these sites (serine 472 in the mouse P1/MASNS isoform) conforms to the more stringent (S/T)PX(K/R) consensus CDK phosphorylation site. To test whether Serine 472 is responsible for the migration shift and hence phosphorylation of RUNX2 at mitosis, we transfected U2OS cells with expression constructs for full length RUNX2 and two deletion mutants (i.e., RUNX2/1–391 and RUNX2/1–376) and carried out λ phosphatase assays. As shown in Figure 6A, similar to the wild-type protein, both mutants exhibit reduced mobility at mitosis, which is reversed by λ phosphatase treatment. However, unlike the wild-type RUNX2 protein, deletion mutants do not exhibit a complete migration shift in mitotically arrested cells. This result suggests that there is a partial loss of phosphorylation, increased dephosphorylation, or enhanced sensitivity to proteases. Alterna-

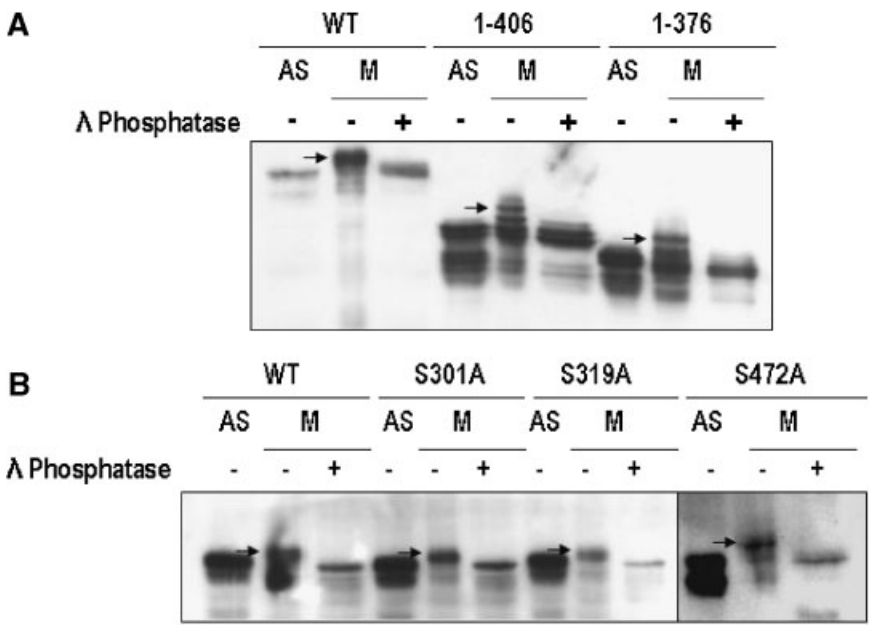

Fig. 6. Analysis of CDK-dependent phosphorylation sites in RUNX2. **A**: Deletion analysis of RUNX2 to define proteins segments required for the mitosis-dependent mobility shift of RUNX2. U2OS cells were transfected using Fugene with constructs expressing full length RUNX2 or the indicated deletion constructs (amino acid 1 refers to the first residue of the P1/MASNS isoform of RUNX2). The cells were blocked 24 h after transfection at mitosis by the administration of nocodazole (100 ng/ml) for 19 h. Lysates from asynchronous or mitotic cells were analyzed by western blotting using a RUNX2 antibody following 8% SDS/PAGE. Samples from mitotic cells were treated with (+) or without (–) 600 U of λ phosphatase for 30 min at 30°C. **B**: Mutational analysis of RUNX2 to assess the requirement of serine residues in the mitotic mobility shift of RUNX2. U2OS cells were transfected and treated as described in panel A with constructs expressing wild-type RUNX2 protein (WT) or the indicated serine mutants.

tively, the ability of these RUNX2 mutants to adopt a phosphorylation-dependent conformation that produces the electrophoretic mobility shift may be compromised.

To assess whether mutations of the CDK related serine 472 and putative MAPK related serine residues at 301 and 319 affect the phosphorylation of RUNX2, we tested alanine substitution mutants of these three serines in our λ phosphatase assay. Interestingly, all three point mutants (S472A, S319A, and S301A) show the reduced mobility and λ phosphatase sensitivity of wild-type RUNX2 at mitosis (Fig. 6B,C). Thus, mitotic phosphorylation may be independent of the conserved CDK phosphorylation site at serine 472, or other cryptic CDK sites may compensate upon mutation of serine 472. Irrespective of the exact serine residue that is phosphorylated by CDK1 at mitosis, the data presented in Figure 6A clearly demonstrate that the C-terminal region of RUNX2 starting at position 391 is required for full penetrance of the mitotic mobility shift phenotype of RUNX2.

DISCUSSION

In this study, we have established that RUNX2 is phosphorylated by CDK1/cyclin B complexes during mitosis in osteoblastic cells resulting in altered electrophoretic mobility of RUNX2. RUNX2 is dephosphorylated by a PP1/PP2A-dependent mechanism as cells enter G_1 following mitosis. Importantly, our research group has shown that RUNX2 is associated with mitotic chromosomes, and that RUNX2 is equally distributed into daughter cells [Zaidi et al., 2003; Young et al., 2004, 2005]. The findings presented here indicate that most if not all RUNX2 is quantitatively phosphorylated during mitosis, and suggest that chromosome-bound RUNX2 may in fact be a CDK1 phosphorylated form. The equal partitioning of the cellular complement of RUNX2 during cell division appears to correspond with the loss of CDK1-dependent phosphorylation.

Our main finding that RUNX2 is phosphorylated during mitosis corroborates previous studies which provided evidence for RUNX2 phosphorylation during interphase by MAPK/ERK and PKA signaling [Selvamurugan et al., 2000; Xiao et al., 2002]. Our data are consistent with findings from Wee et al. [2002], who showed that S472 located within a CDK consensus motif is one of three principal phosphorylation sites in living cells. The novelty of the current finding is that we have established a direct link between the mitotic function of RUNX2 in maintaining osteoblastic phenotype identity [Zaidi et al., 2003; Young et al., 2004, 2005] and the CDK related regulatory machinery that maintains orderly progression through the cell-cycle. Thus, RUNX2 integrates multiple cell signaling pathways that are activated by extracellular cues (e.g., local or systemic growth factors) as well as signals that are connected to the internal cell-cycle clock that regulates the temporal succession of events required for mitotic division.

While this study was being prepared for publication, Passaniti and colleagues have found that RUNX2 expressed in endothelial cells is phosphorylated by CDK1 kinase in vitro [Qiao et al., 2006]. Similar to our results, these authors also established that RUNX2 forms a stable complex with cyclin B1. One unique aspect of our study is the detection of a complete mitotic mobility shift in RUNX2 that is endogenously expressed in osteoblastic Saos-2 cells. The findings presented here complement our previous demonstration that RUNX2 is a quasi-epigenetic regulator that maintains the phenotypic memory of osteoblastic cells during mitosis [Zaidi et al., 2003; Young et al., 2004, 2005]. Consistent with the critical mitotic role for RUNX2, which is based on its ability to associate with mitotic chromosomes, the CDK site at 472 can promote the intrinsic DNA binding activity of RUNX2 [Qiao et al., 2006]. Furthermore, recent findings by Chen and colleagues indicate that S472 phosphorylation by CDK4/cyclin D1 increases the stability of RUNX2 [Shen et al., 2006]. The CDK-dependent phosphorylation of RUNX2 by distinct CDKs as cells progress through the cell-cycle may support specific regulatory functions at different cell-cycle stages. We conclude that CDK1/cyclin B phosphorylation of RUNX2 during mitosis represents a unique post-translational modification that may control the mitotic function of RUNX2 in maintaining cell fate and lineage commitment.

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