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Nuclear translocation of doublecortin-like protein kinase and phosphorylation of a transcription factor JDP2

Tadashi Nagamine, Shohgo Nomada, Takashi Onouchi, Isamu Kameshita, Noriyuki Sueyoshi *

Department of Life Sciences, Faculty of Agriculture, Kagawa University, Kagawa 761-0795, Japan

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ABSTRACT

Doublecortin-like protein kinase (DCLK) is a microtubule-associated protein kinase predominantly expressed in brain. In a previous paper, we reported that zebrafish DCLK2 (zDCLK) was cleaved into two functional fragments; the N-terminal zDCLK(DC + SP) with microtubule-binding activity and the C-terminal zDCLK(kinase) with a Ser/Thr protein kinase activity. In this study, we demonstrated that zDCLK(kinase) was widely distributed in the cytoplasm and translocated into the nucleus when the cells were treated under hyperosmotic conditions with NaCl or mannitol. By two-hybrid screening using the C-terminal domain of DCLK, Jun dimerization protein 2 (JDP2), a nuclear transcription factor, was identified as zDCLK(kinase)-binding protein. Furthermore, JDP2 served as an efficient substrate for zDCLK(kinase) only when histone was present. These results suggest that the kinase fragment of DCLK is translocated into the nucleus upon hyperosmotic stresses and that the kinase efficiently phosphorylates JDP2, a possible target in the nucleus, with the aid of histones.

1. Introduction

Doublecortin-like protein kinase (DCLK), one of the members of the doublecortin (DCX) family, is a Ser/Thr protein kinase expressed in both adult and embryonic brain [1]. The primary structure of DCLK is composed of three functional domains. The N-terminal doublecortin-like (DC) domain is highly homologous to DCX and believed to be involved in microtubule association. The middle part of DCLK is a Ser/Pro-rich (SP) domain and the C-terminal region is a Ser/Thr protein kinase domain, which is highly homologous to multifunctional Ca^{2+}/calmodulin-dependent protein kinases (CaMKs). Both DCLK and DCX are known to bind to microtubules and promote polymerization/stabilization of microtubules [2–6]. The microtubule-binding of DCX is regulated by phosphorylation with various protein kinases such as cAMP-dependent protein kinase, c-Jun N-terminal kinase (JNK) and cyclin-dependent kinase 5 [7–9]. The phosphorylation sites in the DC domain are highly conserved in both DCX and DCLK. Although the microtubule-binding activity of DCLK is assumed to be regulated by phosphorylation/dephosphorylation as in case of DCX, regulatory mechanisms of microtubule-association/dissociation of DCLK are still unknown.

To date, three paralogous genes for DCLK have been reported [10]. Among these DCLKs, amino acid sequences of DCLK1 and DCLK2 are highly homologous to each other and exhibit similar tissue distribution [10,11], though the differences in the function between DCLK1 and DCLK2 still remain unclear. Recently, we reported that proteolytic processing of zebrafish DCLK2 (zDCLK) produced two functional proteins: the N-terminal microtubule-associated protein, zDCLK(DC + SP), and the C-terminal kinase domain, zDCLK(kinase) [12]. Although the kinase fragment thus produced was demonstrated to be widely distributed throughout the cytoplasm, the function or the target of the kinase is not known.

To understand the functional roles of DCLK, it is necessary to elucidate the regulatory mechanisms and the physiological target of the kinase. In the previous study, we demonstrated that zDCLK was activated by removal of the N-terminal (DC + SP) domain and further activation was observed by removal of the C-terminal autoinhibitory domain corresponding to the amino acid residues 678–810 [13]. In the present study, we showed that zDCLK(kinase) in the cytoplasm was translocated into the nucleus upon hyperosmotic stresses and the kinase efficiently phosphorylated a nuclear transcription factor, Jun dimerization protein-2 (JDP2), in the presence of histones.

Abbreviations: CREB, cyclic AMP-response element-binding protein; DCLK, doublecortin-like protein kinase; SP domain, Ser/Pro-rich domain; CaMK, Ca^{2+}/calmodulin dependent protein kinase; DCX, doublecortin; GST, glutathione S-transferase; JDP2, Jun dimerization protein-2; JNK, c-Jun N-terminal kinase; MBP, myelin basic protein.

* Corresponding author. Fax: +81 87 891 3114.
E-mail address: sueyoshi@ag.kagawa-u.ac.jp (N. Sueyoshi).

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2. Materials and methods

2.1. Materials

Cy3-labeled anti-mouse IgG, Cy5-labeled anti-rabbit IgG, anti-FLAG antibody, bovine serum albumin, and histone from calf thymus (Type II-AS) were purchased from Sigma Chemicals. Goat anti-mouse IgG + A + M and goat anti-rabbit IgG, conjugated with horseradish peroxidase, were from Pierce Biotechnology. Anti-myc antibody was purchased from Invitrogen.

2.2. Construction of plasmids

For mammalian cells, the cDNA encoding zDCLK (Accession No. AB266175) and its deletion mutants, were inserted into a pcDNA3.1/myc-HisB (Invitrogen)-backbone plasmid with the FLAG tag (pcFLAG) [11]. The various mutants of zDCLK having a *Hin*dIII site on the 5′-end and an *Xho*I site on the 3′-end were inserted into the pcFLAG vector. GST-tagged zDCLK (pGEX 4T-1) was generated as described previously [12]. The cDNA encoding zebrafish JDP2 (zJDP2, Accession No. NM_001002493) was inserted into *Bam*HI-*Xho*I and *Nhe*I-*Xho*I sites of pcDNA3.1/myc-HisB and that of pET-23a to generate pczJDP2 and pETzJDP2, respectively.

2.3. Expression and purification of recombinant proteins

Recombinant zDCLK and rat CREB were expressed in *Escherichia coli* and purified as described previously [12]. To obtain recombinant zJDP2, the expression plasmid pETzJDP2 was introduced into *E. coli* strain Rosetta (DE3). The transformed bacteria were grown at 37 °C to an A_{600} of 1.0, and then isopropyl-β-D-thiogalactopyranoside was added to a final concentration of 0.1 mM. After incubation at 15 °C for 6 h, the bacteria were harvested by centrifugation and suspended in buffer A [20 mM Tris–HCl (pH 7.5), 150 mM NaCl and 0.05% Tween 40]. After sonication, cell debris was removed by centrifugation (20,000*g*) at 4 °C for 10 min, and the supernatant was loaded on a HiTrap Chelating HP column (GE Healthcare Bio-Sciences) pre-equilibrated with buffer A. The column was sequentially washed with buffer A, buffer A containing 20 mM imidazole and buffer A containing 50 mM imidazole, before being eluted with buffer A containing 200 mM imidazole. The purified fractions were pooled, dialyzed against 20 mM Tris–HCl (pH 7.5) containing 0.05% Tween 40 and 1 mM 2-mercaptoethanol, and used for kinase assay.

2.4. Cell culture and transfection

COS7 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Wako) containing 10% heat inactivated fetal calf serum. Cells were grown at 37 °C in a humidified incubator with a 5% CO_2/95% air atmosphere. Transfection of COS7 cells was performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. COS7 cells were plated at 2×10^5 in a 35-mm dish in 2 ml of DMEM containing 10% fetal calf serum. After 24 h of culture, cells were transfected with 4 μl of Lipofectamine 2000 and 2 μg of plasmid DNA by incubation for 24 h in 0.8 ml of DMEM containing 5% fetal calf serum.

2.5. Immunocytochemistry of zDCLKs

Transfected cells were cultured on poly-L-lysine coated cover glass and treated with 10% formalin in phosphate-buffered saline (PBS) for 20 min. After being rinsed with PBS, formalin-fixed cells were permeabilized with 0.1% Triton X-100 in PBS for 5 min. The cells were washed with PBS, treated with 1% bovine serum albumin in PBS, and then incubated with anti-FLAG antibody (mouse IgG) diluted 1:3000 or anti-tubulin α antibody (rabbit IgG, Epitomics) diluted 1:25 with 1% bovine serum albumin in PBS at room temperature for 2 h. The cell samples were then incubated with Cy3-labelled anti-mouse IgG or Cy5-labeled anti-rabbit IgG, respectively, at room temperature for 2 h. Subsequently, the cells were treated with DAPI (Wako) diluted 1:1000 in PBS at room temperature for 0.5 h and observed by a confocal laser-scanning microscope (FV1000-D, OLYMPUS).

2.6. Immunoprecipitation

Cells were collected at 24 h after transfection and homogenized with IP buffer [10 mM Tris–HCl (pH 8.0), 1% NP-40, 150 mM NaCl, 1 mM EDTA, 10 μg/ml protease inhibitors (antipain, pepstatin, leupeptin and chymostatin) and phosphatase inhibitor cocktail (Cosmo Bio)]. The homogenates were centrifuged at 20,000*g* for 20 min, and the supernatants were mixed with antibodies (0.5 μg of anti-FLAG). After incubation at 4 °C for 1.5 h, 20 μl of protein G-Sepharose 4B Fast Flow (GE Healthcare Bio-Sciences) was added to the samples, and rotated at 4 °C for 1.5 h. The samples were washed three times with IP buffer and three times with kinase assay buffer [40 mM Hepes-NaOH (pH 8.0), 2 mM dithiothreitol, 0.1 mM EGTA and 5 mM $Mg(CH_3COO)_2$], and then determined protein kinase activity using 100 μM [γ-^{32}P]ATP or eluted with SDS–PAGE sample buffer for SDS–PAGE analysis.

2.7. GST pull-down assay

For pull-down experiments, GST or GST-DCLKs included in 200 μl of cell lysate were absorbed on glutathione Sepharose 4B (20 μl) at 4 °C for 1 h. The glutathione sepharose was washed five times with ice cold buffer A and then recombinant JDP2 (20 μg) in 200 μl of buffer A was added. After incubation at 4 °C for 2 h, samples were washed five times with the ice cold buffer A, and eluted with 20 μl of 2 × SDS–PAGE sample buffer and analyzed by SDS–PAGE, followed by Western blotting.

2.8. SDS–PAGE and Western blotting

SDS–PAGE was performed on slab gels consisting of a 10% or 15% acrylamide separation gel and a 3% stacking gel. The resolved proteins were electrophoretically transferred to nitrocellulose membranes (Hybond ECL, GE Healthcare) and immunoreactive protein bands were detected essentially according to the method described previously [14].

2.9. Protein kinase assay

The protein kinase activities of zDCLKs were determined as described previously [12]. Phosphorylation of proteins was carried out at 30 °C for 10 min in a standard reaction mixture (10 μl) consisting of 40 mM Hepes-NaOH (pH 8.0), 2 mM dithiothreitol, 0.1 mM EGTA, 5 mM $Mg(CH_3COO)_2$, 100 μM [γ-^{32}P]ATP and the indicated concentration of protein substrates. After incubation, the reaction was stopped by the addition of 10 μl of 2 × SDS–PAGE sample buffer. Phosphorylated proteins were resolved by SDS–PAGE and detected by autoradiography.

2.10. Bacterial two-hybrid screening

To explore the target protein for DCLK(kinase), the Bacterio-Match II two-hybrid system (Stratagene) was used to screen a zebrafish adult brain cDNA library. A fragment of the zDCLK encoding the C-terminal region (amino acids 678–810) was used as a bait for screening and was cloned in frame into the *Eco*RI/*Xho*I sites of the

pBT vector to generate pBT-zDCLK(678–810). The cDNA library in the pTRG plasmid was screened with pBT-zDCLK(678–810) according to the manufacturer's instructions. Briefly, the screening reporter competent cells were cotransformed with pBT-zDCLK(678–810) and pTRG-cDNA library plasmids and transformants were selected on the selective screening medium containing 5 mM of 3-amino-1,2,4-triazole (3-AT). Positive clones were then subjected to further screening on a dual selective screening medium (5 mM 3-AT and streptomycin). To confirm the interaction between DCLK and the candidate protein, competent cells were retransformed by use of pTRG purified from the positive clones and recombinant pBT-zDCLK(678–810).

2.11. Other methods

Protein concentrations were determined by the method of Bensadoun and Weinstein using bovine serum albumin as a standard [15]. Nucleotide sequences were determined by the dideoxynucleotide chain termination method with a BigDye Terminator Cycle Sequencing Ready Reaction Kit Ver. 3.1 (Applied Biosystems) and a DNA Sequencer (model 3100, Applied Biosystems).

3. Results

3.1. Hyperosmotic stresses induce nuclear translocation of zDCLK(kinase)

DCLK is composed of two functional domains; the N-terminal microtubule-association domain and the C-terminal protein kinase domain (Fig. 1A). In a previous study, we demonstrated that zDCLK was cleaved into two functional proteins, zDCLK(DC + SP) and zDCLK(kinase), by proteolytic processing *in vivo* [11]. Since the N-terminal DC domain has microtubule-binding activity, the C-terminal kinase fragment may be released from cytoskeletal compartment. When the full-length zDCLK was transfected and expressed in COS7 cells, not only DCKL(WT) but also DCKL(kinase) was detected (Fig. 1B). To examine subcellular localization of the kinase fragment, DCLK(kinase) was expressed in COS7 cells. DCLK(kinase), which lacks microtubule-association domain, was found to be distributed throughout the cytoplasm as predicted (Fig. 1C, upper panel, WT). Since zDCLK(kinase) exhibited much higher activity than that of zDCLK(WT) [12], the truncated kinase may play important roles after proteolytic processing.

In the present study, we examined the effect of various stimuli on the subcellular localization of zDCLK(kinase), and found that hyperosmotic conditions, but not other stimuli, caused drastic changes in the localization of zDCLK fragment. When COS7 cells were treated with 0.5 M NaCl, zDCLK(kinase) was clearly translocated into the nucleus (Fig. 1C, lower panel, WT). Nuclear translocation of zDCLK(kinase) was also observed when kinase-dead mutant zDCLK(kinase/K449R) was used, indicating that the kinase activity was not required for the nuclear translocation of zDCLK(kinase). Essentially the same results of nuclear translocation of zDCLK(kinase) were obtained when the cells were treated with 0.2 M–0.5 M NaCl, 0.2 M–0.5 M mannitol or 0.1–0.5 M KCl (not shown). In case of p38 MAP kinase, nuclear translocation was observed when the critical phosphorylation sites in its activation loop were phosphrylated [16,17]. To examine whether zDCLK(kinase) is also the case, translocation of zDCLK(kinase/T576D) and zDCLK(kinase/T576A) were investigated and compared with the result of zDCLK(kinase). The point mutant of zDCLK(kinase/T576D) is the constitutively active kinase that mimics phosphorylated form of zDCLK(kinase), and zDCLK(kinase/T576A) is a non-phosphorylatable mutant of zDCLK(kinase) [12]. These two zDCLK(kinase) mutants, however, translocated to the nucleus upon high salt treatment as in case of wild-type zDCLK(kinase) (Fig. 1C, T576D and T576A), indicating that phosphorylation of the Thr residue in

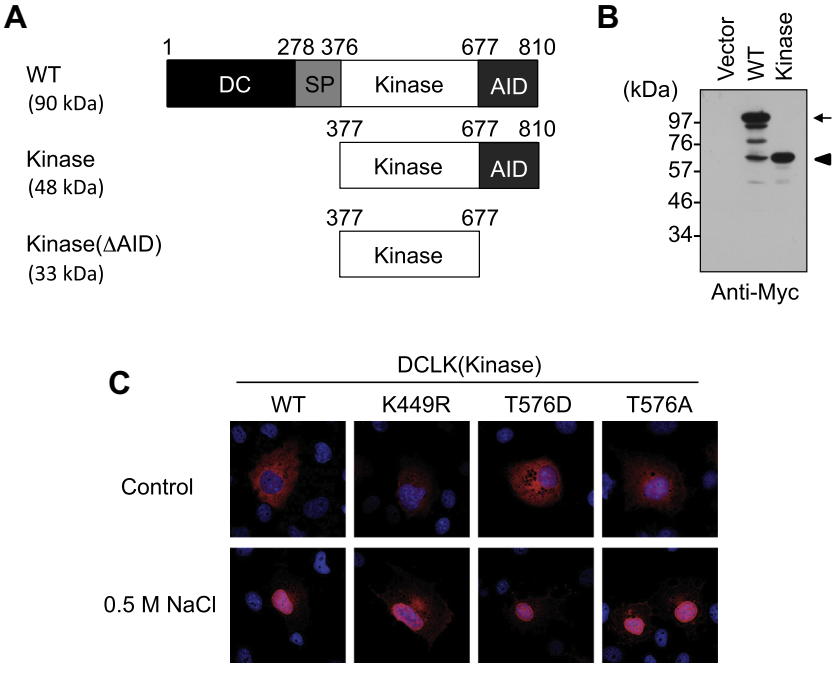

Fig. 1. Nuclear translocation of the kinase domain of zDCLK. (A) Schematic illustration of zDCLK and truncation mutants used in the present study. Amino acid numbers of the truncation mutants are indicated. (B) Transfection and expression of zDCLK(WT) and zDCLK(kinase) in COS7 cells. COS7 cells were transfected with empty vector or expression vectors for myc-DCLK(WT) and myc-DCLK(kinase). Transfected cells were lysed and cell extracts were analyzed by Western blotting with anti-myc antibody. (C) Translocation of zDCLK(kinase) and its mutants after hyperosmotic stress. COS7 cells transiently expressing FLAG-tagged zDCLK(kinase) and its mutants were treated with 0.5 M NaCl (lower panels) or H_2O (upper panels; Control) for 30 min. Wild-type zDCLK(kinase) and its mutants were visualized by indirect immunofluorescence with an anti-FLAG antibody (Cy3) using confocal laser scanning microscopy. The nuclei were counterstained by DAPI.

the activation loop is not necessary for the nuclear translocation of zDCLK(kinase).

3.2. Kinase activity of zDCLK(kinase) was stimulated by a nuclear protein, histone

To examine the activity of zDCLK(kinase) in the nucleus, the kinase was immunoprecipitated with anti-FLAG antibody after stimulation of transfected cells with 0.5 M NaCl. When the kinase activity was detected by the *in vitro* kinase assay using [γ-^{32}P]ATP, neither autophosphorylation nor phosphorylation of DCX was observed (Fig. 2A, lanes 1 and 3). By contrast, when the phosphorylation was carried out in the presence of histones (Type IIA, Sigma), both autophosphorylation and mDCX phosphorylation were clearly observed in addition to significant phosphorylation of histones (Fig. 2A, lanes 2 and 4). No phosphorylation was observed at all when kinase-dead mutant zDCLK(kinase/K449R) was used instead of zDCLK(kinase) (Fig. 2A, lanes 5–8). When zDCLK(kinase/ΔAID) was used, similar stimulatory effect of phosphorylation by histones was observed (Fig. 2A, lanes 9–12), indicating that histones stimulated the kinase activity by interacting with the catalytic domain of zDCLK, but not with the autoinhibitory domain (AID) in the C-terminal region.

Next, we examined the effect of histones on the autophosphorylation and phosphorylation of DCX by the recombinant zDCLK(kinase) *in vitro*. Phosphorylation of DCX by GST-zDCLK(kinase/T576D) and GST-zDCLK(kinase/T576A) were activated by the addition of histones as in case of GST-zDCLK(kinase) (Fig. 2B), indicating that the activation of zDCLK(kinase) by histones is not affected by the phosphorylation in the activation loop. Phosphorylation of Dnmt1 by cyclin-dependent kinase like 5 (CDKL5) is known to be activated by the addition of genomic DNA [18]. Therefore, we examined the effect of genomic DNA on the kinase activity of zDCLK(kinase) in combination with histones. Although kinase activity of zDCLK(kinase) was significantly stimulated by the addition of histones, DNA alone or DNA in combination with histones did not show further activation of the kinase as compared to single addition of histones (Fig. 2C, lanes 1–4). Since histones are typical basic proteins with high pI values, effect of the other basic protein on zDCLK(kinase) activity was examined. When myelin basic protein was added to the phosphorylation mixture, no stimulation of the kinase activity was observed (Fig. 2C, lane 5), suggesting that not all the basic proteins serve as the activators for zDCLK(kinase).

3.3. JDP2 is a potential substrate for zDCLK(kinase) in the nucleus

Although some possible candidates of endogenous substrates for DCLK have been suggested to date [10,13], physiological target for DCLK is still unclear. zDCLK(kinase) was translocated into nucleus under hyperosmotic conditions, and it was markedly activated when it contacted with the histones in the nucleus. In this case, some nuclear proteins may be phosphorylated with the activated zDCLK(kinase) by the coexisting histones. Therefore, to explore the target protein for DCLK(kinase), we performed a two-hybrid screening using the C-terminal region (amino acid residues 678–810) of zDCLK as a bait. By this screening, we found a novel DCLK-binding protein, JDP2, as shown in Fig. 3A. JDP2 is known as a transcription factor that is present in the nucleus [19]. To further confirm interaction between zDCLK and JDP2, we performed GST pull-down experiments. When various truncation mutant of zDCLK was used for pull-down assay of JDP2, not only the C-terminal region zDCLK(678–810) but also the N-terminal region zDCLK(1–376) clearly showed binding affinity for JDP2 (Fig. 3B).

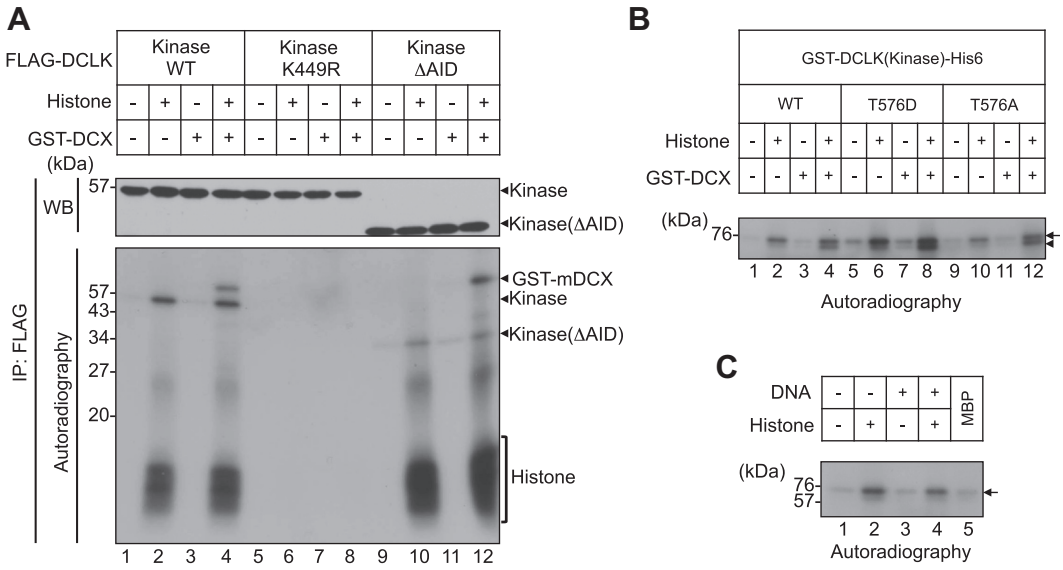

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Fig. 2. Activation of zDCLK by histone. (A) COS7 cells transfected with FLAG-tagged zDCLK(kinase) (lanes 1–4) or FLAG-tagged zDCLK(kinase/K449R) (lanes 5–8) or FLAG-tagged zDCLK(kinase/ΔAID) (lanes 9–12) were cultured for 24 h and then treated with 0.5 M NaCl for 30 min. FLAG-tagged zDCLK mutants in the cells were immunoprecipitated with an anti-FLAG antibody and detected by Western blotting using an anti-FLAG antibody (upper panel). The kinase activity of immunoprecipitated zDCLK was measured *in vitro* kinase assay using GST-mDCX (200 ng) as a substrate in the standard phosphorylation mixture (10 μl) containing 100 μM [γ-^{32}P]ATP in the presence (+) or absence (−) of histone (3.5 μg) (lower panel). (B) The point mutants and wild-type zDCLK(kinase) were assayed using GST-mDCX (200 ng) as a substrate in the standard phosphorylation mixture (10 μl) containing 100 μM [γ-^{32}P]ATP in the presence (+) or absence (−) of histone (3.5 μg). (C) Autophosphorylation activity of zDCLK(kinase). Recombinant zDCLK(kinase) (150 ng) was incubated with the standard phosphorylation mixture (10 μl) containing 100 μM [γ-^{32}P]ATP in the presence (+) or absence (−) of mouse genomic DNA (365 ng), histone (3.5 μg) and MBP (3.5 μg) as indicated. Autophosphorylation of zDCLK(kinase) was analyzed by SDS–PAGE followed by autoradiography. An arrow indicates the migration position of GST-zDCLK(kinase).

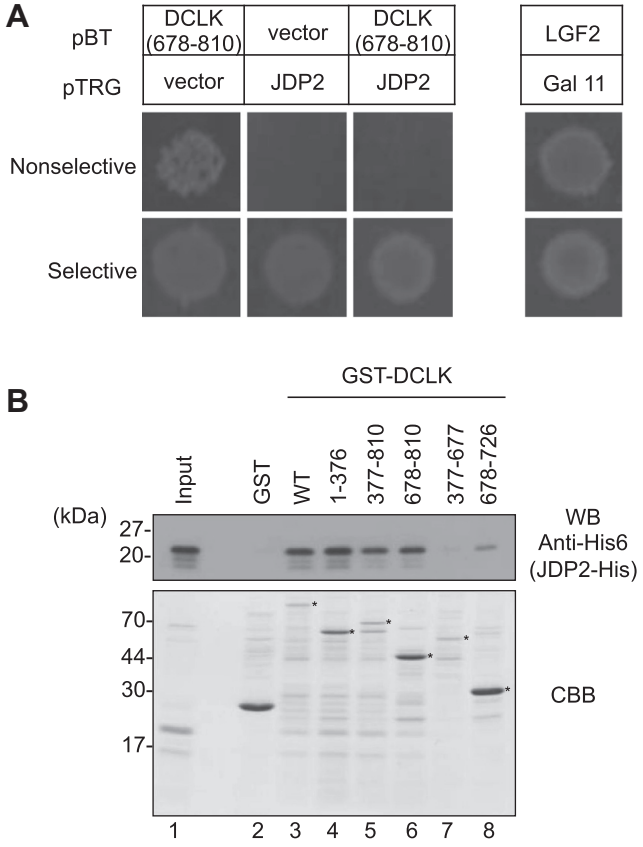

Fig. 3. JDP2 is a novel binding protein of DCLK. (A) BacterioMatch II Screening Reporter competent cells were transfected with 50 ng of pBT zDCLK(678–810) and pTRG JDP2. After incubation, aliquots (4 μl) of culture solution were spotted on the nonselective or selective plate. (B) GST or GST-DCLKs included in 200 μl of cell lysate were absorbed on glutathione sepharose 4B (20 μl). The glutathione Sepharose was washed with ice cold buffer A, and recombinant JDP2 (20 μg) in 200 μl of buffer A was added. After incubation, samples were washed with the ice cold buffer A and eluted with 20 μl of 2 × SDS sample buffer, and then analyzed by SDS–PAGE followed by Western blotting with anti-His_6 antibody.

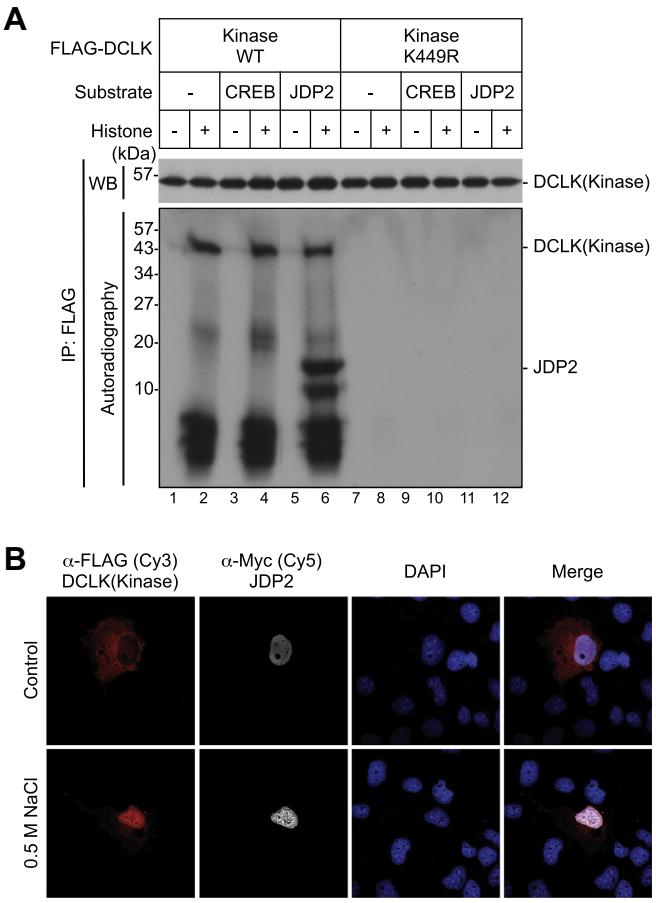

Fig. 4. JDP2 is a potential substrate for zDCLK. (A) FLAG-tagged zDCLK(kinase) or zDCLK(kinase/K449R) was immunoprecipitated from 0.5 M NaCl-treated COS7 cells using an anti-FLAG antibody. Immunoprecipitates were analyzed by Western blotting with anti-zDCLK antibody (upper panel). Alternatively, immunoprecipitated zDCLK was incubated in a standard phosphorylation mixture containing 100 μM [γ-^{32}P]ATP in the presence of CREB or JDP2, or in the absence of substrate (−) (lower panel). Phosphorylation was carried out either in the presence (+) or absence (−) of histone. (B) Subcellular localization of JDP2. COS7 cells transiently coexpressing FLAG-tagged zDCLK(kinase) and Myc/His-tagged JDP2 were treated with 0.5 M NaCl (lower panels) or H_2O (upper panels; Control) for 30 min. Then the recombinant proteins were visualized by indirect immunofluorescence with an anti-FLAG antibody (Cy3) or anti-myc antibody (Cy5) using confocal laser scanning microscopy. Nuclei were counterstained with DAPI.

Next, we examined phosphorylation of two transcription factors, JDP2 and cAMP-response element-binding protein (CREB), as substrates for zDCLK(kinase). JDP2 was efficiently phosphorylated by zDCLK(kinase) when it was incubated with histones (Fig. 4A, lane 6), while CREB was not phosphorylated by the kinase even in the presence of histones (Fig. 4A, lane 4). The kinase-dead mutant zDCLK(kinase/K449R) did not show any kinase activity under the conditions used (Fig. 4A, lanes 7–12). Transfected JDP2 in COS7 cells was found to be exclusively localized in the nucleus either under the normal or hyperosmotic conditions. These results implicate possible colocalization of zDCLK(kinase) with JDP2 in the nucleus under hyperosmotic conditions (Fig. 4B).

4. Discussion

DCLK is a Ser/Thr protein kinase that is specifically expressed in the brain and binds to microtubules through its N-terminal domain. The N-terminal region of DCLK, which is highly homologous to DCX, is involved in the stability of microtubules and migration of neuronal cells, similar to the case of DCX. Although the C-terminal kinase domain is highly homologous to multifunctional CaMKs, its physiological function and physiological targets are still unknown. To understand the functional roles of DCLK, it is necessary to elucidate the regulatory mechanisms for its activity. Although full length zDCLK shows quite low kinase activity [12], at least three different mechanisms for activation of zDCLK have been reported. (1) Proteolytic processing of zDCLK generates highly active C-terminal fragment, zDCLK(kinase) [11,12]. (2) Phosphorylation of Thr residue in the activation loop stimulates the kinase activity of zDCLK(kinase) [12]. (3) Removal of autoinhibitory domain (AID) corresponding to the amino acid residues 678–810 enhances the kinase activity [12]. In the present study, we found that autophosphorylation and phosphorylation of DCX by zDCLK(kinase/ΔAID) was activated by the addition of histones, suggesting that histones directly stimulated zDCLK(kinase) by interacting with the catalytic domain of the enzyme. Furthermore, the kinase activity of zDCLK(kinase/T576D), a constitutively active phospho-mimic mutant, was also stimulated by the addition of histones. These results, taken together, implicate that the activation of zDCLK(kinase) by histones is another regulatory mechanism that had not been reported previously.

The C-terminal kinase domain of zDCLK was separated from the N-terminal microtubule-association domain by proteolytic processing, and zDCLK(kinase) was found to be widely distributed in the cytoplasm. We also reported that the kinase fragment of zDCLK

was activated after separation from the N-terminal zDCLK(DC + SP) domain [12]. zDCLK(kinase) present in the cytoplasm was translocated into the nucleus when the cells were transferred to the hyperosmotic conditions such as 0.5 M NaCl or mannitol. In the nucleus, zDCLK(kinase) phosphorylates nuclear proteins such as JDP2 and histones. It is of special interest, however, that phosphorylation of JDP2 by zDCLK(kinase) occurs only in the presence of histones. Therefore, it is rational to speculate that JDP2, a nuclear transcription factor, is a physiological substrate for zDCLK(kinase) in the nucleus.

In the previous study, we identified synapsin II, a synaptosomal protein in the presynaptic neuron, as a possible target of zDCLK in the central nervous systems [13]. In this study, we found that DCX and JDP2 as possible targets of zDCLK(kinase) in the neuronal cells. Phosphorylation of DCX by DCLK may regulate association/dissociation of DCX to microtubules as in case of phosphorylation by JNK. Consequence of phosphorylation of JDP2 by DCLK(kinase) is not unclear, because physiological function of JDP2 itself is not fully characterized yet. In consideration of the fact that JDP2 is phosphorylated by zDCLK(kinase) when it is translocated into the nucleus after hyperosmotic stresses, it may induce various genes that protect the cells from osmotic stresses. Detailed mechanisms of translocation of zDCLK(kinase) to the nucleus after hyperosmotic stresses and the effects of phosphorylation of JDP2 by zDCLK(kinase) will be the next question to be solved.

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