dMi-2 Chromatin Binding and Remodeling Activities Are Regulated by dCK2 Phosphorylation*

Karim Bouazoune1 and Alexander Brehm2

From the Adolf-Butenandt-Institut, Lehrstuhl für Molekularbiologie, Ludwig-Maximilians-Universität, Schillerstrasse 44, 80336 München, Germany

A plethora of ATP-dependent chromatin-remodeling enzymes have been identified during the last decade. Many have been shown to play pivotal roles in the organization and expression of eukaryotic genomes. It is clear that their activities need to be tightly regulated to ensure their coordinated action. However, little is known about how ATP-dependent remodelers are regulated at the molecular level. Here, we have investigated the ATP-dependent chromatin remodeling enzyme Mi-2 of Drosophila melanogaster. Radiactive labeling of S2 cells reveals that dMi-2 is a phosphoprotein in vivo. dMi-2 phosphorylation is constitutive, and we identify dCK2 as a major dMi-2 kinase in cell extracts. dCK2 binds to and phosphorylates a dMi-2 N-terminal region. Dephosphorylation of recombinant dMi-2 increases its affinity for the nucleosome substrate, nucleosome-stimulated ATPase, and ATP-dependent nucleosome mobilization activities. Our results reveal a potential mechanism for regulation of the dMi-2 enzyme and point toward CK2 phosphorylation as a common feature of CHD family ATPases.

ATP-dependent chromatin remodelers play important roles in the regulation of eukaryotic genome organization and activity. All ATP-dependent chromatin remodelers known to date share a conserved SNF2 ATPase/helicase domain. The growing list of chromatin-remodeling ATPases is divided into several subfamilies. Four of these have been characterized to some extent and are distinguished by the presence of additional protein domains (1): SWI2/SNF2 ATPases possess a bromodomain that is important for stable binding to acetylated chromatin (2). SWI1-related ATPases share characteristic SANT-like domains some of which form nucleosome recognition modules (3).

CHD proteins harbor a pair of chromodomains, which have been implicated in binding of nucleosomal DNA and in the recognition of the methylated lysine 4 mark on histone H3 (4, 5). INO80 factors contain a distinctive insertion in their “split” ATPase/helicase domain (6).

Mi-2/CHD3/CHD4 ATPases (from hereon referred to as Mi-2) are members of the CHD subfamily. They contain a pair of PHD fingers, in addition to the chromodomains present in all CHD family members. Mi-2 ATPases associate with other proteins to form multisubunit complexes with a type II histone deacetylase, MeCP1, or Mi-2 complexes: S2, Drosophila Schneider line 2 cells; LyBu, lysis buffer; GST, glutathione S-transferase; Tricine, N-(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)glycine; CIP, calf intestine phosphatase; NCP, nucleosome core particle; CDK, cyclin-dependent kinase; HMGI, high mobility group.

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1 To whom correspondence should be addressed. Tel.: 49-(0)89-2180-75-435; Fax: 49-(0)89-2180-75-425; E-mail: abrehm@lmu.de.

2 The abbreviations used are: Mi-2, Mi-2/CHD3/CHD4 ATPases; NCP, nucleosome core particle; CDK, cyclin-dependent kinase; HMGI, high mobility group.

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nucleosome-dependent ATPase and ATP-dependent nucleosome mobilization activities. Dephosphorylation of dMi-2, therefore, provides a potential mechanism to rapidly and reversibly control dMi-2 activity at a step subsequent to chromatin association.

**MATERIALS AND METHODS**

*Preparation of Whole Cell Extracts—Drosophila Schneider line 2 (S2) cells were lysed in lysis buffer (LyBu) 200 (20 mM Heps, pH 7.6, 10 mM β-glycerophosphate, 0.1% Nonidet P-40, 10% glycerol, and protease inhibitors) containing 200 mM KCl by subjecting the cells to two freeze-thaw cycles followed by sonication. Extracts were then cleared by centrifugation before use.*

*In Vivo 32P Labeling of S2 Cells—S2 cells were washed in phosphate-free Tris-buffered saline and then incubated in phosphate-free medium in the presence of 1 mCi of [32P]orthophosphate for 4 h. Cells were washed with phosphate-buffered saline, lysed in HEMG 500 (20 mM Heps, pH 7.6, 500 mM KCl, 12.5 mM MgCl2, 0.5 mM EDTA) containing 1% Nonidet P-40. Immunoprecipitations using antisera directed against dMi-2 N and C termini (dMi-2N and dMi-2C, respectively) were performed. After extensive washes, immunoprecipitates were subjected to SDS-PAGE followed by autoradiography.*

*Immunoprecipitations and Western Analysis—Immunoprecipitations were carried out in HEMG 500 supplemented with 0.1% Nonidet P-40. Antiserum and 500 µl of cell extracts, prepared from 106 S2 cells in LyBu 200, were subjected to SDS-PAGE and stained with Coomassie Blue.*

*Expression and Purification of Recombinant Proteins from Insect Cells—The generation of FLAG-tagged dMi-2 WT and mutant constructs has been described before (4, 7). dMi-2 932–1158 (referred to as dMi-2 ΔATPase) was generated by digesting pVL1392-dMi-2-FLAG baculovirus transfer vector with Stul, removing the 678-bp restriction fragment, and re-ligating. Recombinant baculovirus was produced as described (4, 7). For expression, exponentially growing S9 cells were infected with recombinant viruses and harvested 48 h post-infection. Infected cells were collected by centrifugation, resuspended in LyBu 200, lysed by two cycles of freeze-thaw in liquid nitrogen, and sonicated. Lysates were cleared by centrifugation prior to adding M2-affinity gel (Sigma) to the lysate. After incubation, for at least 4 h at 4 °C, the affinity gel was cleared by centrifugation prior to adding M2-affinity gel (Sigma) to the lysate. After incubation, for at least 4 h at 4 °C, the affinity gel was recovered by centrifugation and washed twice with 10 resin-volumes of LyBu 500, twice with 10 resin-volumes of LyBu 1000, once with 10 resin-volumes of LyBu 500, once with 10 resin-volumes of LyBu 200, and finally once with 1 ml of elution buffer (20 mM Tris, pH 8.0, 150 mM KCl, 10% glycerol) without FLAG-peptide. Bound proteins were eluted with FLAG peptide. Amounts of phosphorylated and dephosphorylated dMi-2 were monitored by SDS-PAGE and Coomassie Blue staining. Gels were dried and phosphorylation was detected by phosphorimaging.*

*Purification of dMi-2 Kinase by Affinity Chromatography—5 ml of GSTrap columns (Amersham Biosciences) was saturated with GST or GST-dMi-2 (4–560) proteins and washed according to the manufacturer’s instructions. About 50 ml of whole cell extracts (prepared from 106 S2 cells in LyBu 200) were loaded onto the GST column, and the flow-through was subsequently applied to the GST-dMi-2 (4–560) resin. After extensive washing with LyBu 200, bound proteins were eluted by increasing the KCl concentration to 1 M.*

*Purification of dCK2 by Conventional Chromatography—dCK2 was purified as described before (39) with the following modifications: Whole cell extracts were prepared from 5 × 108 S2 cells in LyBu 200 and adjusted to 100 mM KCl prior to loading onto the first column (Q-Sepharose). After each step of this purification, 4 µl of the collected fractions was assayed for kinase activity and presence of dCK2 by Western blot. 10 µl of the Mono Q fractions was subjected to SDS-PAGE followed by silver staining or Western blotting.*

*dMi-2 Sequence Analysis—CK2 consensus sites were identified using Pseearch (www.ebi.ac.uk/pssearch/). Sequence alignments were performed with MulAlin (prodes.toulouse.inra.fr/multalin/) using default parameters.*

*Phosphatase Treatment for SDS-PAGE Analysis—About 500 ng of proteins was incubated with 0.5 unit of calf intestine phosphatase (CIP) for 30 min at 26 °C in the dephosphorylation buffer provided by the supplier (CIP buffer, Roche Applied Science), in the presence or absence of 20 mM phosphatase inhibitor (β-glycerophosphate). Proteins were next subjected to SDS-PAGE and stained with Coomassie Blue.*

*Phosphatase Treatment for Activity Assays—Baculovirus-expressed FLAG-tagged dMi-2 proteins were purified and immobilized on M2 affinity gel as described above. One-half of the material was treated with CIP (0.25 unit per µl of M2-gel) for 45 min at 26 °C, and the other half (mock treatment) was treated identically except that phosphatase was replaced by chicken-egg albumin (Sigma). FLAG beads were then subjected to extensive washes to remove the phosphatase, and dMi-2 proteins were eluted with FLAG peptide. Amounts of phosphorylated and dephosphorylated dMi-2 were monitored by SDS-PAGE and Coomassie Blue staining.*

*ATPase Assays—ATPase assays were performed and analyzed as in a previous study (4).*

*Nucleosome Mobilization Assay—Nucleosome mobilization assay were carried out as described before (40). Briefly, mononucleosomes were assembled using a mixture of Drosophila histones and polyglutamic acid (Sigma P4886) on a radiolabeled 247-bp DNA fragment derived from the mouse rDNA promoter (~231 to +16, relative to transcription start site). Centrally and end-positioned nucleosomes were resolved by native gel electrophoresis through 4.5% polyacrylamide gels in 0.4X TBE (90 mM Tris borate pH 8.3, 2 mM EDTA). Separated mononucleosomes were eluted from the gel slices by diffusion for at least 1 h at 4 °C in EX50 buffer (10 mM HEPS, pH 7.6, 50 mM KCl, 2.5 mM MgCl2, 10% glycerol, 10 mM β-glycerophosphatase, 1 mM dithiothreitol) containing 400 ng/µl bovine serum albumin. Isolated mononucleosomes were positioned at the end of the 247-bp DNA fragment were incubated with dMi-2 proteins (90–450 fmol) for 90 min at 26 °C in a final volume of 10 µl of EX50 buffer containing 1 mM ATP and 400 ng/µl bovine serum albumin. The reaction was stopped by the addition of 700 ng of unlabeled plasmid DNA and further incubation for 10 min on ice. Histone octamer mobilization was monitored by native gel electrophoresis on 4.5% polyacrylamide gels in 0.4X TBE (at 100 V for 3 h). Gels were dried and exposed to film overnight at ~80 °C.
**RESULTS**

We have shown previously, that recombinant dMi-2 expressed in insect cells possesses low basal ATPase activity that is strongly stimulated in the presence of nucleosomes (4, 7). By contrast, recombinant dMi-2 expressed in *Escherichia coli* displays high basal activity that does not change in the presence of nucleosomes (data not shown). We hypothesized that the observed lack of ATPase regulation was due to a difference in post-translational modifications in bacterially expressed dMi-2. Many eukaryotic proteins display post-translational modifications that are usually absent from bacterially expressed recombinant proteins resulting in the loss of enzymatic and biological activity. In contrast, expression of a recombinant *D. melanogaster* protein in insect cells can be expected to result in a correct modification. To test if dMi-2 is indeed post-translationally modified in insect cells, we sought to determine its phosphorylation status *in vivo*. For that purpose, we incubated *Drosophila* S2 cells in the presence of [32P]orthophosphate to radioactively label proteins that became phosphorylated during the incubation period. Cells were lysed and immunoprecipitations using antisera directed against the dMi-2 N or C terminus (α-dMi-2N and α-dMi-2C, respectively) were performed. The precipitated material was subjected to SDS-PAGE and phosphoproteins were detected by autoradiography (Fig. 1A).

Both dMi-2 antisera specifically precipitated two phosphoproteins with apparent molecular masses of 220 and 150 kDa, respectively (Fig. 1A, lanes 3 and 4). These two proteins were not efficiently precipitated when antisera was omitted (lane 1) or when preimmune serum was used (lane 2). Western blot analysis confirmed the presence of the 220-kDa dMi-2 protein in both anti-dMi-2 immunoprecipitates (Fig. 1B, lanes 3 and 4) strongly suggesting that the 220-kDa phosphoprotein is dMi-2. The identity of the 150-kDa phosphoprotein is at present unclear. Taken together, these results demonstrate that dMi-2 is phosphorylated *in vivo*.

We next assessed whether we could detect dMi-2 kinase activity in S2 cell extracts, *in vitro*. We incubated recombinant dMi-2 in the presence of cell extract and [γ-32P]ATP and detected incorporation of [32P]phosphate by SDS-PAGE and phosphorimaging analysis. The extract showed robust and specific dMi-2 kinase activity (Fig. 2B, compare lanes 2 and 3). This allowed us to map the region phosphorylated by the dMi-2 kinase using a panel of dMi-2 deletion mutants (Fig. 2A). Phosphorylation in mutants lacking amino acid residues 1–483 was weak (Fig. 2B, lanes 4 and 5) or undetectable (lane 7). In contrast, the full-length protein and all proteins containing the N-terminal region of dMi-2 were efficiently phosphorylated *in vitro* (Fig. 2B, lanes 2, 6, 8, and 9). We also expressed a fusion between GST and the N-terminal region of dMi-2 (amino acids 4–560 (17)) and tested whether this protein was a kinase substrate. The fusion protein, but not GST alone, was efficiently phosphorylated (Fig. 2B, lanes 10 and 11). This analysis defines residues 4–483 as being both required and sufficient for phosphorylation by dMi-2 kinase activity *in vitro*.

We reasoned that, if the interaction between the kinase and dMi-2 N terminus is sufficiently stable, it might be possible to use the GST fusion protein to affinity-purify dMi-2 kinases from cell extracts. To this end, GST and the GST-dMi-2-(4–560) fusion protein were immobilized on different glutathione resins. We first loaded S2 extract onto the GST resin to deplete proteins that would bind nonspecifically to the GST moiety (Fig. 3A). The flow-through was then applied to the GST-dMi-2-(4–560) resin. Bound proteins were eluted by an increase of the salt concentration, and eluate fractions were assayed for dMi-2-kinase activity (Fig. 3B). This procedure resulted in a robust enrichment of dMi-2 kinase activity (compare fraction 4 (lane 5) to input (lane 1)). These results demonstrate that dMi-2 kinase activity present in S2 cell extract can stably interact with the N terminus of dMi-2.

Next, we used a candidate approach to identify the dMi-2-interacting kinase. Several studies have suggested links between Mi-2 ATPases, cell cycle regulation, and transformation in mammalian cells (7, 20, 23). For this reason, we tested GST-dMi-2 affinity-purified fractions for the presence of the cell cycle-regulating cyclin-dependent kinases dCDK1 and dCDK2 by Western blot. We used an antibody recognizing the PSTAIR epitope, which is present in both CDKs (41). The antibody detected two major proteins none of which was enriched in fractions eluting from the GST-dMi-2 column.
We therefore consider it unlikely that these CDKs are responsible for dMi-2 phosphorylation in our assays.

A physical association between yeast CHD1, the sole Saccharomyces cerevisiae member of the Mi-2/CHD ATPase family, and CK2 (formerly designated as casein kinase II) has been reported (42). Western blot using an antiserum recognizing both Drosophila CK2/H9251 and H9252 subunits (37) revealed a robust enrichment of both dCK2 subunits in the purified fractions containing dMi-2 kinase activity (Fig. 3C, lower panel). These results suggest that dCK2 binds to and phosphorylates the dMi-2 N terminus.

Moreover, sequence analysis of the dMi-2 N-terminal region reveals 12 putative CK2 phosphorylation sites ((S/T)XX(D/E), Fig. 3D). The positions of at least eight of these appear to be conserved between the Mi-2 proteins of D. melanogaster and Anopheles gambiae. Vertebrate Mi-2 proteins likewise show a high abundance of putative CK2 phosphorylation sites in this region: mouse and human Mi-2β contain 12, and frog Mi-2 harbors 10 CK2 sites. The high prevalence of N-terminal CK2 phosphorylation sites appears to be a common feature of Mi-2 ATPases.

Taken together, these results support the idea that dCK2 is a dMi-2 kinase but do not rule out the possibility that other kinases contribute to dMi-2 phosphorylation. To address this issue, we purified dCK2 from Drosophila S2 cell extract by conventional chromatography (Fig. 4A).

We followed an established protocol that combines ion exchange, hydroxylapatite, and gel filtration chromatography (39). At each step of this purification, we monitored whether dMi-2 kinase activity copurified with dCK2. We found that S2 extract contained a single detectable dMi-2 kinase activity, which precisely cofractionated with dCK2 (Fig. 4B and data not shown). Therefore, it is likely that dCK2 is a major if not the only dMi-2 kinase in these Drosophila cells.

Having established that dMi-2 is a phosphoprotein and substrate of dCK2, we next sought to determine the functional consequences of
phosphorylation. In contrast to dMi-2 expressed in insect cells, dMi-2 expressed in *E. coli* displays high basal ATPase activity that does not respond to the presence of nucleosomes. We therefore asked whether phosphorylation of *E. coli*-expressed dMi-2 was sufficient to restore regulation of ATPase activity. We did not detect any changes in ATPase activity following incubation with recombinant CK2 and ATP suggesting that additional modifications are required or that bacterially expressed dMi-2 is not properly folded (data not shown).

In a complementary approach, we incubated recombinant dMi-2 proteins expressed in insect cells with calf intestine phosphatase. This treatment did not allow us to detect changes in the electrophoretic mobility of the 220-kDa dMi-2 protein during SDS-PAGE, most likely owing to the large size of the protein (Fig. 5A, compare lanes 1 and 2). By contrast, the electrophoretic mobility of a smaller dMi-2 deletion mutant retaining the N-terminal dCK2 binding region (amino acids 1–1271) increased significantly upon phosphatase treatment (compare lanes 5 and 6). The phosphatase-treated mutant comigrated with the same protein expressed in *E. coli* suggesting that dephosphorylation was quantitative (Fig. 5B, compare lanes 2 and 4). Importantly, phosphatase treatment in presence of phosphatase inhibitors did not result in mobility changes verifying that the observed effects were due to dephosphorylation (compare lanes 1–3).

We also expressed a similarly sized deletion mutant lacking the N-terminal CK2 binding region (dMi-2-(691–1982)) in insect cells. In this case, no phosphatase-induced shift in electrophoretic mobility was detected (Fig. 5A, lanes 3 and 4). These results confirm that the dMi-2 N terminus is phosphorylated *in vivo*. Furthermore, they demonstrate that recombinant dMi-2 can be efficiently dephosphorylated *in vitro*. This allowed us to investigate the functional consequences of dephosphorylation.

We compared phosphorylated and dephosphorylated dMi-2 proteins purified from insect cells in ATPase assays. Consistent with our previous results, mock treated dMi-2 showed weak basal ATPase activity that was stimulated 4-fold by addition of nucleosomes (but not by addition of free DNA) to the reaction (Fig. 6A). Dephosphorylated dMi-2 displayed similar basal ATPase activity. Addition of nucleosomes to the reaction resulted in a 12- to 16-fold activation suggesting that dephosphorylation significantly enhances dMi-2 nucleosome-stimulated ATPase activity.

Importantly, a dMi-2 mutant with a defective ATPase domain did not show significant ATPase activity irrespective of nucleosome addition and phosphatase treatment. This verified that the increased nucleosome-stimulated ATPase activity of dMi-2 was not a consequence of contamination by residual phosphatase carried over into the ATPase reactions.

We then investigated whether the dephosphorylation-induced changes in dMi-2 ATPase activity were directly translated into changes in dMi-2-mediated nucleosome remodeling (43). Our previous work demonstrated that recombinant dMi-2 catalyzes mobilization of...
FIGURE 4. Copurification of dCK2 and dMi-2 kinase activity from S2 cells. A, schematic representation of dCK2 purification. A silver-stained SDS-PAGE gel of the Mono Q peak fraction 23 is shown below. In B: Upper panel, fractions eluting from the Mono Q column were subjected to in vitro kinase assays as described in Fig. 3B. Number of fractions tested are indicated at the top, position of dMi-2 kinase substrate on the left. IN, input; FT, flow-through. Lower panel, fractions eluting from the final Mono Q column were subjected to Western analysis using α-dCK2 antibody. Positions of dCK2α and dCK2β are indicated on the left.
nucleosomes along a DNA fragment in an ATP-dependent manner (4, 7). Repositioning of the histone octamer from the end to the center of a 247-bp DNA fragment resulted in the appearance of a slower migrating band. As expected, incubation of positioned mononucleosomes with increasing amounts of dMi-2 resulted in nucleosome mobilization in an ATP-dependent manner (Fig. 6, lanes 2–5). At high dMi-2 concentrations ATP-dependent destabilization of nucleosomes (causing an increase in the amount of free DNA) was also detected (compare lanes 4 and 5). Pre-treatment of dMi-2 with phosphatase lead to a significant ATP-dependent stimulation of nucleosome mobilization activity that was most pronounced when dMi-2 amounts were limiting (compare lanes 2, 3, 6, and 7). Approximately half of the nucleosomes were relocated to the central position when the highest concentration of dMi-2 was used (compare lanes 4 and 8). In addition, ATP-dependent destabilization of the nucleosome was strongly stimulated by phosphorylation of dMi-2 (compare free DNA lanes 2–4 with lanes 6–8). These results are consistent with and further strengthen our hypothesis that dMi-2 functions are regulated by phosphorylation.

Because the early steps of the nucleosome-remodeling process likely involve nucleosome binding followed by ATP hydrolysis, we sought to determine whether changes in affinity for its nucleosomal substrate contributed to modulation of dMi-2 enzymatic activity. We have shown in a previous study that dMi-2 and mononucleosomes form complexes that are stable during native gel electrophoresis (7). As shown in Fig. 6C, addition of increasing amounts of mock treated or dephosphorylated dMi-2 to radiolabeled nucleosome core particles resulted in the formation of complexes that migrated slower than the free nucleosome. At least 50 fmol of mock treated dMi-2 were required to detect nucleosome binding in this assay (lane 5). By contrast, 25 fmol of dephosphorylated dMi-2 were sufficient for complex formation (lane 9). This result suggests that phosphorylation increases the affinity of dMi-2 for its nucleosome substrate, which might, at least in part, explain the observed differences in nucleosome stimulated ATPase and remodeling activities.

**DISCUSSION**

The eukaryotic nucleus contains a large number of ATP-dependent chromatin remodeling complexes. Their activities need to be tightly regulated to prevent remodeling catastrophes. For example, a mutation in *Drosophila* ISWI leads to grossly abnormal chromosome structure, whereas overexpression of the CHD family member Hrp1 in *Schizosaccharomyces pombe* results in chromosome decondensation and segregation defects (44, 45). Most investigations into how ATP-dependent chromatin remodelers are regulated have focused on targeting mechanisms that ensure their correct chromosomal localization. It is clear, however, that additional ways of regulation must exist that operate subsequent to chromatin binding. Mechanisms for regulation of the enzymatic activity of CHD ATPases have not been described so far. Here, we have uncovered a potential mechanism to modulate dMi-2 nucleosome remodeling activity by phosphorylation.

The 32P-labeling demonstrated that dMi-2 exists as a phosphoprotein in vivo. Recombinant dMi-2 proteins also become phosphorylated when expressed in insect cells. Phosphatase treatment of such a recombinant dMi-2 protein results in a quantitative shift in electrophoretic mobility during SDS-PAGE. This argues that it is not only a subpopulation of dMi-2 molecules that becomes phosphorylated in insect cell lines. Rather, our results are consistent with the hypothesis that dMi-2 phosphorylation is a constitutive event that is carried out by a constitutively active protein kinase.

We have identified dCK2 as a dMi-2 kinase in cell extracts. CK2 is a ubiquitously expressed and constitutively active serine/threonine protein kinase (46). More than 300 protein substrates for CK2 have been described, many of which function to regulate transcription, chromatin structure, or other aspects of genome activity. Two observations suggest that dCK2 is a major dMi-2 kinase. First, dMi-2 kinase activity precisely cofractionates with dCK2 during purification of the enzyme from crude S2 extracts by conventional chromatography. We have been unable to detect dMi-2 kinase activity in any fractions that did not also contain dCK2, arguing against the presence of other dMi-2 kinases in S2 extracts. Second, addition of the CK2 inhibitor DRB to S2 extract efficiently abrogated dMi-2 phosphorylation in vitro (data not shown).

Nevertheless, we cannot formally exclude that other kinases contribute to dMi-2 phosphorylation. Indeed, a genome-wide, high throughput yeast two-hybrid screen has recently identified a putative serine/threonine protein kinase (CG8173) as a potential dMi-2-binding protein (47). However, the physiological relevance of this finding is unclear, and there is no experimental evidence to support the notion that the CG8173 gene product phosphorylates dMi-2.

dCK2 binds to and phosphorylates an N-terminal dMi-2 region (residues 1–484), which harbors a high mobility group (HMG)-like domain and a tandemly arranged pair of PHD fingers. Our previous analysis has demonstrated an essential role for the dMi-2 chromodomains and the ATPase domain in nucleosome binding, nucleosome-regulated ATPase, and nucleosome mobilization assays (4). It is possible that phosphorylation modulates intramolecular interactions between domains that influence enzyme activity.
Recombinant dMi-2 expressed in E. coli exhibits high constitutive ATPase activity that is not further stimulated by the presence of nucleosomes (data not shown). In an attempt to restore nucleosome-dependent ATPase activity we incubated bacterially expressed dMi-2 with recombinant CK2 and ATP. However, this treatment had no effect on ATPase activity. Several explanations could account for this observation: bacterially expressed dMi-2 might be irreversibly misfolded, the in vitro phosphorylation by CK2 might be inefficient, and/or additional modifications might be required to impose nucleosome regulation. At present, we cannot distinguish between these possibilities.

Calf intestine phosphatase treatment of a baculovirus-expressed 170-kDa dMi-2 protein harboring N terminus and ATPase domain leads to a quantitative change in electrophoretic mobility that is readily detectable by standard SDS-PAGE. We were unable to detect changes in electrophoretic mobility upon phosphatase treatment of the full-length 220-kDa dMi-2 protein. Similar results were obtained using other phosphatases (data not shown). We attribute this to the difficulty to resolve phosphorylation induced mobility changes in very large proteins. In support of this hypothesis we find that full-length recombinant dMi-2 proteins expressed in insect cells (phosphorylated) and E. coli (unphosphorylated), respectively, display no difference in mobility even after prolonged electrophoresis (data not shown).

Dephosphorylation of dMi-2 positively modulates three related biochemical activities of the enzyme: binding to the nucleosome substrate, nucleosome-stimulated ATPase activity, and ATP-dependent nucleosome mobilization. We have also observed an increased affinity for free DNA upon dephosphorylation of dMi-2 (data not shown). It is unlikely that phosphorylation reduces the affinity for nucleosomal DNA exclusively by increasing the number of negative charges on dMi-2. Numerous studies have demonstrated that phosphorylation by CK2 can increase, decrease, or not affect the DNA binding activity of transcription factors and in many cases phosphorylation has been demonstrated to induce conformational changes in protein structure (Refs. 48–51 and references therein). It is conceivable that dMi-2 likewise undergoes conformational changes upon phosphorylation and dephosphorylation, which impinge on its enzymatic activity.

In all assays employed, dephosphorylation leads to an increase of dMi-2 activity. This suggests that constitutive phosphorylation of dMi-2 in insect cells serves to restrain enzymatic activity and raises the possibility that dMi-2 is fully activated by a phosphatase following recruitment to chromatin. Like dMi-2, histone deacetylase components of mammalian NuRD complexes are also targeted by CK2 (52). In this case, however, phosphorylation was reported to have an activating effect on enzymatic activity. It is therefore possible that CK2 phosphorylation influences both enzymatic activities of NuRD complexes. We speculate that phosphorylation is a mechanism to modulate the ATP-dependent chromatin remodeling and histone deacetylase activities of NuRD complexes. This “fine-tuning” of
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NuRD would allow gene activity at a particular chromosomal site to be adjusted to the required level. In this respect, it would be interesting to investigate if phosphorylation patterns of dNuRD subunits correlate with target gene activity (18, 29).

Previously, mammalian members of the SWI/SNF subfamily of ATP-dependent chromatin remodelers have been shown to be phosphorylated with functional consequences (35, 36): hBRM and BRG-1 are independent chromatin remodelers that have been shown to be phosphorylated and inactivated by hSWI/SNF complexes in vitro (35). However, the kinase responsible for hSWI/SNF phosphorylation in vivo is still unknown.

In contrast to hBRM and BRG1, we find dMi-2 phosphorylation not to be cell cycle-regulated but to be constitutive and have identified the constitutively active dCK2 as a major dMi-2 kinase. Interestingly, a proteomic screen aimed at identifying multisubunit protein complexes in S. cerevisiae has revealed a physical association between CK2 and the ATP-dependent chromatin remodeler CHD1, the only CHD family member present in S. cerevisiae. Moreover, analysis of the yeast phosphoproteome has identified a CHD1-derived phosphopeptide containing two putative CK2 sites (53). Several CHD4 phosphopeptides were recently identified in a proteome-wide characterization of HeLa cell nuclear proteins (54). A subset of these carries phosphate groups within putative CK2 recognition sites. Taken together with our observation that multiple CK2 sites are found in several CHD subfamily members, it is tempting to speculate that phosphorylation by CK2 is a general mechanism to regulate CHD ATPase functions.

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