Dyrk1A Phosphorylates α-Synuclein and Enhances Intracellular Inclusion Formation*

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Lewy bodies (LBs) are pathological hallmarks of Parkinson disease (PD) but also occur in Alzheimer disease (AD) and dementia of LBs. α-Synuclein, the major component of LBs, is observed in the brain of Down syndrome (DS) patients with AD. Dyrk1A, a dual specificity tyrosine-regulated kinase (Dyrk) family member, is the mammalian ortholog of the Drosophila minibrain (Mnb) gene, essential for normal postembryonic neurogenesis. The Dyrk1A gene resides in the human chromosome 21q22.2 region, which is associated with DS anomalies, including mental retardation. In this study, we examined whether Dyrk1A interacts with α-synuclein and subsequently affects intracellular α-synuclein inclusion formation in immortalized hippocampal neuronal (H19-7) cells. Dyrk1A selectively binds to α-synuclein in transformed and primary neuronal cells. α-Synuclein overexpression, followed by basic fibroblast growth factor-induced neuronal differentiation, resulted in cell death. We observed that accompanying cell death was increased α-synuclein phosphorylation and intracytoplasmic aggregation. In addition, the transfection of kinase-inactive Dyrk1A or Dyrk1A small interfering RNA blocked α-synuclein phosphorylation and aggregate formation. In vitro kinase assay of anti-Dyrk1A immunocomplexes demonstrated that Dyrk1A could phosphorylate α-synuclein at Ser-87. Furthermore, aggregates formed by phosphorylated α-synuclein have a distinct morphology and are more neurotoxic compared with aggregates composed of unmodified wild type α-synuclein. These findings suggest α-synuclein inclusion formation regulated by Dyrk1A, potentially affecting neuronal cell viability.

α-Synuclein is a major component of Lewy bodies (LBs)* found in Parkinson disease (PD), dementia with LB, Alzheimer disease (AD), and multiple system atrophy (1). In these neurodegenerative disorders (collectively referred to as synucleinopathies), LBs are characterized by fibrillar, cytoplasmic α-synuclein aggregates within selective populations of neurons and glial cells (2). α-Synuclein inclusion formation is clearly involved in the pathogenic process of PD. α-Synuclein was first identified as a partial fragment in AD amyloid plaques (41), and subsequently three missense mutations in the α-synuclein gene were reported in early onset familial PD of some kindred (3, 4).

Down syndrome (DS) is the most common genetic disorder, with a frequency of 1 in every 700–800 live births, and is caused by an extra copy of all or part of chromosome 21 (5). In addition to characteristic physical features, DS individuals have congenital heart defects, gastrointestinal malformations, immune and endocrine system defects, a high incidence of leukemia, and early onset of Alzheimer-like dementia. DS individuals also exhibit mild to severe mental retardation (6–8). Efforts to isolate the gene(s) responsible for DS mental retardation identified Dyrk1A as a candidate gene (9, 10).

The Drosophila melanogaster minibrain (Mnb) gene encodes a serine/threonine protein kinase essential in cell proliferation and neuronal differentiation during postembryonic neurogenesis (10). Dual specificity tyrosine-regulated kinase-1A (Dyrk1A), the Mnb kinase human homolog, maps to the DS critical region on chromosome 21. Dyrk1A is thought to be responsible for the DS neurological defects. In DS fetal brains, Dyrk1A expression increases 1.5-fold, and transgenic mice overexpressing Dyrk1A exhibit neurodevelopmental delays, motor abnormalities, and cognitive deficits (11, 12).

α-Synuclein post-translational modifications include nitration, glycosylation, and phosphorylation and are likely to influence α-synuclein aggregation. Constitutive α-synuclein phosphorylation at C-terminal Ser-87 and Ser-129 residues occurs in neuronal and nonneuronal cell lines (13). α-Synuclein aggregates in brain tissue from individuals with synucleinopathy are extensively phosphorylated at Ser-129 (14). Furthermore,
α-synuclein Ser-129 phosphorylation promotes eosinophilic fibril formation in vitro and in SH-SY5Y cells (14, 15). α-Synuclein is a substrate for G protein-coupled receptor kinases, and the protein-tyrosine kinase Pyk2/RAFTK phosphorylates α-synuclein at the Tyr-125 residue in response to hyperosmotic stress (16, 17). These findings suggest that filamentous protein phosphorylation, including α-synuclein phosphorylation, is important in the pathogenesis of neurodegenerative disorders.

Interestingly, α-synuclein-positive LBs and neuritic processes frequently occur in DS brains with AD phenotypes (18). In addition, LB formation frequency in DS patient brains with AD is greater than in sporadic AD cases (19). To study the molecular mechanisms leading to LB formation in DS patients, we examined whether Dyrk1A interacts with α-synuclein and affects cytoplasmic inclusion formation in hippocampal neuroprogenitor cells. Our data show that Dyrk1A phosphorylates α-synuclein at the Ser-87 residue. Additionally, Dyrk1A-mediated α-synuclein phosphorylation facilitates its aggregation.

**EXPERIMENTAL PROCEDURES**

**Materials**—Peroxidase-conjugated anti-rabbit and antiserum Gs were purchased from Zymed Laboratories Inc. (San Francisco, CA); Dulbecco’s modified Eagle’s medium, fetal bovine serum, Lipofectamine Plus reagent, and cell culture reagents were from Invitrogen; glutathione-Sepharose 4B and Protein A-Sepharose were from Amersham Biosciences; ECL reagents and [γ-32P]ATP were from PerkinElmer Life Sciences; anti-α-synuclein IgG was from AbCam; anti-Dyrk1A antibodies were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA); and anti-phosphoserine/phosphothreonine and anti-tyrosine antibodies were from Sigma. Anti-phospho-Ser-129 α-synuclein antibody was developed as described previously (14). Plasmids encoding HA-tagged Dyrk1A (pSVL-HA-Dyrk1A) and its K188R mutant (pSVL-HA-Dyrk1A-K188R) were kindly provided by W. Becker (Institute of Pharmacology and Toxicology, RWTH, Aachen, Germany). Wild type α-synuclein plasmid was provided by R. Jakes (Medical Research Council Laboratory of Molecular Biology, Cambridge, UK). FLAG-tagged α-synuclein with a serine to alanine point mutation (S9A, S42A, S87A, and S129A) cDNAs were generously provided by C. A. Ross (Johns Hopkins University School of Medicine, Baltimore, MD) and J. L. Benovic (Thomas Jefferson University, Philadelphia, PA). Bacterially recombinant α-synuclein proteins were either purchased from ATGen (Seongnam-si, Gyeonggi-do, Korea) or purified as described previously (20).

**Cell Culture and DNA Transfection**—Conditionally immortalized hippocampal (H19-7) cell lines were cultured as described previously (21). The neuroblastoma SH-SY5Y cells were maintained in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum with penicillin and streptomycin. Rat fetal brain lysates and primary cortical neurons were prepared as described previously (22). The cells were transfected with Lipofectamine Plus reagent (Invitrogen), according to the supplier’s instructions. To prepare cell lysates, cells were rinsed with ice-cold phosphate-buffered saline and solubilized in lysis buffer (10 mM Tris, pH 7.9, containing 1% Nonidet P-40, 150 mM NaCl, 1 mM EGTA, 1 mM EDTA, 10% glycerol, 1 mM Na3VO4, 1 μg/ml leupeptin, 1 μg/ml aprotinin, 10 mM NaF, and 0.2 mM phenylmethanesulfonyl fluoride). Cells were scraped, and supernatants were collected after centrifugation for 10 min at 14,000 × g at 4°C. Protein concentrations were determined using the detergent- compatible protein assay kit (Bio-Rad).

**Cell Viability**—Cell survival quantitation was performed using the tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-2,5-di-phenyltetrazolium bromide extraction method, as described previously (23). Statistical analyses were completed with the aid of the StatView II program (Abacus Concepts, CA). All data were analyzed by one-way analysis of variance and preplanned comparisons with the control were performed by means of Dunnett’s T-statistic.

**Immunoprecipitation and Western Blot Analysis**—One microgram of suitable antibodies was incubated with 0.5—1 mg of cell extracts in cell lysis buffer overnight at 4°C. Fifty microliters of a 1:1 protein A-Sepharose bead suspension was added and incubated for 2 h at 4°C with gentle rotation. Beads were pelleted and washed extensively with cell lysis buffer. Bound proteins were dissociated by boiling in SDS-PAGE sample buffer, and samples were separated on SDS-polyacrylamide gel and transferred to a nitrocellulose membrane (Millipore). Membranes were blocked in TBST buffer (20 mM Tris, pH 7.6, 137 mM NaCl, 0.05% Tween 20) plus 3% nonfat dry milk for 3 h and then incubated overnight at 4°C in TBST buffer with 3% nonfat dry milk and the appropriate antibodies. Membranes were washed several times in TBST and then incubated with secondary IgG-coupled horseradish peroxidase antibody (Zymed Laboratories Inc.). After 60 min, the blots were washed several times with TBST and visualized by ECL.

**Immunocytochemistry**—Cells were seeded overnight, at 70% confluence, onto coverslips in 6-well dishes and transfected with the appropriate plasmids the following day for 24 h. After washing with phosphate-buffered saline, the cells were fixed with neutral buffered 4% (w/v) paraformaldehyde and permeabilized with 1% bovine serum albumin containing 0.1% Triton X-100 for 1 h. Cells were incubated at 4°C for 24 h with the appropriate primary antibody and diluted in phosphate-buffered saline containing 1% bovine serum albumin. After washing with phosphate-buffered saline, either rhodamine- or fluorescein isothiocyanate-coupled secondary antibodies were added and incubated for 2 h at room temperature. Fixed cells were analyzed by confocal or fluorescence microscopy.

**Hematoxylin and Eosin Staining**—Hematoxylin and eosin staining were performed according to the manufacturer’s instructions (Sigma). We counted cells with eosinophilic inclusions in six different fields, ~1000 cells/experimental condition.

**In Vitro Kinase Assay**—Confluent cells were harvested in lysis buffer. Soluble cell lysate fraction was incubated for 2 h at 4°C with suitable antibodies. After the addition of protein A-Sepharose beads, the reaction mixture was incubated for 2 h at 4°C and rinsed with lysis and kinase buffers. Immunocomplex kinase assays were performed by incubating the cell lysates for 2 h at 30°C with the substrate in the reaction buffer (0.2 mM sodium orthovanadate, 2 mM dithiothreitol, 10 mM MgCl2, 2 mM T32P[γ-32P]ATP, and 20 mM HEPES, pH
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FIGURE 1. Dyrk1A binds to α-synuclein in hippocampal H19-7 cells and neuroblastoma SH-SY5Y cells. A, H19-7 cell lines were mock-transfected (−) or transiently transfected with α-synuclein (Syn). After 24 h, immunoprecipitation (IP) was performed with either anti-α-synuclein or anti-Dyrk1A antibodies and subsequently examined by Western blot analysis with anti-Dyrk1A or anti-α-synuclein antibodies. β-Tubulin expression showed equal loading. B, α-Synuclein (Syn) or HA-tagged Dyrk1A was transfected separately or together into SH-SYSY cells. Immunoprecipitation was performed with either anti-α-synuclein or anti-HA antibodies, and the immunocomplexes were analyzed by Western blot analysis with either anti-HA or anti-α-synuclein antibodies. These results are representative of three independent experiments.

RESULTS

Dyrk1A Binds to and Phosphorylates α-Synuclein in H19-7 Cells—We examined whether the α-synuclein and Dyrk1A interaction occurs in mammalian neuronal cells, such as in immortalized hippocampal H19-7 cells. We previously reported that the endogenous α-synuclein protein levels in H19-7 cells were undetectable (25, 26). Therefore, we transiently transfected H19-7 cells with α-synuclein cDNA, immunoprecipitated α-synuclein, and immunoblotted for Dyrk1A. Exogenously expressed α-synuclein binds to endogenous Dyrk1A (Fig. 1A). We confirmed this interaction by reverse co-immunoprecipitation and detecting the HA-tagged Dyrk1A-α-synuclein interaction in the dopaminergic neuroblastoma SH-SY5Y cell line (Fig. 1, A and B). These data demonstrate that α-synuclein interacts with Dyrk1A in H19-7 and SH-SY5Y cells.
Next, we asked if endogenous Dyrk1A interacts with α-synuclein in the mammalian central nervous systems. α-Synuclein and Dyrk1A are highly expressed in rat brain lysates and primary cortical neurons (Fig. 2, A and B). As shown in Fig. 2, α-synuclein selectively associates with Dyrk1A, and we did not observe nonspecific interaction with preimmune IgG or protein A beads (Fig. 2, A and B). Moreover, immunostaining of primary cortical neurons showed that Dyrk1A and α-synuclein co-localize in the cytoplasm (Fig. 2C). These data suggest that the Dyrk1A and α-synuclein interaction is not an artifact observed in transformed cell lines but occurs in the mammalian central nervous system.

Dyrk1A Phosphorylates α-Synuclein upon the Stimulation with bFGF in H19-7 Cells—Previously, we showed that basic fibroblast growth factor (bFGF) addition to H19-7 cells causes Dyrk1A activation, which plays an important role during neuronal differentiation of H19-7 cells (21). Based on these findings, we tested whether active Dyrk1A directly phosphorylates α-synuclein. Transient α-synuclein expression in H19-7 cells, followed by bFGF stimulation under the neuronal differentiation conditions, enhanced Ser/Thr phosphorylation within 6 h, reaching a maximum at 1 h (Fig. 3A). Since Dyrk1A acts as a dual specificity protein kinase, which catalyzes the tyrosine-directed autophosphorylation as well as serine/threonine residue phosphorylation in exogenous substrates (27), α-synuclein could also be phosphorylated at tyrosine residues. We performed a similar experiment to determine whether α-synuclein was phosphorylated on tyrosine residue(s). As shown in Fig. 3B,
we did not detect tyrosine-phosphorylated α-synuclein within 6 h of bFGF treatment, in H19-7 cells.

To clarify whether α-synuclein phosphorylation is due to active Dyrk1A, we tested whether a kinase-deficient Dyrk1A mutant or Dyrk1A siRNA duplex affects α-synuclein phosphorylation. As shown in Fig. 3C, α-synuclein phosphorylation was significantly diminished by expressing a kinase-dead Dyrk1A mutant and Dyrk1A siRNA, as compared with control cells. As a negative control, the transient expression of nonsilencing siRNAs did not affect α-synuclein phosphorylation (Fig. 3C). As expected, the Dyrk1A siRNA duplex, but not nonspecific siRNAs, blocked the endogenous Dyrk1A expression in a dose-dependent manner (Fig. 3C).

To verify the specific role of Dyrk1A on α-synuclein phosphorylation, we utilized an in vitro kinase assay. We used immunoprecipitated Dyrk1A, from H19-7 cells, to phosphorylate recombinant α-synuclein (GST-α-synuclein). As shown in Fig. 3D, the serine/threonine phosphorylation(s) of α-synuclein was significantly enhanced upon bFGF stimulation. As a control, GST alone was not phosphorylated by anti-Dyrk1A immunocomplexes (Fig. 3D). Furthermore, anti-Dyrk1A immunocomplexes prepared after α-synuclein transfection with either kinaseinactive Dyrk1A or competitive Dyrk1A siRNAs significantly reduced α-synuclein phosphorylation (Fig. 3E). Transient, noncompetitive Dyrk1A siRNAs had no effect on α-synuclein phosphorylation (Fig. 3E). Taken together, these data suggest that active Dyrk1A selectively phosphorylates α-synuclein at serine/threonine residue(s).

**Dyrk1A Phosphorylates Ser-87 Residue within α-Synuclein**—To identify the specific α-synuclein residues phosphorylated by Dyrk1A, we used recombinant α-synuclein fragments as substrates for an in vitro kinase assay. As shown in Fig. 4A, active Dyrk1A phosphorylated α-synuclein peptides consisting of amino acids 1–140 and 61–95. However, Dyrk1A did not phosphorylate the C-terminal peptide (residues 96–140) or the N-terminal peptide (residues 1–60) (Fig. 4A), suggesting that the critical phosphorylated amino acid(s) resided between amino acids 61 and 95. Sequence analysis identified four possible phosphorylatable serines at positions 9, 42, 87, and 129. Therefore, we hypothesized that Dyrk1A phosphorylated α-synuclein at serine 87. To test this hypothesis, we mutated each serine residue (positions 9, 42, 87, and 129) to an alanine and examined α-synuclein phosphorylation after bFGF stimulation. The α-synuclein mutants S9A and S42A were readily phosphorylated in response to bFGF-induced active Dyrk1A, indicating that these residues were probably not involved in Dyrk1A-mediated phosphorylation (Fig. 4B). However, when cells were transfected with the α-synuclein S87A mutant, bFGF-induced α-synuclein phosphorylation was abolished (Fig. 4B). Interestingly, the α-synuclein S129A also blocked α-synuclein phosphorylation (Fig. 4B), suggesting that active Dyrk1A selectively phosphorylates α-synuclein at the serine 87 residue, whereas neurogenic bFGF appears to activate other protein kinase(s) to modify α-synuclein at the serine 129 residue. This finding was further confirmed by using the antibody against phospho-Ser-129-α-synuclein (PSer-129). Based on our previous finding (13), PSer-129 antibody was first verified by in vitro phosphorylation of α-synuclein by casein kinase 1 (Fig. 4C). Then active Dyrk1A immunocomplexes, which were validated through CREB phosphorylation at Ser-133 in vitro (21), was shown not to phosphorylate α-synuclein at Ser-129 (Fig. 4D).

**The Kinetics and Neurotoxicity Induced by Intact α-Synuclein Inclusions Are Different from the Phosphorylated Forms by Dyrk1A**—We monitored protein aggregation of recombinant or phosphorylated wild type α-synuclein samples by measuring...
We next asked whether the enhanced aggregation of phosphorylated α-synuclein proteins exhibited greater cytotoxicity. Phosphorylated α-synuclein protein aggregates were more cytotoxic than unphosphorylated α-synuclein aggregates (Fig. 5C). Transmission electron microscopy analysis of the aggregated granular structure formation during the α-synuclein aggregation in the presence of protein phosphorylation was microscopically distinct from those aggregates from unphosphorylated α-synuclein (Fig. 5D). Intact α-synuclein aggregates contained only the fibrillar forms, whereas the inclusions obtained with the Dyrk1A-induced phospho-α-synuclein showed that spherical granular forms are also present, in addition to the fibrillar aggregates (Fig. 5D).

The α-Synuclein Phosphorylation via Active Dyrk1A Promotes Intracellular α-Synuclein Inclusion Formation—To validate the consequences of α-synuclein Ser-87 phosphorylation, we tested whether Dyrk1A could influence the insoluble α-synuclein aggregate formation, in H19-7 cells. Previously, we showed that transient α-synuclein expression in H19-7 cells leads to neuronal cell death. This effect is closely associated with the formation of intracytoplasmic α-synuclein-positive inclusions, which have similar composition to LBs found in PD patients (26). After H19-7 cells were transfected with α-synuclein, the expression pattern of α-synuclein within the cells was compared in the absence or presence of bFGF. Consistent with our previous finding, the distribution of α-synuclein was uneven and took the form of granular aggregates (Fig. 6A). Stimulation with bFGF enhanced intracytoplasmic α-synuclein inclusion formation (Fig. 6A). Additionally, α-synuclein co-expression with the dominant negative Dyrk1A diminished α-synuclein aggregates, as compared with α-synuclein alone (Fig. 6A). Cells expressing the α-synuclein S87A mutant also exhibited reduced aggregate formation (Fig. 6B). Quantification of intracellular eosinophilic protein aggregates found a 2-fold increase when cells were transfected with α-synuclein plus GFP and stimulated with bFGF (Table 1). However, we did not observe this increase in cells transfected with either α-synuclein S87A or kinase-deficient Dyrk1A in the absence of bFGF stimuli (Table 1).

Following transient α-synuclein expression, we measured cell viability after 24 h. As shown in Table 1, α-synuclein over-expression significantly decreased the cell viability by ~42% in H19-7 cells. Consistent with a previous report that H19-7 cells

![Image](https://example.com/image.png)

**Figure 6.** α-Synuclein phosphorylation at Ser-87 by Dyrk1A enhances aggregate formation and neuronal cell death in H19-7 cells. A, cells were mock-transfected (Mock) or transfected with α-synuclein alone or transfected with α-synuclein and kinase-defective Dyrk1A plasmid (mDyrk) for 24 h. Cells were untreated (Mock, Con) or stimulated with bFGF (bFGF or F) for 48 h. Immunocytochemical analysis was performed with anti-α-synuclein antibodies. Representative images of cells expressing α-synuclein are shown by confocal microscopy. B, cells were transfected with either wild type α-synuclein (wt-Syn) or α-synuclein S87A mutant (Syn-S87A) for 24 h and left untreated (No T) or stimulated with bFGF for 48 h. These data are representative of three independent experiments.

**Table 1.** α-Synuclein phosphorylation effects on cell viability and eosinophilic inclusion formation in hippocampal H19-7 cells

| Treatment | Transfection | GFP-expressing cells with hematoxylin and eosin-positive inclusions | Cell viability |
|-----------|--------------|-----------------------------------------------------------------|--------------|
|            |              | %                                                               | %            |
| None       | GFP          | ND      | 100 ± 0.7            |
| bFGF       | GFP          | 28.1 ± 2.6          | 58.1 ± 3.9           |
|            | GFP + WT Syn | 16.9 ± 1.8          | 77.2 ± 4.5           |
|            | GFP + Syn S87A | 11.8 ± 1.6          | 83.3 ± 2.6           |
|            | GFP + mDyrk1A | ND            | 75.4 ± 3.4           |
|            | GFP + WT Syn | 51.9 ± 5.5          | 41.2 ± 4.9           |
|            | GFP + Syn S87A | 26.7 ± 2.9          | 72.4 ± 3.5           |
|            | GFP + WT Syn + mDyrk1A | 22.2 ± 1.6 |

* Number of GFP-positive cells containing eosinophilic inclusions was counted in six different fields among approximately 250 cells/experimental condition. Data are shown as the means ± S.E. from three separate experiments performed in duplicate (*p < 0.01 versus cells transfected with wild type α-synuclein alone).  
* Cell viability was measured by the tetrazolium salt, 3-(4,5-dimethylthiazol-2-yf)-2,5-diphenyltetrazolium bromide extraction method.  
* ND, not determined.  
* ND, not determined.  
* p < 0.01 versus cells transfected with wild type α-synuclein alone.
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also undergo apoptosis upon differentiation (28), bFGF addition resulted in a 25% loss of the total cell population, within 24 h (Table 1). However, co-transfection with wild type α-synuclein plus dominant-negative Dyrk1A diminished the neuronal cell death (Table 1). Furthermore, when cells were transfected with α-synuclein S87A, cytotoxicity was significantly reduced in response to bFGF. As a control, transfection of either kinase-dead Dyrk1A or α-synuclein S87A mutant in the absence of fibroblast growth factor stimulation reduced the toxic effect (Table 1). These data indicate that active Dyrk1A increases intracellular α-synuclein aggregates and potentiates its cytotoxicity in H19-7 cells.

DISCUSSION

Several examples of α-synuclein post-translational modifications have been reported in the human brain, including phosphorylation (14), nitration (29), glycosylation (30), and SUMO modification (31). α-Synuclein phosphorylation reduces lipid binding and enhances fibril formation (15). Supporting this finding, phosphorylated forms of α-synuclein at its Ser-129 and Ser-87 residues are found in LBs (13). Furthermore, α-synuclein post-translational modifications by tyrosine nitration and oxidation can also promote intracytoplasmic inclusion formation (32, 33).

α-Synuclein can be phosphorylated in vitro at several residues, including serines 87 and 129 and three C-terminal tyrosine residues (tyrosines 125, 133, and 136). Several protein kinases were reported to phosphorylate α-synuclein in vitro and/or in vivo. For example, G protein-coupled receptor kinase-2 phosphorylates Ser-129 in vivo and enhances α-synuclein toxicity (16). Casein kinase 1 and 2 can also phosphorylate Ser-129 and α-synuclein in cultured cells (13). In addition, the α-synuclein Tyr-125 residue is phosphorylated by c-Src and Fyn (34, 35). Although α-synuclein is constitutively phosphorylated at Ser-87 as well as at Ser-129 residues (13), the kinase targeting serine 87 residue and its physiological role have not been described. We examined whether α-synuclein is a Dyrk1A phosphorylation target. The current study shows that Dyrk1A can phosphorylate α-synuclein at Ser-87, and this enhances cytoplasmic aggregate formation. In addition to Ser-129, the Ser-87 residue plays an important role modulating cytoplasmic α-synuclein inclusion formation. Dyrk1A catalyzes the tyrosine-directed autophosphorylation and serine/threonine phosphorylation in exogenous substrates (27). Therefore, we could not exclude the possibility that Dyrk1A can phosphorylate α-synuclein at tyrosine residues. Western blot analysis of anti-α-synuclein immunocomplexes with anti-phosphotyrosine IgGs revealed that the tyrosine residues within the α-synuclein are not probably phosphorylated in response to bFGF-induced active Dyrk1A, in H19-7 cells.

Previously, we reported that active Dyrk1A phosphorylates CREB, which subsequently leads to the stimulation of cAMP-response element-mediated gene transcription, during neuronal differentiation (21). These data strongly suggest that Dyrk1A may play an important role during the neurogenic factor-induced differentiation of central nervous system neuronal cells. In addition, our recent findings show that huntingtin-interacting protein-1 phosphorylation by Dyrk1A has an important role in neuronal differentiation and cell death (36).

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