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Phosphatidylinositol 3-kinase (PI3K) has numerous cellular functions, including cell survival and proliferation. In this study, we demonstrated that the expression of the active form of PI3K induced dorsal differentiation and axis duplication and strongly induced the expression of neural markers. In contrast, the inhibition of PI3K activity by its dominant negative mutant induced the phenotype of losing posterior structures and the expression of ventral markers. Akt is an essential target of PI3K for neurogenesis. The expression of the active form of Akt induced axis duplication and increased the expression of neural markers. Inhibition of the Akt activity abolished the PI3K-induced double heads and axes. This signal transmits through its target, glycogen synthase kinase 3β, which is known to mediate Wnt signaling for *Xenopus* development. These results identify a new function of PI3K/Akt signaling in axis formation and neurogenesis during *Xenopus* embryonic development and provide a direct link between growth factor-mediated PI3K/Akt signaling and Wnt signaling during embryonic development.

Phosphatidylinositol 3-kinase (PI3K)[1] is a heterodimeric enzyme composed of a 110-kDa catalytic and an 85-kDa regulatory subunit (1). PI3K phosphorylates the D3 hydroxyl of phosphoinositides and produces phosphatidyl-inositol-3-phosphates. PI3K is activated by several receptor and nonreceptor protein tyrosine kinases (2, 3). The best known downstream target of PI3K is the serine-threonine kinase Akt, which transmits survival signals from growth factors (4, 5). Activation of Akt requires the following two factors: (i) the binding of the lipid second messengers to its intact pleckstrin homology domain; and (ii) the phosphorylation of the pleckstrin homology domain by the phosphoinositide-dependent kinase (6, 7). Akt has multiple downstream targets, including nitric oxide synthase (8–10), NF-κB (11–13), BAD (14), and glycogen synthase kinase 3β (15). PI3K and Akt are involved in multiple cellular functions such as cell survival, proliferation, transformation, myogenic differentiation, angiogenesis, and cytoskeletal changes (6, 7, 16–22). However, the roles of PI3K and Akt in embryonic development still remain to be elucidated. We hypothesize that PI3K signaling plays an important role in embryonic development because PI3K is commonly activated by growth factors, some of which display dorsalizing activity to induce a secondary axis in *Xenopus* embryos. Here we used *Xenopus* as a model system to investigate the roles of PI3K and Akt in embryonic development. To determine whether PI3K is sufficient to induce a dorsal phenotype, an active form of PI3K was expressed in *Xenopus* embryos, and its effect on development was studied; to determine whether PI3K activity is required, a dominant negative form of PI3K was expressed to specifically inhibit endogenous PI3K activity in the embryos. To further understand the downstream signaling molecules involved in PI3K-mediated embryo development, we analyzed the effects of Akt and identified Akt as an essential target through the forced activation or inhibition of its activity in the embryos. We also used a similar approach to identify GSK-3β as a potential target of Akt to mediate the PI3K-initiated developmental cue in *Xenopus* embryos.

EXPERIMENTAL PROCEDURES

Embryo Manipulation—Adult pigmented *Xenopus laevis* specimens were obtained from *Xenopus* I, Inc. (Dexter, MI). *Xenopus laevis* embryos were obtained by artificial insemination after females were injected with 500 units of human chorionic gonadotropin. The embryos were chemically dejellied using 2% cysteine and then washed and transferred to Petri dishes containing 0.3× MMR solution (0.1 M NaCl, 2 mM KCl, 1 mM $MgSO_4$, 2 mM $CaCl_2$, 5 mM HEPES, and 0.1 mM EDTA) and 3% Ficoll (Amersham Biosciences). The embryos were staged according to Nieuwkoop and Faber's table (23).

Plasmid Construction and RNA Preparation—cDNA clones encoding β-galactosidase (β-gal) have been described previously (24). The cDNA constructs encoding an active form of the PI3K catalytic subunit, p110α*, and a dominant negative form of the PI3K regulatory subunit, p85ΔiSH2, were inserted into pSG5 vector and linearized by SalI for *in vitro* synthesis of capped sense mRNA using the T7 transcription kit (Ambion, Austin, TX). Myr-Akt, an active form of Akt, was inserted into pBluescript II KS±, and linearized by NotI for *in vitro* synthesis of capped sense mRNA using the T7 transcription kit. DN-Akt constructed in PBS-Sfi vector was linearized by SalI for *in vitro* synthesis of capped sense mRNA using the T3 transcription kit (Ambion). Cellular GSK-3β and a dominant negative mutant of GSK-3β, DN-GSK-3β, were sub-

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[1] The abbreviations used are: PI3K, phosphatidylinositol 3-kinase; β-gal, β-galactosidase; BMP-4, bone morphogenetic protein 4; GSK-3β, glycogen synthase kinase 3β; DN, dominant-negative; EF-1α, elongation factor 1α; NCAM, neural cell adhesion molecule; RT, reverse transcription.

cloned into pSP64TEN vector and linearized by XbaI for *in vitro* synthesis of capped sense mRNA using the Sp6 transcription kit (Ambion), according to the manufacturer's instruction. The synthetic RNAs were quantitated by ethidium bromide staining with reference to standard RNA (24).

Embryo Injection and Explant Culture—Embryos at the two-cell stage were injected in the animal poles with various mRNAs. The embryos were cultured up to 45 stages after injection. Animal caps were dissected from the injected embryos at stages 8.5–9 and cultured at 22 °C in 67% Leibovitz's L-15 medium (Invitrogen) with 7 mM Tris-HCl (pH7.5) and gentamicin (50 μg/ml) to various stages before being harvested for RT-PCR for further analysis.

RT-PCR—Total RNAs were extracted from cultured animal cap explants with TRIzol reagent (Invitrogen) in accordance with the manufacturer's instructions. RT-PCR amplification was performed using a Superscript preamplification system (Invitrogen). The primer sets and PCR condition for EF-1α, NCAM, BMP-4, Xvent-1, Xmsx1, Otx2, and Hoxb9 were as described previously (24–26). The primers designed for Xvent-2 5′-GGA CTA TAC TAA AGG CTG GA-3′ (forward) and 5′-ATT ACT CAT AGA ATA TAC AC-3′ (reverse). PCR conditions for Xvent-2 were 95 °C for 5 min followed by 32 cycles of 94 °C for 1 min, 60 °C for 45 s, and 72 °C for 1 min. PCR products were analyzed by data from replicate experiments.

RESULTS

Ectopic Expression of PI3K Induces Double Heads and Dorsal Axes—The PI3K pathway has been demonstrated to play a significant role in cell survival, proliferation, protein synthesis, myogenic differentiation, and angiogenesis. However, the role of PI3K in development still remains to be defined. To understand the role of PI3K signaling during early *Xenopus* development, we overexpressed an active form of the PI3K catalytic subunit, p110α* (27, 28). The expression of p110α* increased PI3K activity in the embryos dose-dependently (Fig. 1*A*). The embryos expressing p110α* developed with double heads and dorsal axes (Fig. 1*B*). This result indicated that the activation of PI3K is sufficient to induce axis duplication and is a potent inducer for neurogenesis during *Xenopus* embryonic development. To test whether PI3K is required for embryo development, a truncated form of the p85 regulatory subunit of PI3K, p85ΔiSH2, was expressed. p85ΔiSH2 is unable to bind to the catalytic subunit of PI3K but retained its ability to bind to the receptor tyrosine kinases, thereby specifically inhibiting endogenous PI3K activation by growth factors (12, 21). Ectopic expression of p85ΔiSH2 during *Xenopus* embryonic development produced a losing posterior structure phenotype at the tadpole stage, which lacked apparent axial structures and had a short tail and well patterned ventral tissues (Fig. 1*C*). These data suggested that PI3K activation is required for dorsal axis development in *Xenopus* embryos. To investigate whether ectopic expression of the active form of PI3K, p110α*, may reverse the inhibitory effect of p85ΔiSH2 on the activation of PI3K by receptor tyrosine kinases, both p110α* and p85ΔiSH2 were co-expressed in the *Xenopus* embryos. The expression of p110α* bypassed the p85ΔiSH2 inhibitory effect and restored *Xenopus* phenotypes similar to those of the control (Fig. 1*D*). Taken together, these results suggested that PI3K activity is essential for axis duplication in *Xenopus* development.

PI3K Signaling Is Required for Neural Tissue Induction in Animal Caps—The blastula animal cap is composed of pluripotent cells that can be induced to form endodermal, mesodermal, or ectodermal cell types and is a useful tissue for assessing the roles of various inducing factors in development. To study further the role of PI3K activity in neurogenesis, *Xenopus* embryos at the two-cell stage were injected with mRNAs coding for β-gal as a control or p110α*. Animal caps were dissected at stages 8.5–9 and cultured in 67% Leibovitz's L-15 medium until the equivalent of stage 22. Expression of p110α* induced a neural like extension (Fig. 2*A*). Co-expression of p85ΔiSH2 inhibited p110α*-induced neural development, and the phenotype was similar to that of the control and the caps treated with p85ΔiSH2 alone. RT-PCR analysis demonstrated that p110α* induced pan-neural marker NCAM expression, whereas p85ΔiSH2 inhibited the p110α*-induced NCAM expression. Expression of the mesoderm tissue marker *Xbra* was negative, which implied no mesoderm tissue induction (Fig. 2*B*). This result suggests that activation of PI3K is sufficient to mediate neural development in *Xenopus* embryos. To study the role of PI3K activity in neural development mediated by other upstream inducers, we tested whether the inhibition of endogenous PI3K activity by LY294002 or p85ΔiSH2 affected neural development in animal caps induced by retinoic acid. Retinoic acid is a derivative of vitamin A and a potent teratogen. In vertebrates, retinoic acid is involved in the development of the central nervous system and is a neural inducing factor (25). *Xenopus* embryos at the two-cell stage were injected with mRNAs coding for β-gal control or p85ΔiSH2. Animal caps were dissected at stages 8.5–9 and cultured in 67% Leibovitz's L-15 medium with retinoic acid in the absence or presence of LY294002 until the equivalent of stage 22. Animal caps induced by retinoic acid and injected with β-gal developed a neural like phenotype. Their appearance was slightly swollen and elongated, with one side white and the other side black. In contrast, animal caps injected with p85ΔiSH2 or treated with LY294002 developed an epidermal like phenotype. They had a

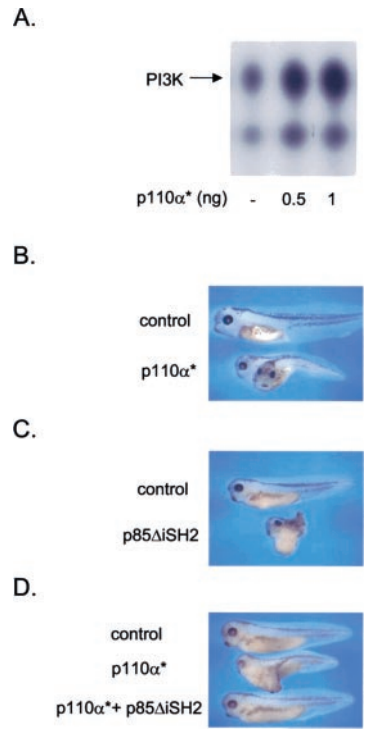

FIG. 1. **Activation or inhibition of PI3K by expression of an active form of PI3K, p110α*, or a dominant negative form of PI3K, p85ΔiSH2, affects *Xenopus* embryo development.** The embryos at the two-cell stage were injected into the animal pole area with 1 ng of mRNA encoding β-gal (a negative control), p110α*, or p85ΔiSH2. The embryos were cultured in 30% MMR solution until the equivalent of stage 45 after the injection. *A*, cellular extracts were prepared from the embryos and used for a PI3K activity assay *in vitro*, as we described previously (19). *Left lane*, vector control; *middle* and *right lanes*, injection of 0.5 and 1 μg of p110α*, respectively. *B*, compared with the β-gal control, the embryos injected with p110α* produce a dorsalizing phenotype (double heads and axes 36/50, 72%). *C*, the embryos injected with a dominant negative construct of PI3K, p85ΔiSH2, produce a ventralizing phenotype with a short tail and a small head (34/50, 68%). *D*, the co-expression of p110α* and p85ΔiSH2 in the embryos results in a normal phenotype similar to the control (double heads and axes 4/50, 8%).

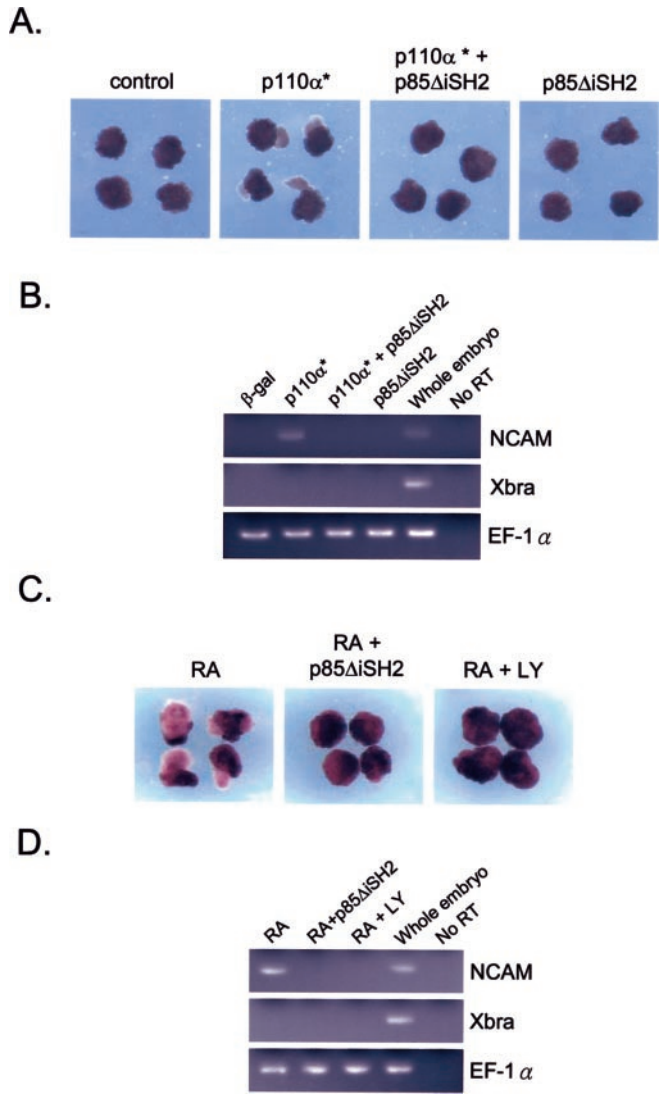

FIG. 2. **PI3K activity mediates neural tissue induction.** *A*, embryos at the two-cell stage were injected with mRNA coding for β-gal (control), p110α*, p110α* plus p85ΔiSH2, or p85ΔiSH2 as indicated. The animal caps were dissected at stages 8.5–9 and then cultured in 67% Leibovitz's L-15 medium until the equivalent of stage 22 for photography. The ectopic expression of p110α* induced neural tissue, which was inhibited by the co-expression of p85ΔiSH2. *B*, animal caps were harvested at the equivalent of stage 22 for RT-PCR and analysis of the expression of the pan-neural marker NCAM and the mesoderm marker *Xbra*. EF-1α expression was used as an internal control. *C*, retinoic acid (*RA*)-induced neural tissue development was inhibited by LY294002 and p85ΔiSH2. The embryos at the two-cell stage were injected with β-gal (control) or p85ΔiSH2 into the animal pole area. The animal caps were dissected at stages 8.5–9 and incubated in 67% Leibovitz's L-15 medium with retinoic acid (10^{-5}m) in the absence or presence of 50 μM LY294002 until the equivalent of stage 22 for photography. *D*, animal caps were also harvested at the equivalent of stage 22 for analysis of the expression of NCAM and *Xbra* by RT-PCR. *No RT*, absence of reverse transcriptase.

round shape and were evenly brownish in color (Fig. 2*C*). LY294002 and p85ΔiSH2 inhibited retinoic acid-induced NCAM expression. Mesoderm induction was also excluded as a negative *Xbra* expression (Fig. 2*D*). These data further suggest that PI3K is required for neural development of the *Xenopus* embryo.

Akt Is a Sufficient Downstream Target of PI3K for Axis Duplication—The serine-threonine kinase Akt is a well known target of PI3K, which mediates PI3K-induced multiple functions in response to growth factors *in vitro*. To test whether Akt activity is regulated by PI3K in the embryos, relative Akt activity in response to the activation of PI3K activity was determined in these cells. The expression of p110α* induced the phosphorylation of Akt at Ser-473, which determines Akt activation in the cells (Fig. 3*A*). To test whether Akt is sufficient to mediate the process, an active form of Akt, Myr-Akt, was expressed in the embryos by microinjection of its mRNA. When compared with the β-gal control, ectopic expression of Myr-Akt also induced axis duplication (Fig. 3*B*). This phenotype was similar to that observed in the embryos by the expression of PI3K, indicating that Akt is a target of PI3K for the process. To further determine whether Akt activity is required, endogenous Akt activity was inhibited by the expression of a dominant negative mutant of Akt, DN-Akt. The expression of DN-Akt inhibited axis formation, resulting in a phenotype with a shorter tail and smaller head than those of the β-gal control (Fig. 3*C*). These data suggest that Akt is required for axis induction during embryo development. To determine whether Akt activity is required for PI3K-induced axis duplication, DN-Akt was co-expressed with the active form of PI3K, p110α*. The co-expression of DN-Akt inhibited PI3K-induced axis duplication, resulting in a normal phenotype similar to that of the control (Fig. 3*D*). Similarly, the expression of DN-Akt also inhibited Myr-Akt-induced axis duplication in embryo development (Fig. 3*E*). These data further confirmed that Akt is an essential downstream target of PI3K for axis formation and neurogenesis during *Xenopus* embryonic development.

PI3K and Akt Induced Axis Formation through the Inhibition of GSK-3β during Xenopus Development—The activation of Akt is known to induce the phosphorylation and inactivation of GSK-3β in the cells. To determine whether GSK-3β is a functional downstream target of Akt for inducing dorsal axis formation, cellular GSK-3β was co-expressed with active forms of PI3K or Akt in the embryos. Co-injection of cellular GSK-3β reversed the effects by the expression of PI3K and Akt on axis induction and resulted in a normal phenotype similar to that of the control (Fig. 4). This result suggests that PI3K and Akt induced axis formation through the inhibition of GSK-3β.

PI3K and Akt Signaling Is Required for the Expression of Neural and Ventral Markers during Embryo Development—To study the roles of PI3K and Akt signaling in neural development in *Xenopus* embryos, the expression of neural and ventral markers was analyzed by performing RT-PCR using RNA extracted from the initial neural stage of embryos. *Xenopus* embryos at the two-cell stage were injected with mRNAs encoding, respectively, β-gal, p110α*, p85ΔiSH2, Myr-Akt, DN-Akt, GSK-3β, or DN-GSK-3β. Animal caps were dissected at stages 8.5–9 and cultured until the equivalent of stage 13 for the analysis of neural and ventral markers. p110α*, Myr-Akt, and DN-GSK-3β induced the expression of the pan-neural marker NCAM (29), the anterior neural marker Otx2 (30, 31), and the posterior neural marker HoxB9 (32), whereas p85ΔiSH2, DN-Akt, and GSK-3β induced the expression of the ventral markers Xvent1, Xvent2, BMP-4, and Xmsx1 (Fig. 5*A*). EF-1α mRNA expression was used as an internal control to indicate that similar RNA amounts and reaction conditions were used. This result further confirmed that PI3K and Akt were involved in the neurogenesis of *Xenopus* embryonic development.

To further elucidate the signaling pathway leading to the expression of neural markers and neural development, we explored whether the inhibition of PI3K and Akt activity or the ectopic expression of GSK-3β could inhibit the induction of neural marker expression. The expression of the PI3K dominant negative mutant p85ΔiSH2 inhibited PI3K-induced, but not Akt- and DN-GSK-3β-induced, neural marker expression (Fig. 5*B*). This result is consistent with a position for PI3K upstream of Akt and GSK-3β for mediating neural marker

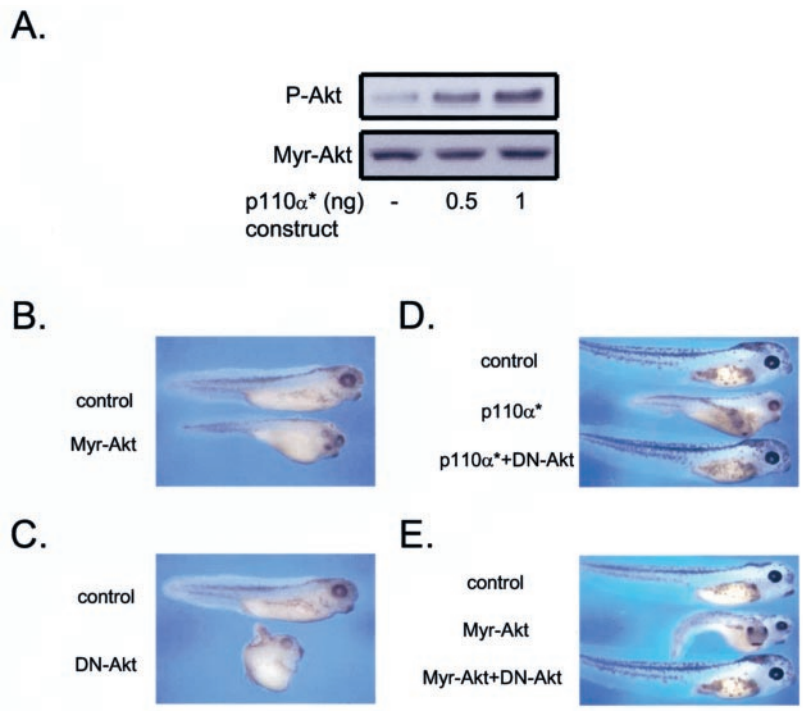

FIG. 3. **Akt is an essential downstream target of PI3K in mediating axis duplication.** *A*, the activation of Akt by p110α*. Protein extracts were prepared from the embryos as described in Fig. 1 and used for an immunoblot assay using specific antibodies against phospho-Akt at Ser-473 (19, 20). *B*, ectopic expression of an active form of Akt, Myr-Akt, induces dorsal axis duplication. The embryos at the two-cell stage were injected in the animal pole area with 1 ng of mRNA encoding Myr-Akt or β-gal (*control*). The embryos were cultured in 30% MMR solution until the equivalent of stage 45 after the injection. Embryos injected with Myr-Akt generated a dorsalizing phenotype with double heads and axes (35/50, 70%). *C*, the embryos injected with a dominant negative construct of Akt, DN-Akt, produced a ventralizing phenotype with a short tail and a small head (31/50, 62%). *D*, Akt is required for PI3K-induced axis induction. The embryos were injected with mRNAs for β-gal (*control*), p110α*, or p110α* and DN-Akt and cultured in 30% MMR solution until the equivalent of stage 45. The phenotypes were observed and photographed at stage 45. *E*, the embryos were injected with mRNAs for β-gal (*control*), Myr-Akt, or Myr-Akt and DN-Akt and cultured as above. Expression of DN-Akt inhibited p110α*- and Myr-Akt-induced axis duplication and restored to embryos phenotypes similar to those of the control.

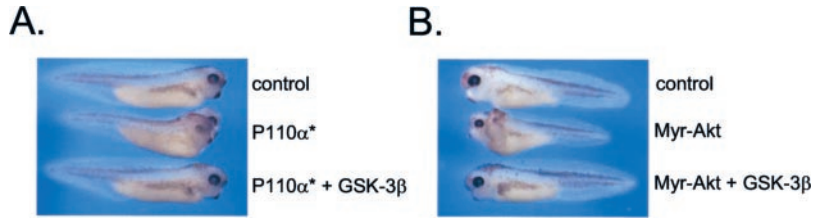

FIG. 4. **GSK-3β is a downstream negative regulator in PI3K- and Akt-induced axis duplication.** *A*, embryos were injected with mRNA coding for β-gal (*control*), p110α*, or p110α* and GSK-3β and cultured in 30% MMR solution until the equivalent of stage 45 as above. *B*, embryos were injected with mRNA coding for β-gal (*control*), Myr-Akt, or Myr-Akt and GSK-3β and cultured in 30% MMR solution until stage 45. Axis duplication was observed by the expression of p110α* and Myr-Akt, and the co-expression of GSK-3β completely inhibited PI3K- and Akt-mediated axis induction, resulting in normal phenotypes.

expression and axis induction. The expression of Akt dominant negative mutant DN-Akt inhibited both PI3K- and Akt-induced neural marker expression but not DN-GSK-3β-induced neural marker expression. However, the expression of the ventral markers (*i.e.* Xvent1, Xvent2, BMP4, and Xmsx1) induced by p85ΔiSH2, DN-Akt, and GSK-3β were blocked by p110α*, Myr-Akt, and DN-GSK-3β, respectively. These data further confirmed that Akt mediated neural marker expression and axis formation through the inhibition of GSK-3β.

In conclusion, this study has demonstrated a novel role of PI3K in mediating axis formation and neural development in *Xenopus* embryos. The activation of PI3K is transmitted through its downstream target, Akt, which inhibits the activation of GSK-3β for the process (Fig. 5*C*).

DISCUSSION

PI3K and the Akt signaling pathway play an important role in many cellular functions, including survival, proliferation, protein synthesis, and differentiation. In this study, we demonstrated that ectopic expression of an active form of PI3K was sufficient to induce axis duplication and neurogenesis through the increased expression of neural markers such as NCAM, Otx2, and HoxB9 and that the inhibition of PI3K activity by a PI3K inhibitor or a PI3K dominant negative mutant inhibited axis formation and neural development. PI3K was also found to transmit retinoic acid-induced mesoderm induction and a neural like phenotype (Fig. 2). This result is consistent with recent observations that PI3K, Ras, and mitogen-activated protein kinase/extracellular signal-regulated protein kinase kinase (MEK) have a synergistic effect in mesoderm induction by the fibroblast growth factor signaling pathway (33). This study also showed that overexpression of an active form of Akt induced double heads and axes and the expression of neural markers such as NACM, Otx2, and Hoxb9 (Figs. 3 and 5). The inhibition of Akt activity by DN-Akt induced *Xenopus* to produce a ventral

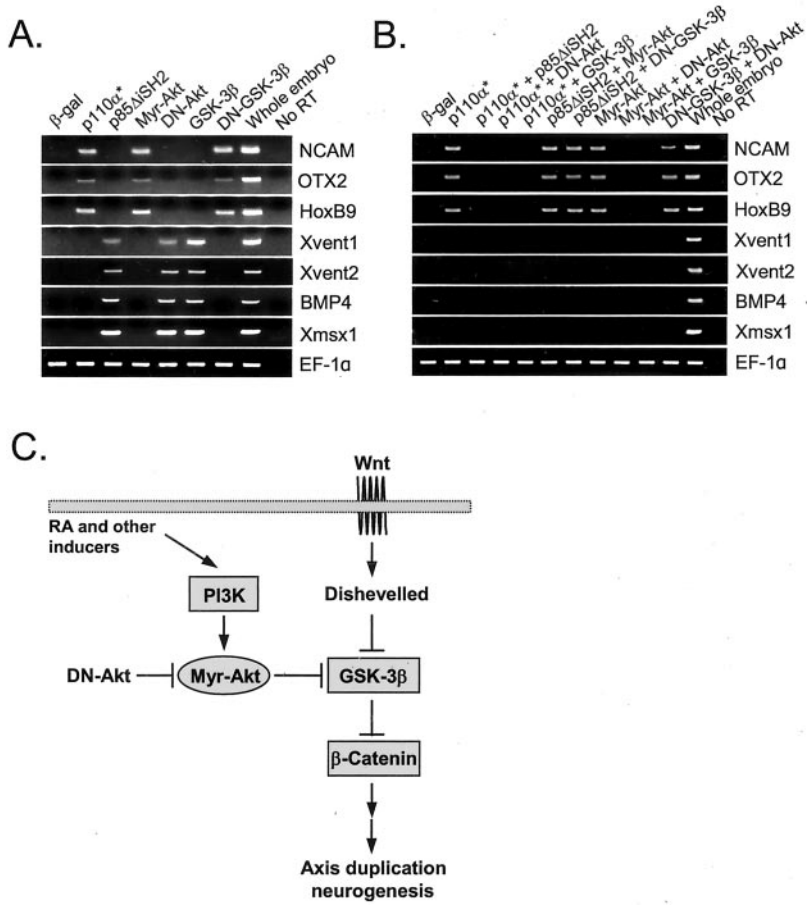

FIG. 5. **Effects of PI3K, Akt, and GSK-3β on the expression of neural and ventral markers.** *A*, embryos at the two-cell stage were injected into the animal pole with mRNAs encoding β-gal, p110α*, p85ΔiSH2, Myr-Akt, DN-Akt, GSK-3β, or DN-GSK-3β respectively. Animal caps were dissected at stages 8.5–9 and cultured in 67% Leibovitz's L-15 medium until the equivalent of stage 13. Total RNA was isolated from the animal caps and assayed for the expression of NCAM, Otx2, HoxB9, Xvent1, Xvent2, BMP-4, Xmsx1, and EF-1α by RT-PCR. EF-1α mRNA expression was used as an internal control. Total RNAs from a whole embryo at the equivalent stage were used as a positive control (*second lane* from the *right*), and the reaction without the addition of reverse transcriptase (*No RT*) was used as a negative control (*far right lane*). *B*, embryos at the two-cell stage were injected into the animal pole with mRNAs encoding β-gal, p110α*, p110α* plus p85ΔiSH2, p110α* plus DN-Akt, p110α* plus GSK-3β, p85ΔiSH2 plus Myr-Akt, p85ΔiSH2 plus GSK-3β-DN, Myr-Akt, Myr-Akt plus DN-Akt, Myr-Akt plus GSK-3β, and DN-GSK-3β plus DN-Akt, respectively. Animal caps were dissected at stages 8.5–9 and cultured in 67% Leibovitz's L-15 medium until the equivalent of stage 13. Total RNA was isolated from the animal caps and analyzed for mRNA levels of NCAM, Otx2, HoxB9, Xvent1, Xvent2, BMP-4, Xmsx1, and EF-1α. Total RNAs from a whole embryo at the equivalent stage were used as a positive control (*second lane* from the *right*). Negative control reactions (*far right lane*) were performed with total RNAs obtained from the embryos in the absence of reverse transcriptase (*No RT*) to confirm the absence of contaminating genomic DNA. *C*, schematic presentation of PI3K/Akt signaling in axis induction and neurogenesis.

phenotype and expression of the ventral markers, including Xvent1, Xvent2, BMP-4, and Xmsx1. These data indicated that Akt is an essential downstream target of PI3K for mediating axis formation and neural development. Our results provide the first evidence that PI3K and Akt are involved in axis formation and neurogenesis in vertebrate embryonic development. Dorsoventral patterning in *Xenopus* embryos requires multiple inductive events involving Niewkoop center action, mesoderm induction, and formation of the Spemann organizer (34–36). The effects of PI3K and Akt on *Xenopus* embryogenesis are similar to those of noggin (37), goosecoid (38), chordin (39), follistatin (40), siamois (41), Xwnt-1 (42), and Xwnt-8 (37, 44), which can function as dorsalizing signals. Unlike Xwnt8, noggin, and chordin, however, PI3K activity is restricted to the equatorial region of the embryo during normal embryogenesis and is required for trunk mesoderm induction (34).

Akt is required for PI3K-mediated cell growth and survival. Overexpression of constitutively active forms of PI3K or Akt induces oncogenic transformation in culture cells and tumors in animals (18, 45). We have previously reported that PI3K activity is required for myogenic differentiation (19). Akt is an essential downstream target for mediating PI3K-induced myogenesis (20). Similarly, Akt also stimulates the differentiation of adipocytes. PI3K and Akt also share a common effect in mediating angiogenesis, as we have observed previously (21). The effects of PI3K and Akt signaling on axis formation and neurogenesis are very similar to the effects reported for skeletal myogenesis and angiogenesis (19–21).

GSK-3β is a downstream target of Akt and an important component of Wnt signaling. Akt inhibited GSK-3β activation by inducing its phosphorylation (15, 45). We showed that GSK-3β expression inhibited PI3K- and Akt-induced dorsal phenotype and neural marker expression. In contrast, the expression of DN-GSK-3β induced dorsal phenotype and neural marker expression. These results are consistent with previous reports showing that overexpression of GSK-3 suppresses dorsal differentiation, whereas inhibition of GSK-3 is necessary and sufficient to initiate dorsal axis specification (46). Mammalian GSk-3β can rescue the sgg/zw3 mutant phenotype connected with wg signal transduction and Notch signaling (47). Genetic evidence suggests that sgg/zw3 behaves as a constitutively active kinase and is inhibited by wg signaling (48). Therefore, our results provide the direct link between PI3K/Akt signaling mediated by growth factors and the GSK-3β/β-cate-

nin pathway regulated by Wnt signaling and will help to elucidate the molecular mechanism of neurogenesis and dorsal axis formation.

In dorsoventral patterning, BMP-4 blocks the dorsalizing action of the DN-GSK-3β and appears to function downstream of GSK-3β in dorsoventral axis specification (46). Based on a number of previous studies, PI3K may activate two separate signal transduction pathways, one in mesoderm induction involving the activation of extracellular signal-regulated kinase in response to the fibroblast growth factor (33) and the other in neural development involving the inhibition of GSK-3 (43, 46). In this study, we demonstrated that PI3K is involved in neurogenesis during *Xenopus* embryonic development and that the PI3K signal transmits through Akt and GSK-3β. Taken together, our results reveal a novel role for PI3K signaling in both mesoderm induction and neural development during *Xenopus* embryogenesis. It remains to test the potential upstream signals that depend on PI3K activity to elicit neural tissue differentiation and to study the cross-talk between PI3K/Akt signaling and other signaling pathways in neurogenesis.

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