Regulation of Gli1 Transcriptional Activity in the Nucleus by Dyrk1*

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Although the developmental importance of the Dyrk gene family remains largely unknown, studies with Yak1 in yeast and mini-brain (mnb) in Drosophila suggest their involvement in regulating cell proliferation (7, 8). As the name suggests, mnb mutant flies have a smaller brain size as a result of a defect in neurogenesis (8). There is evidence in humans that the Dyrk1 class, a homolog of the Drosophila mnb gene, contains three separate genes, Dyrk1a, Dyrk1b, and Dyrk1c (9). Interestingly, Dyrk1a has been mapped to the Down’s syndrome critical region of chromosome 21, and transgenic mice harboring an extra copy of this gene retain deficits in learning (10–12). This strongly suggests that the Dyrk1/mnb class has evolved a conserved role that is essential for proper brain development.

Cell transfection studies on GFP-tagged versions of Dyrk1a and homeodomain-interacting protein kinase 2 have confirmed their nuclear localization (2, 4). Interestingly, the staining pattern of GFP fluorescence in the nucleus has revealed that Dyrk1a and homeodomain-interacting protein kinase 2 associate with nuclear speckles, potential sites of alternative splicing or transcriptional regulation. Recently, studies with homeodomain-interacting protein kinase 2 have demonstrated its ability to associate with nuclear bodies, co-localize with PML3, and bind p53 and CREB-binding protein, revealing its role in transcriptional regulation (13, 14). Consistent with the idea that nuclear localized Dyrks may regulate transcriptional activity, many of the protein substrates identified in protein interactor screens involving Dyrk have been transcription factors. Biochemical studies with Dyrk1a have demonstrated its ability to interact with the transcription factors Forkhead, CREB, and signal transducers and activators of transcription 3 (15–17).

Cell transfection studies carried out in our laboratory have revealed that Dyrk1a can dramatically enhance the transcriptional activity of Gli1. Gli1, Gli2, and Gli3 are vertebrate homologs to the Drosophila cubitus interruptus (ci) gene, a five zinc finger transcription factor whose function is highly regulated by the hedgehog signaling pathway (18–20). Hedgehog genes encode for secreted signaling proteins that can function as growth factor and morphogen important for cell proliferation and pattern formation (21–23). Analogous to Ci in Drosophila, Gli transcription factors are key downstream signaling components of the hedgehog pathway in vertebrates.

Increasing evidence suggests that Gli/Ci proteins are highly regulated in the cell cytoplasm and nucleus. In the absence of hedgehog signaling, Ci protein is retained in the cell cytoplasm as part of a protein complex associated with microtubules con-
taining the negative regulators Costal2 and suppressor of fused (Su(F)) (24–27). Here full-length Ci protein is proteolytically processed into a shortened transcriptional repressor form (28, 29). In the presence of hedgehog signaling, full-length Ci protein is transported into the nucleus to activate hedgehog target genes (30). Once inside the nucleus, Gli transcription factor activity is still modulated by a number of different factors. CREB-binding protein, a general transcriptional co-activator, can directly bind Ci or Gli3 and is believed to enhance transcription in part through its acetylase activity (31–33). Recent biochemical data also suggest that SuF may associate with SAP18 and mSin3, forming a deacetylase complex, resulting in Gli/Ci transcriptional repression (34).

In this report, we describe our findings that Dyrk1 activated Gli1-dependent gene transcription, but not LEF-1, c-jun-, or Elk-dependent gene transcription. Dyrk1 does this by retaining Gli1 in the nucleus as well as by enhancing the transcriptional activity. In addition, we show that Dyrk1 functionally interacts with the Shh pathway to induce gene transcription and differentiation in C3H10T1/2 cells.

MATERIALS AND METHODS

Cell Culture, Transfection, and Reporter Gene Assay—NIH3T3 cells and Cos-7 cells were maintained in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum. C3H10T1/2 cells were cultured in minimum essential medium α with 10% heat-inactivated fetal calf serum. Transfection was carried out using LipofectAMINE Plus as suggested by the manufacturer (Invitrogen). The transfection was stopped after 3 h, and cell extracts were collected 24 h later for luciferase assays, kinase assays, and Western analysis. Luciferase assays were performed using the Roche Molecular Biochemicals Luciferase Assay Kit as instructed. Cell lysates were first taken to determine the fluorescence intensity emitted by GFP in a Wallac multilcourter, which is capable of counting fluorescence and luminescence. Then, luciferase substrate was added to the cell lysates, and luciferase activities were determined by measuring luminescence intensity using the same counter. Luminescence intensity was normalized against fluorescence intensity. DNA concentrations were adjusted if transfection of any of the cDNAs resulted in significant differences between normalized and non-normalized data.

Immunoprecipitation, Kinase Assay, and Western Analysis—Immunoprecipitation and Western analysis were performed as described previously (35). For in vitro Dyrk kinase assay, Gli1-HA or its mutants and Cos-7 cells were separately expressed in Cos-7 cells and immunoprecipitated by anti-HA antibodies. 24 h later, cells were lysed, and HA-tagged proteins were immunoprecipitated with an anti-HA antibody. The immunocomplexes were mixed and then divided into two portions. One portion was washed, separated by SDS-PAGE, and analyzed by Western blotting using an anti-HA antibody, and the other was incubated at 30 °C for 30 min in the presence of 10 μCi of [γ-32P]ATP and 10 μM ATP. The kinase reactions were terminated by the addition of 4× SDS sample buffer, and the samples were separated by SDS-PAGE and visualized using a phosphorimager.

Immunofluorescence Staining—NIH3T3 cells transfected with appropriate expression plasmids were fixed for 15 min in phosphate-buffered saline containing 2% paraformaldehyde, followed by permeabilization in phosphate-buffered saline containing 0.2% Triton X-100. HA-tagged Dyrk1 or Dyrk1KR was detected by immunofluorescence staining using a red fluorescent Alexa dye-conjugated anti-HA antibody, whereas the intracellular localization of GFP fusion proteins was directly visualized under a fluorescence microscope simultaneously. Nuclei were stained with Hoechst dye.

Alkaline Phosphatase Assay—C3H10T1/2 cells were lysed 3 days after transfection. The cell lysates were mixed with the CDP-Star chemiluminescent substrate for alkaline phosphatase as instructed by the manufacturer (Tropix). After a 1-h incubation at room temperature, the luminescence intensity was measured using the Wallac multilcourter.

RESULTS

Effects of Dyrk1 and Mnb on Gene Transcription—To investigate the possible involvement of Dyrk1 in gene transcriptional regulation, we examined the effects of Dyrk1 overexpression in a number of reporter gene assay systems. We found that co-expression of Dyrk1 and Gli1 strongly induced Gli1-dependent gene transcription in the presence of the 3′GliBS-Luc reporter construct, but not in the presence of the mutant reporter construct, m3′GliBS-Luc (Fig. 1A). The 3′GliBS-Luc reporter construct, as described previously (19), contains Gli DNA binding sequences through which the production of the reporter, luciferase, is regulated. In addition, we found that Dyrk1 enhanced the binding of Gli1 to its response element in an electrophoretic mobility shift assay (data not shown). The involvement of Dyrk1 in gene transcriptional regulation of Gli1 appears to be specific because expression of Dyrk1 did not activate Elk-, c-Jun-, or LEF-1-mediated gene transcription in NIH3T3 cells (Fig. 1, B–D). In addition, many other protein kinases, including mitogen-activated protein kinase/extracellular signal-regulated kinase, Src, and casein kinase Iε, while stimulating Elk-, c-Jun-, and LEF-1-dependent gene transcription, respectively, failed to induce Gli1-dependent gene transcription (Fig. 1A). The Dyrk1 Drosophila homolog mnb, which shares 85% amino acid sequence homology with Dyrk1 in its kinase domain (1, 8), was also tested, and it was found to be a potent activator of Gli1 (Fig. 1A).

To determine whether activation of Gli1-dependent gene transcription by Dyrk1 depends on its kinase activity, we obtained a kinase-null mutant, Dyrk1KR, which contains a Lys-188 to Arg point mutation at the ATP binding region of its kinase domain. Unlike wild-type Dyrk1, this kinase-deficient mutant did not show any synergistic effect with Gli1 (Fig. 2A). In addition, activation of Gli1-mediated gene transcription seems to be independent of Dyrk1 C-terminal sequences because deletion of C-terminal residues 498–763 located after the kinase domain did not affect its capacity to induce Gli1-dependent gene transcription (data not shown).

Dyrk1 Regulates Gli1 Nuclear Export—Regulation of Ci proteolysis has been proposed as a mechanism for regulation of its transcriptional activity (36). Because there are no significant changes in the levels of full-length Gli1 protein when Gli1 and Dyrk1 were co-expressed (Fig. 2B), the processing of Gli1 protein may not play an important role in Dyrk1-mediated Gli1 activation. In the past several years, it has been reported that regulation of nuclear trafficking represents a novel mechanism for the control of the activities of gene transcriptional regulators (37). Two recent studies (38, 39) as well as our data (see below) demonstrate that Gli1 and Ci are nuclear shuttling proteins. Hedgehog signaling appears to regulate the import of Ci (38). In addition, vertebrate Sufu, a homolog of the Drosophila hedgedehog signaling protein suppressor of fused, binds to Gli proteins (25, 39) and may retard Gli1 nuclear import (39, 42). We investigated whether Dyrk1, which is localized in the nucleus (2), has any effect on the subcellular localization of Gli1. To determine the intracellular localization of Gli1, we constructed the expression plasmid Gli1-GFP. The intracellular localization of Gli1-GFP fusion protein was visualized under fluorescence in the presence or absence of Dyrk1-HA. Dyrk1-HA and nuclei were visualized after Gli1-GFP in the same cell and stained with red fluorescent Alexa dye-conjugated anti-HA antibody and Hoechst dye, respectively. Consistent with previous reports (9, 39), Dyrk1 and its mutant, Dyrk1KR, were primarily localized in the nucleus (Fig. 3, E and H), whereas the Gli1 protein was predominantly localized in the cytoplasm in the absence of co-expressed Dyrk1 (Fig. 3A). However, co-expression of Dyrk1-HA caused significant accumulation of Gli1-GFP in the nucleus (Fig. 3D). More importantly, Dyrk1-mediated nuclear accumulation of Gli1-GFP protein depended on Dyrk1 kinase activity because Dyrk1KR could not induce nuclear accumulation of Gli1 protein (Fig. 3G). The levels of Gli1 in the nuclear fraction from cells expressing
Dyrk1 were also found to be higher than those from cells expressing control protein β-galactosidase (data not shown).

**Dyrk1 Phosphorylates Gli1**—Phosphorylation has recently emerged as a major mechanism for regulating protein nuclear transport (37). Because Dyrk1 kinase activity is essential for Gli1 nuclear retention, we wanted to know whether Dyrk1 could phosphorylate Gli1 protein, and which domains might be phosphorylated. Gli1-HA or its truncated mutants (Fig. 4A) and Dyrk1-HA were separately expressed in Cos-7 cells and immunoprecipitated by anti-HA antibodies. Then the immunocomplexes were mixed in the presence of [γ-32P]ATP. Significant phosphorylation of Gli1-HA, Gli1N-HA, and Gli1C-HA, but not Gli1ZF-HA, was observed in the presence of Dyrk1 (Fig. 4B). No phosphorylation was detected in the absence of Dyrk1 (Fig. 4B) or in the presence of Dyrk1KR (data not shown). Although Dyrk1 is a dual specificity kinase, the phosphorylation of Gli1 by Dyrk1 appeared to be predominantly on Ser/Thr residues because we could not detect phosphotyrosines in Dyrk1-treated Gli1 or its mutants by Western analysis using an anti-phosphotyrosine antibody (data not shown). However, we were able to detect Dyrk1 autophosphorylation by using the same anti-phosphotyrosine antibody (Fig. 2C). A previous report has demonstrated that tyrosine autophosphorylation of Dyrk1 is required for its kinase activity (1). These results, together with the observation that Dyrk1-mediated nuclear accumulation of Gli1 depends on its kinase activity, suggest that Dyrk1 may regulate nuclear transport of Gli1 proteins via phosphorylation.

Dyrk1 phosphorylates both the N-terminal and C-terminal portions of Gli1. To determine which portions of Gli1 are required for Dyrk1-mediated nuclear retention, two Gli1 mutants, Gli1ΔN-GFP (amino acids 195–1107) and Gli1ΔC-GFP (amino acids 1–410), were generated, and the intracellular localization was determined in the presence or absence of co-expressed Dyrk1. We found that both Gli1ΔN-GFP and Gli1ΔC-GFP were predominantly localized in the cytoplasm despite the presence of Dyrk1. However, we noticed that Gli1ΔC-GFP was detected in the nuclei of a small fraction of cells that co-express both Gli1ΔC-GFP and Dyrk1 (data not shown). Nevertheless, we believe that both the N-terminal and C-terminal portions of Gli1 are important for efficient nuclear retention of Gli1 by Dyrk1.

**Dyrk1 Stimulates Transcriptional Activity of Gli1**—With the knowledge that Dyrk1 phosphorylates and retains Gli1 protein in the nucleus, the next question is whether nuclear retention of Gli1 is the sole mechanism for Dyrk1-induced Gli-dependent transcriptional activation. To address this question, we generated another Gli1 mutant, called Gli1-AHA-GFP, in which three amino acids (Leu-Arg-Leu) in the core tetramer of the nuclear export sequence (NES) were changed to Ala-His-Ala (Fig. 5A). As we expected, this mutant accumulated in the nucleus independently of Dyrk1 (Fig. 5B). This result further confirmed that Gli1 NES is involved in Gli1 export, probably by interacting with the nuclear export receptor Crm1 (46). The transcriptional activity of Gli1-AHA was determined using the 3′GliBS-Luc reporter assay. We found that Gli1-AHA showed a higher basal transcriptional activity than the wild-type Gli1 (Fig. 5C). This augmented basal activity is probably due to the increased accumulation of Gli1 mutant proteins in the nucleus. The fact that basal activity of Gli1-AHA is less than the activity of the wild-type Gli1 in the presence of Dyrk1 and that Gli1-AHA can be further stimulated by co-expressed Dyrk1 suggests that Dyrk1 can also stimulate the transcriptional activity of Gli1 in addition to retaining Gli1 in the nucleus. The expression levels of wild-type Dyrk1 and its AHA mutant are shown in Fig. 5D.

**Dyrk1 Interacts with the Shh Signaling Pathway**—In the presence of hedgehog signaling, increased levels of Gli1 protein get imported into the nucleus and activate downstream target genes (36, 47). Therefore, we wanted to know whether
Dyrk1 could potentiate a Gli response in cells undergoing Shh signaling. It was previously shown that Shh could induce Gli-dependent transcriptional activation of the 3′GliBS-Luc reporter gene and differentiation in C3H10T1/2 embryonic fibroblast cells (48). Consistent with previous reports, when C3H10T1/2 cells were co-transfected with cDNA encoding Shh and 3′GliBS-Luc reporter gene, the cells produced more luciferase activity than cells transfected with 3′GliBS-Luc reporter gene alone (Fig. 6A). Co-expression of Dyrk1 with Shh further increases reporter gene activity, which is more than the sum of those in cells expressing Dyrk1 or Shh alone, suggesting that Shh and Dyrk1 act synergistically. The effect of Dyrk1 on Shh-induced differentiation of C3H10T1/2 cells was also examined by assaying changes in alkaline phosphatase activity as described previously (48, 49). Again, co-expression of Dyrk1 with Shh demonstrated a synergistic effect, this time in stimulating alkaline phosphatase activity (Fig. 6B).

To determine whether the Shh-activated pathway directly regulates Dyrk1, we examined whether Shh could stimulate Dyrk1 phosphorylation. Dyrk1 was immunoprecipitated from cells expressing Dyrk1 alone or from cells co-expressing Dyrk and Shh. The phosphotyrosine content of precipitated Dyrk was detected using a phosphotyrosine-specific antibody. Shh did not stimulate phosphorylation of Dyrk (Fig. 6D). In agreement with this, the Dyrk kinase-deficient mutant did not inhibit Shh-induced production of alkaline phosphatase in C3H10T1/2 cells (Fig. 6C). Thus, Dyrk does not appear to be directly regulated by Shh.

**DISCUSSION**

In this study, we provide biochemical evidence that Dyrk1, a nuclear protein kinase, and its Drosophila homolog mnb can enhance the transcriptional activity of Gli1. In part, this increase in transcriptional activity is a result of elevated Gli1 protein levels in the nucleus. However, the ability of Dyrk1 to induce transcriptional activity of Gli1-AHA, a nuclear export mutant, also suggests that Dyrk1 may be more directly involved in modulating Gli1 transcriptional activity. We also show that Dyrk1 is phosphorylated in the presence of Dyrk1 and that Dyrk1 kinase activity is essential for Gli1 nuclear retention and its increased transcriptional activity. In addition, Dyrk1 can synergistically function with Shh to stimulate Gli reporter gene activity and differentiate C3H10T1/2 cells.

**FIG. 4. Phosphorylation of Gli1 by Dyrk1.** A, a schematic representation of Gli1 and Gli1 mutants. Some Gli1 structural motifs, including zinc finger (ZF), nuclear localization signal (NLS), NES, and transcriptional activation domain (TAD), are denoted. B, Cos-7 cells were transfected with Gli1-HA, its mutants, Dyrk1-HA, or Dyrk1KR-HA expression plasmids. 24 h later, cells were lysed, and HA-tagged proteins were immunoprecipitated with an anti-HA antibody. The immunocomplexes were mixed and then divided into two portions. One was incubated with [γ-32P]ATP for in vitro kinase assay, and the other was washed, separated by SDS-PAGE, and analyzed by Western blotting using an anti-HA antibody.
protein-protein interactions. In support of this idea, recent work has shown that co-expression of Zic proteins, nuclear localized transcription factors, with members of the Gli family also results in accumulation of Gli proteins in the nucleus (44). Our studies demonstrate that Dyrk1 kinase activity does more than retain Gli1 in the nucleus—it also stimulates Gli1 transcriptional activity. Reporter gene studies in which Dyrk1 and Gli1-AHA were co-expressed clearly show that Gli1 transcriptional activity can be further enhanced by Dyrk1. This suggests that Dyrk1 may be more directly involved in modulating the transcriptional activity of Gli1 in the nucleus. Therefore, it is possible that Dyrk1 phosphorylation of Gli1 results in more efficient association of Gli1 with other transcriptional cofactors, thus delaying its nuclear export.

Consistent with our observation that Dyrk1 can stimulate Gli1 transcriptional activity, previous work on Dyrk1 has suggested that it may also regulate the function of other transcription factors such as CREB, Forkhead, and signal transducers and activators of transcription 3 (15–17). There is also evidence that homeodomain-interacting protein kinase 2, a Dyrk-like kinase, can bind p53 and CREB-binding protein to enhance transcription (13, 14). Collectively, these studies provide increasing evidence that one possible role of nuclear localized Dyrk kinases may be to modulate transcription factor function. However, how specific or general a role remains to be determined.

The distinct effect of Hh and Dyrk on the nuclear transport of the Ci/Gli proteins suggests that Dyrk1 may not be a direct signaling component within the Hh pathway. This is consistent with our observations that Shh did not stimulate phosphorylation of Dyrk1 and that the kinase-deficient mutant of Dyrk1 could not inhibit Shh-induced effects in C3H10T1/2 cells. However, the synergistic effect between Dyrk1 and Shh in enhancing Gli-dependent gene transcription and increasing alkaline phosphatase activity in C3H10T1/2 cells suggests that these two pathways may functionally interact. Although Dyrk1 is not essential for Hh signaling, levels of Dyrk1 kinase activity in the nucleus may determine how robust cells respond to Hh signaling. Thus, Gli/Ci proteins may be the cellular targets for at least two distinct signaling pathways: one regulated by the Hh proteins in the cytoplasm, and the other mediated by the Dyrk kinases in the nucleus.

The inability of Shh to stimulate Dyrk1 phosphorylation still leaves the following question: what regulates Dyrk kinase activity? Similar to mitogen-activated protein kinases, Dyrks contain an activation loop within the catalytic domain (2). Therefore, early studies hypothesized that Dyrk activation may be analogous to mitogen-activated protein kinase activation, via phosphorylation by an upstream kinase (1). However, recent transfection studies in mammalian cells favor the possibility that, unlike mitogen-activated protein kinases, Dyrk1 can activate itself through auto-phosphorylation (43). This may explain why overexpression of Dyrk1 alone results in vigorous kinase activity. It is also interesting to speculate that if Dyrk1 can activate itself through autophosphorylation, then gene dosage and transcriptional control may be important regulatory mechanisms. Another possibility is that Dyrk, like glycogen synthase kinase 3, might be negatively regulated by yet unknown upstream kinases or even phosphatases.

Developmental studies of the Dyrk1mnb class of Dyrk kinases suggest an important role in proper brain development. Mutations in the Drosophila mnb gene that cause a reduction...
protein expression were associated with defects in neuroblast proliferation and specific behavioral abnormalities (8). The mammalian homolog of mnb, Dryk1a, has been mapped to the Down’s syndrome critical region of chromosome 21, and transgenic mice retaining an extra copy of Dryk1 have learning deficits (10–12). Several studies have also demonstrated the involvement of the Hh-Gli signaling pathway in neuronal proliferation (47). In the vertebrate central nervous system, Shh acts as a mitogen for a number of neuronal precursor cells, including those located in the spinal cord and retina, oligodendrocyte cell precursors in the neural tube, and granule neuronal cell precursors in the cerebellum (40, 41, 47). Studies on the hedgehog receptor Patched1 also reveal the importance of repressing the Hh pathway during brain development. Loss of function mutations in Patched1, which aberrantly activates hedgehog signaling, result in overproliferation of granule cell precursors in the cerebellum (41). Because Hh and Dryk1/mnb pathways are important for central nervous system development, it is interesting to speculate whether or not both these pathways interact with each other during neuronal development in vivo. Future studies will focus on the potential for Hh and Dryk1 pathways to interact with each other during neuronal development through modulating Gli transcriptional activity.

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REFERENCES
1. Kentrup, H., Becker, W., Heukelbach, J., Wilmes, A., Schurmann, A., Huppertz, C., Kainulainen, H., and Joost, H. G. (1996) J. Biol. Chem. 271, 3488–3495
2. Becker, W., and Joost, H. G. (1999) Prog. Nucleic Acid Res. Mol. Biol. 62, 1–17
3. Miyata, Y., and Nishida, E. (1999) Biochem. Biophys. Res. Commun. 266, 291–295
4. Kim, Y. H., Choi, C. Y., Lee, S. J., Conti, M. A., and Kim, Y. (1998) J. Biol. Chem. 273, 25787–25789
5. Kainulainen, A. M., Karvonnen, U., Poukkla, H., Janne, O. A., and Palvimo, J. J. (1998) Mol. Biol. Cell 9, 2527–2543
6. Shang, E., Wang, X., Huang, J., Yoshida, W., Kuriwai, A., and Weidemuth, D. J. (2000) Mol. Reprod. Dev. 55, 372–378
7. Hartley, A. D., Ward, M. P., and Garrett, S. (1994) Genetics 136, 465–474
8. Tejedor, F., Zhu, X. R., Kaltenbach, E., Ackermann, A., Baumann, A., Canal, I., Heisenberg, M., Fischbach, K. F., and Pongs, O. (1996) Neuron 14, 287–301
9. Beck, R., Weber, Y., Wetzel, K., Eimbircher, K., Tejedor, F. J., and Joost, H. G. (1998) J. Biol. Chem. 273, 25983–25992
10. Song, W. J., Sternberg, L. R., Kasten-Sportes, C., Keuren, M. L., Chang, S. H., Slack, A. C., Miller, D. E., Glover, T. W., Chiang, P. W., Lou, L., and Kurnit, D. M. (1996) Genomics 38, 331–339
11. Shindoh, N., Kudoh, J., Maeda, H., Yamaki, A., Minoshima, S., Shimizu, Y., and Shimizu, N. (1996) Biochem. Biophys. Res. Commun. 225, 92–99
12. Alatalo, J., Dierssen, M., Baamonde, C., Marti, E., Visa, J., Guinera, J., Oset, M., Gonzalez, J. R., Florez, J., Fillat, C., and Estivill, X. (2001) Hum. Mol. Genet. 10, 1915–1923