Erratum

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The affiliation given for these two authors in the printed version of the journal is incorrect.
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The role of an endogenous PKA inhibitor, PKIα, in organizing left-right axis formation

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SUMMARY

Protein kinase inhibitor (PKI) is an endogenous inhibitor of cAMP-dependent protein kinase A (PKA). We have found that the α-isoform of PKI (PKIα) is asymmetrically expressed along the left-right (L-R) axis in chick embryos. At stage 6, PKIα is expressed on the right side of the node, and this asymmetric expression continues until stage 7+. After stage 8, PKIα expression returns symmetric. Treatment of embryos with antisense PKIα oligonucleotides increased the incidence of reversed heart looping. Antisense oligonucleotides also induced ectopic expression of the left-specific genes *Nodal* and *Pitx2*, and suppressed the expression of the right-specific gene *SnR* in the right lateral plate mesoderm. Similarly, treatment with PKA activators forskolin and Sp-cAMPs resulted in both reversed heart looping and bilateral expression of *Nodal*. Ectopic activin induced PKIα on the left side of the node, while ectopic Shh and anti-Shh antibody had no effect on PKIα expression. Taken together, these data suggest that PKIα induced by an activin-like molecule, through the inhibition of PKA activity, suppresses the *Nodal-Pitx2* pathway on the right side of the body.

Key words: Left-right axis formation, Vertebrate, Embryogenesis, Patterning, Chick

INTRODUCTION

PKI is a peptide of 76 amino acids that was originally isolated from rabbit skeletal muscle by its ability to inhibit the activity of cAMP-dependent protein kinase (PKA; Walsh et al., 1971). In its inactive state, PKA is a tetramer composed of two regulatory (R) subunits and two catalytic (C) subunits. Binding of cAMP by the R subunits leads to dissociation of the R_2C_2 complex, resulting in the release of active C subunits. PKI contains a pseudo-substrate sequence for PKA (Scott et al., 1985), and inhibits PKA enzymatic activity by binding to free C subunits. In rodents, three distinct isoforms of PKI, designated α, β and γ, have been identified (Olsen and Uhler, 1991; Van Patten et al., 1991; Collins and Uhler, 1997). In adults, PKIα mRNA is expressed in skeletal muscle, heart and brain (Olsen and Uhler, 1991; Seasholtz et al., 1995; Van Patten et al., 1992), the β isoform is expressed in testis (Van Patten et al., 1992), and the γ mRNA is found in heart, skeletal muscle and testis (Collins and Uhler, 1997).

Despite the ability of PKI to potently inhibit PKA activity, its physiological roles are not well understood. In adult heart, the level of PKI is ~20% of that necessary to inhibit fully activated PKA (Walsh et al., 1990). Therefore, the effect of PKI on PKA activity after cAMP production may be marginal. For this reason, it has been suggested that PKI may act as a regulator of basal PKA activity, or the activity that persists independent of adenylate cyclase activation (Walsh et al., 1990). In addition to the pseudo-substrate sequence, PKI contains a nuclear export signal (NES) (Wen et al., 1994; Wen et al., 1995). The binding of the C subunit to PKI exposes the NES, triggering its exit from the nucleus. Thus, it is possible that PKI may also regulate the intracellular distribution of C subunits.

PKA activity has been implicated in signal transduction pathways that underlie cell-type specification in invertebrate and vertebrate embryos. In *Drosophila* imaginal discs, cells that lack the C subunit exhibit pattern respecifications similar to those generated by ectopic *hedgehog* (*hh*) expression (Jiang and Struhl, 1995; Lepage et al., 1995; Li et al., 1995; Pan and Rubin, 1995; Strutt et al., 1995). In zebrafish, expression of a variant PKA-R subunit that constitutively inhibits PKA activity induces the expansion of proximal fates in the eye, ventral fates in the brain, and adaxial fates in somites and head mesenchyme (Hammerschmidt et al., 1996; Concordet et al., 1996). In embryonic limb, BMP-2-mediated stimulation of chondrogenesis is dependent on PKA (Lee and Chuong, 1997), and a pharmacological agent that inhibits PKA activity stimulates renal branching morphogenesis (Gupta et al., 1999). These experiments demonstrate that ectopic inhibitions of PKA activity can lead to alterations in cell-type specificity.

Recent studies have identified molecules involved in the formation of L-R asymmetry in the vertebrate embryo (Varlet and Robertson, 1997; Levin, 1998; Yost, 1999; Capdevila et al., 2000). Several genes are asymmetrically expressed along the L-R axis. In the chick, these include *Sonic hedgehog* (*Shh*), *activin receptor IIa* (*ActRIIa*), *Nodal* (Levin et al., 1995), *lefty*

(Meno et al., 1996), *SnR* (Isaac et al., 1997), *Pitx2* (Logan et al., 1998; Yoshioka et al., 1998; Piedra et al., 1998; Ryan et al., 1998), *FGF8* (Boetgger et al., 1999; Meyers and Martin, 1999), *Caronte* (Rodriguez-Esteban et al., 1999; Yokouchi et al., 1998), *NKX3.2* (Schneider et al., 1999), *N-cadherin* (Garcia-Castro et al., 2000), *FGF18* (Ohuchi et al., 2000) and *BMP4* (Monsoro-Burq and Le Douarin, 2000). Between stages 4 and 6, *Shh* is expressed on the left side of the node, while the expression of *ActRIIa* and *FGF8* is confined to the right. Ectopic *Shh* delivered to the right side of the node induces expression of a TGFβ family member *Nodal* (Levin et al., 1995), and anti-Shh antibody placed on the left side of the node abolishes *Nodal* expression (Pagán-Westphal and Tabin, 1998). Furthermore, *Nodal* seems to suppress expression of the right-sided gene *SnR*, a zinc-finger molecule, in lateral plate mesoderm (Pagán-Westphal and Tabin, 1998). *SnR*, in turn, is a repressor of another left-sided gene *Pitx2* (Patel et al., 1999). These experiments suggest that side-specific gene expression is established by positive and negative interactions among the molecules. Finally, experimental manipulations of these side-specific genes lead to abnormal morphogenesis along the L-R axis (reviewed by Capdevila et al., 2000).

We hypothesized that PKI might play a role in cell patterning through the inhibition of PKA activity. To test this idea, we examined the expression pattern of PKIα in chick embryos. PKIα expression is developmentally and regionally restricted in chick embryos. Interestingly, PKIα is expressed more strongly on the right side of Hensen's node than the left side between stages 6 and 7+. Treatment of embryos with antisense PKIα oligonucleotides led to an increase in the incidence of reversed heart looping and bilateral expression of *Nodal* and *Pitx2*. The same treatment suppressed the expression of *SnR* on the right side of the posterior lateral plate. Similarly, treatment with PKA activators induced bilateral expression of *Nodal*. Ectopic activin induced PKIα expression on the left side of the node, while ectopic Shh and anti-Shh antibody had no effect on PKIα expression. We propose a model in which PKIα interferes with the propagation of the *Shh-Nodal* pathway on the right side of the embryo.

MATERIALS AND METHODS

Whole-mount in situ hybridization

Pathogen-free white Leghorn chick embryos were purchased from SPAFAS (Norwich, CT, USA). Eggs were incubated at 37.5°C, and staged according to Hamburger and Hamilton (Hamburger and Hamilton, 1951). Embryos were fixed in 4% paraformaldehyde, and whole-mount in situ hybridization was performed as described in Levin et al. (Levin et al., 1995). The probe for chick PKIα was prepared by RT-PCR, based on the published sequence (Marchetto and Henry, 1995).

Antisense oligonucleotide application

Phosphorothioate oligodeoxynucleotides were used in this study. The sequence of antisense oligonucleotides (shown in Fig. 3B) corresponds to nucleotides 374 to 355, numbered from the A of the start methionine codon of chick PKIα. We chose this sequence for antisense targeting, based on the thermodynamic algorithm developed by Toagosei, Tokyo, Japan. Oligonucleotide applications were performed as described in Nieto et al. (Nieto et al., 1994). Briefly, blastoderms were detached from their membranes, incubated with 80 μm oligonucleotides in protein-free medium (50% Hanks balanced salt solution/50% L-15 tissue culture medium with Ca^{2+} and Mg^{2+}) for 3 hours at 37°C, and placed back on the original membranes. The blastoderms reattached and continued to spread on the membranes. In situ hybridization was performed to analyze expression of *Shh*, *Nodal* and *Pitx2*, using embryos at stages 6, 9, and 10 respectively. Heart looping was observed at stage 12.

Drug application

PKA activators were applied to embryos prepared by the same method used for oligonucleotide treatments (Nieto et al., 1994). Drug concentrations used in this study are as follows: forskolin and 1,9-forskolin, 90 μM; Rp-cAMPS and Sp-cAMPs, 100 μM. These concentrations were chosen based on previous reports (Fan et al., 1995; Frey et al., 1993).

Bead implantation

Activin bead implantation was performed as described in Levin et al. (Levin et al., 1995). Affigel blue beads were washed with PBS and soaked in activin for 1 hour on ice. Then beads were picked up and implanted between the epiblast and endoderm of stage 4 embryo in New culture (New, 1955). The materials for bead implantation using anti-Shh monoclonal antibody 5E1 and Shh protein have been described by Pagán-Westphal and Tabin (Pagán-Westphal and Tabin, 1998). Control beads were soaked in the buffer 5 mM NaP/150 mM NaCl/0.5 mM DTT (pH 5.5) in which Shh was diluted.

RESULTS

PKIα is asymmetrically expressed in chick embryos

We studied PKIα expression in chick embryos by whole-mount in situ hybridization, beginning at early stages of development. At stage 4, PKIα expression is observed on both sides of Hensen's node and along the primitive streak (Fig. 1A,B). At stage 6, PKIα expression becomes stronger on the right side of the node (Fig. 1C,D). PKIα expression at the node continues to be asymmetric at stage 7+ (Fig. 1E,F), and returns symmetric at stage 8 (Fig. 1G,H). In addition to the expression at the node, symmetric expression is observed in the emerging head process at stage 6 (Fig. 1C,D), in the head fold at stage 7+ (Fig. 1E), and in the notochord and somites at stage 8 (Fig. 1G,H). At stage 10+, PKIα is expressed in the notochord, ventricle of the heart and somites (Fig. 1I). Later, PKIα is expressed in the second branchial arch, heart, notochord and somites (Fig. 1J).

Treatment with PKIα antisense oligonucleotides perturbs the establishment of heart situs and Nodal expression

To investigate the role of PKIα in L-R axis formation, we treated embryos with antisense oligonucleotides. Embryos were isolated at stage 4+, incubated for 3 hours with 80 μM oligonucleotides at 37°C, and examined at stage 12 for the direction of cardiac looping. The treatment with antisense oligonucleotides resulted in a higher incidence of reversed heart looping compared to sense treatment (Table 1; Fig. 2A,B). This result shows that the antisense oligonucleotides perturbed the development of proper heart situs.

We next examined the expression of three asymmetric genes *Shh*, *Nodal* and *Pitx2* in embryos treated with PKIα antisense oligonucleotides. *Nodal* and *Pitx2* are components of signaling cascades induced by *Shh*. Treatment with antisense oligonucleotide did not change the expression pattern of *Shh*,

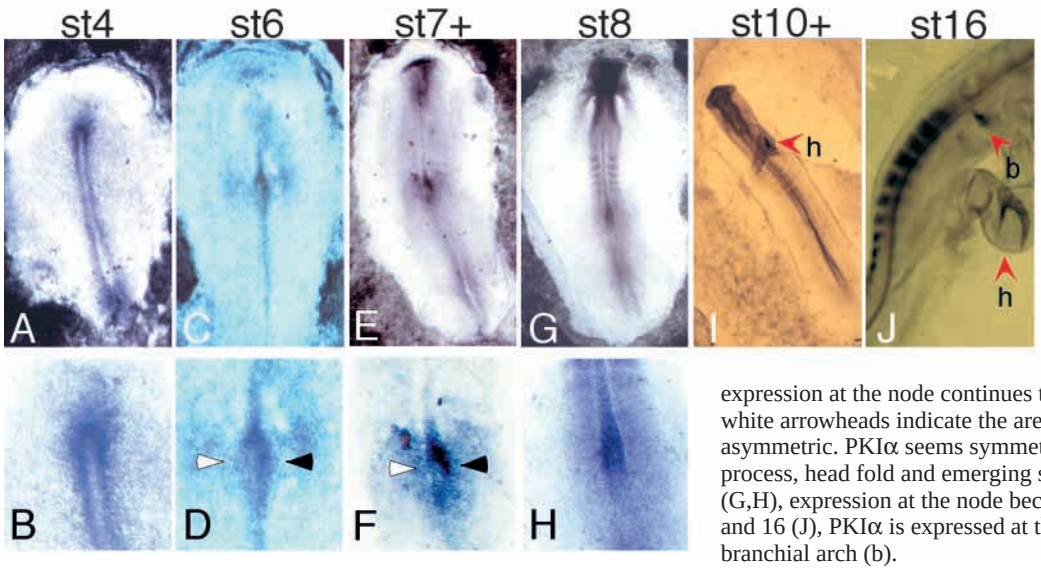

Fig. 1. Expression of PKIα in chick embryos. Whole-mount in situ hybridization with a PKIα probe. (A,C,E,G) Dorsal views of chick embryos from stages 4 to 8. (B,D,F,H) Close-ups of Hensen's node. (I,J) Stage 10+ and stage 16 embryos. At stage 4 (A,B), expression is uniform on both sides of the node and primitive streak. At stage 6 (C,D), expression becomes stronger in the right side of the node. The right-handed expression at the node continues through stage 7+ (E,F). Black and white arrowheads indicate the areas where PKIα signal is asymmetric. PKIα seems symmetrically expressed in the head process, head fold and emerging somites at these stages. At stage 8 (G,H), expression at the node becomes symmetric. At stage 10+ (I) and 16 (J), PKIα is expressed at the notochord, somites, heart (h) and branchial arch (b).

which continued to be on the left side of the node at stage 6 (Table 1; Fig. 2D). In contrast, antisense treatment induced *Nodal* and *Pitx2* expression on the right side of body at stages 9-10, resulting in bilateral expression of these genes (Fig. 2F,H,J,L).

Nodal appears to suppress expression of a right-sided gene *SnR* in lateral plate mesoderm (Pagán-Westphal and Tabin, 1998). Thus, we examined the effect of PKIα antisense oligonucleotides on the expression of *SnR*. If PKIα is upstream of *Nodal*, as indicated from the results shown above, then

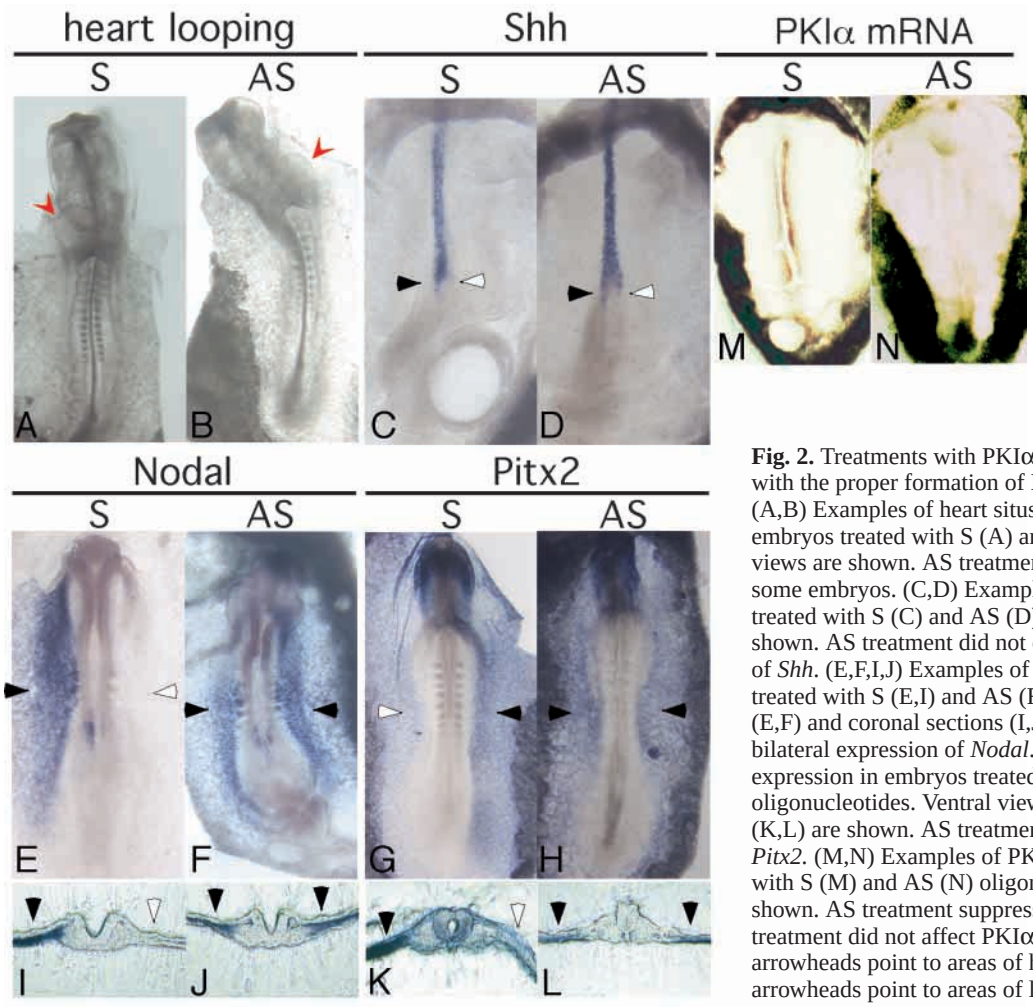

Fig. 2. Treatments with PKIα antisense oligonucleotides interfere with the proper formation of L-R axis. S, sense; AS, antisense. (A,B) Examples of heart situs (indicated by red arrowheads) in embryos treated with S (A) and AS (B) oligonucleotides. Ventral views are shown. AS treatment caused reversed heart looping in some embryos. (C,D) Examples of *Shh* expression in embryos treated with S (C) and AS (D) oligonucleotides. Dorsal views are shown. AS treatment did not change the asymmetric expression of *Shh*. (E,F,I,J) Examples of *Nodal* expression in embryos treated with S (E,I) and AS (F,J) oligonucleotides. Dorsal views (E,F) and coronal sections (I,J) are shown. AS treatment induced bilateral expression of *Nodal*. (G,H,K,L) Examples of *Pitx2* expression in embryos treated with S (G,K) and AS (H,L) oligonucleotides. Ventral views (G,H) and coronal sections (K,L) are shown. AS treatment induced bilateral expression of *Pitx2*. (M,N) Examples of PKIα expression in embryos treated with S (M) and AS (N) oligonucleotides. Dorsal views are shown. AS treatment suppressed the expression of PKIα, while S treatment did not affect PKIα mRNA expression. Black arrowheads point to areas of high expression, and white arrowheads point to areas of low expression.

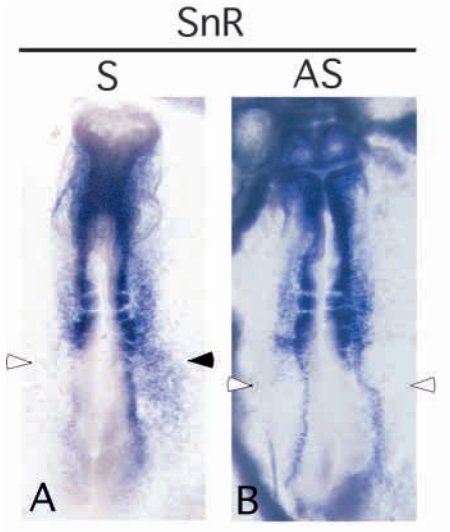

Fig. 3. Treatment with PKIα antisense oligonucleotides suppressed right sided expression of *SnR*. Examples of *SnR* expression in embryos treated at stage 4 with sense (A) and antisense (B) oligonucleotides. Dorsal views at stage 9 are shown. Note that *SnR* signal is symmetrical within somites. Suppression of *SnR* signal by AS treatment is seen most prominently in the posterior area of lateral mesoderm. Black arrowheads point to areas of high expression and white arrowheads point to areas of low expression.

antisense treatment would suppress right-sided expression of *SnR*. In embryos treated with sense oligonucleotides, *SnR* expression is bilateral within somites, while it is asymmetric in lateral plate mesoderm (Fig. 3A). This expression pattern is similar to those in normal embryos (Isaac et al., 1997; Pagán-Westphal and Tabin, 1998). Antisense oligonucleotides drastically reduced the expression of *SnR* in the posterior area of lateral mesoderm (Fig. 3B).

In order to assess the effect of oligonucleotides, we examined the expression of PKIα in embryos treated with PKIα sense and antisense oligonucleotides. Antisense treatment drastically diminished the signal for PKIα mRNA (Fig. 2N), while sense treatment had no effect (Fig. 2M). This finding is consistent with the previous report that antisense oligodeoxynucleotides can lead to degradation of mRNAs by RNaseH-like activity (Dash et al., 1987). Next, we synthesized mutant antisense oligonucleotides with one (M1), three (M3), or five (M5) mismatches (Fig. 4) in order to verify the specificity of the antisense oligonucleotides. We then examined

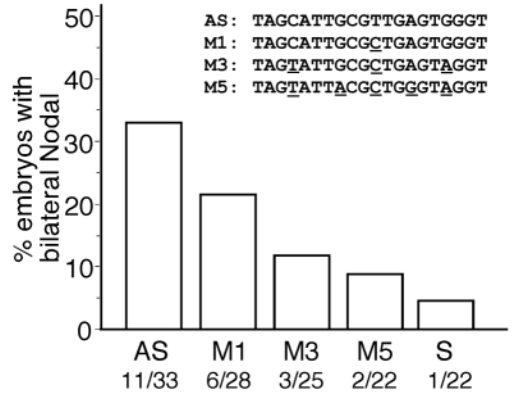

Fig. 4. Sequence specificity of antisense oligonucleotides. Percentages of embryos exhibiting bilateral expression of *Nodal* following treatments with oligonucleotides. Values below treatments represent the number of embryos with bilateral *Nodal* expression/ the number of embryos treated with oligonucleotides. The nucleotide sequences of oligonucleotides are shown in inset. Mismatches with chick PKIα are underlined. AS, antisense; S, sense.

the effect of these oligonucleotides on bilateral expression of *Nodal* at stage 9. As shown in Fig. 4, antisense oligonucleotides with increased mismatches exhibited decreased effects on bilateral expression of *Nodal*. These data suggest that the antisense oligonucleotides perturbed L-R development in a sequence-specific manner. Taken together, endogenous PKIα appears to suppress the *Nodal-Pitx2* pathway in the right lateral plate mesoderm.

Effects of PKA activators on L-R asymmetry

The only known target of PKI is PKA, thus we next asked whether suppression of the *Nodal-Pitx2* pathway by PKIα was mediated by PKA. To this end, we treated embryos with PKA activators forskolin and Sp-cAMPS. If PKIα acts on PKA in the process of suppressing the *Nodal-Pitx2* pathway, then the treatment with PKA activators should alleviate PKIα function.

In the first set of experiments, we treated stage 4+ embryos with medium only (no drug), 1,9-forskolin, forskolin, Rp-cAMPS and Sp-cAMPS. We then performed in situ hybridization to analyze *Nodal* expression at stage 9. Forskolin and Sp-cAMPS induced bilateral expression of *Nodal*, while an inactive compound 1,9-forskolin and a PKA inhibitor Rp-cAMPS did not alter the left-sided expression of *Nodal* (Fig. 5A). In the second set of experiments, we examined the

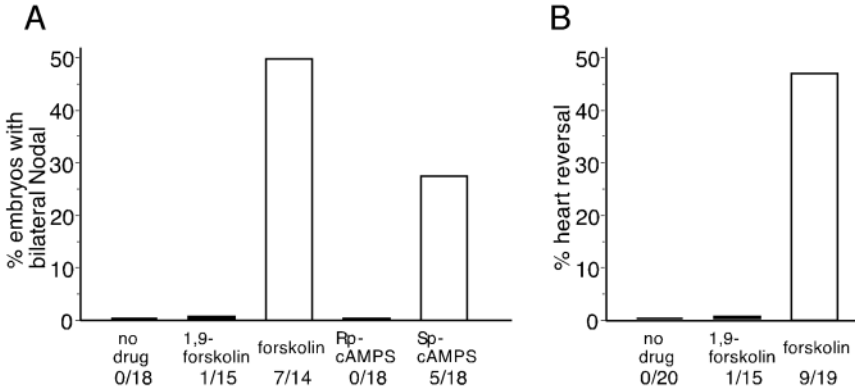

Fig. 5. Effects of PKA activators on *Nodal* expression and heart looping. (A) Percentages of embryos exhibiting bilateral *Nodal* expression after drug treatments. (B) Percentages of embryos exhibiting heart reversal after drug treatments.

Table 1. Effects of PKIα antisense oligonucleotides

Observation	Sense	Antisense
Bilateral expression of Shh	0/12	0/12
Bilateral expression of Nodal	1/22	11/33
Bilateral expression of Pitx2	1/19	7/19
Beversed heart looping	1/21	7/26

After sense and antisense treatments, embryos were scored according to the listed phenotypes. Each value represents the number of embryos exhibiting the phenotype/the number of embryos treated with oligonucleotides.

direction of heart looping in chick embryos treated with forskolin and 1,9-forskolin. Forskolin induced reversed heart looping at the frequency significantly higher than that by 1,9-forskolin (Fig. 5B). These results suggest that activation of PKA in whole embryos leads to abnormal L-R development, and are consistent with the notion that PKIα acts upstream of PKA in the signal transduction pathway leading to the suppression of the *Nodal-Pitx2* pathway.

PKIα is induced by ectopic activin, but unchanged by Shh

We then attempted to identify the upstream molecule(s) of PKIα. A previous study showed that a right-handed gene *ActRIIa* was induced by ectopic activin (Levin et al., 1995). We thus implanted activin-coated beads on the left side of the node on stage 4 embryos, and examined the effect on PKIα expression. The embryos that received buffer-soaked beads exhibited the normal right-sided expression of PKIα (Fig. 6A; *n*=10). In contrast, 12 of 16 embryos in which an activin bead was implanted showed the induction of PKIα on the left side of the node, although this induction did not lead to the fully symmetric expression of PKIα at the node (Fig. 6B). These results suggest that PKIα is downstream of the activin-like molecule at the node.

The *Shh-Nodal* pathway not only induces left-handed genes, but also suppresses the expression of a late right-handed gene *SnR* (Pagán-Westphal and Tabin, 1998). We therefore asked whether right-handed expression of PKIα is controlled by *Shh*. We placed beads soaked in an anti-*Shh* blocking monoclonal antibody on the left side of the node at stage 5. This procedure has previously been shown to induce bilateral expression of *SnR* (Pagán-Westphal and Tabin, 1998). As shown in Fig. 6C, anti-*Shh* antibody had no effect on asymmetric expression of PKIα (*n*=6). We next implanted a bead soaked in Shh protein on the right side of the node at stage 5. Again, asymmetric expression of PKIα was unchanged by this treatment (Fig. 6D; *n*=7). Separate experiments in which nodal expression was examined showed that the anti-*Shh* antibody and SHH protein used in our study were active (data not shown). These data suggest that PKIα expression is not downstream of *Shh*.

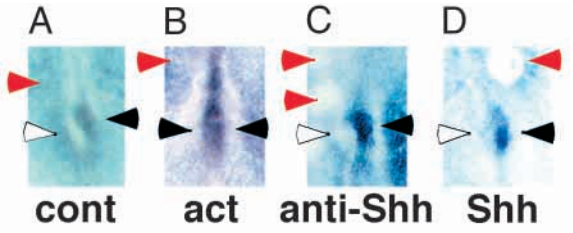

Fig. 6. PKIα is regulated by ectopic activin, but not by *Shh*. Whole-mount in situ hybridization with PKIα probe after beads implantation adjacent to the node. Beads were placed in embryos at stage 4 at the location indicated by red arrowheads, and in situ hybridization was performed at stages 6-7. In one set of experiments, embryos received a buffer-soaked bead (A) or an activin-soaked bead (B) at the left side of the node. An activin-soaked bead induced the expression of PKIα mRNA on the left side of the node. In the second set of experiments, embryos received beads soaked in anti-Shh antibody (C), and a bead soaked in Shh protein (D). Black arrowheads point to areas of high expression, and white arrows point to areas of low expression.

DISCUSSION

Asymmetric expression of PKIα along the L-R axis

PKIα is expressed on the right side of the node in the chick embryo. The asymmetric expression of PKIα occurs as early as stage 6 and continues until stage 7+. We used antisense oligonucleotide techniques to identify the role of endogenous PKIα in the establishment of the L-R axis. PKIα antisense oligonucleotides induced bilateral expression of *Nodal*/*Pitx2* and reversed heart looping. The same treatment also suppressed expression of *SnR*. We then treated whole embryos with PKA activators, which induced *Nodal* expression on the right side of body. Taken together, we propose that PKIα, through the inhibition of PKA, is involved with signaling pathways underlying L-R asymmetry. Specifically, both PKIα activity and PKA inhibition appear required to suppress *Nodal/Pitx2* expression in the right side. It is likely that the relationship between PKIα, PKA and *Nodal* is linear: PKIα is upstream of PKA and PKA is upstream of *Nodal* (Fig. 7A). If this is the case, as PKI should operate cell autonomously, our data is most consistent with the node being the likely place where PKA signaling operates. However, owing to the absence of techniques that allow us to deliver small-molecule drugs in a side-specific manner, we have been unable to localize the action of PKA in embryos. Therefore, we have not eliminated the possibility in which PKIα suppresses *Nodal* without involving PKA activity, while PKA can independently activate *Nodal* (Fig. 7B).

Although ectopic activin induced PKIα in this study, the endogenous ligand(s) that activates activin receptor(s) has not been identified. *ActRIIa*, is expressed in the right side of the node, and its asymmetric expression precedes right-handed

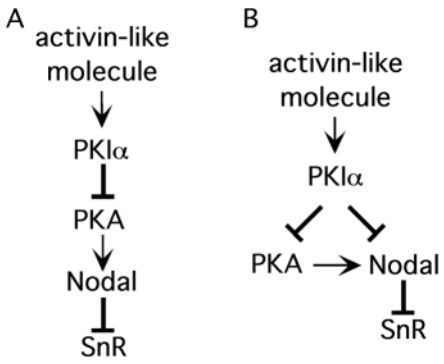

Fig. 7. The role of PKIα in signal transduction cascades determining L-R asymmetry. See text for explanation.

expression of PKIα (Levin et al., 1995). Experiments using mice that lack *ActRIIA*, *ActRIIB* and *Smad2* also support the notion that the *ActRII* pathway is involved in the formation of the L-R axis (Oh and Li, 1997; Nomura and Li, 1998). A recent finding revealed that *FGF8* is downstream of the ActRII pathway (Boetgger et al., 1999). Our data show that PKIα is also downstream of the activin receptor pathway. *FGF8* asymmetric expression starts from stage 6 and continues until at least stage 8 (Boetgger et al., 1999), while PKIα asymmetric expression occurs between stages 6 and 7+. Thus, judging from the timing of expression, PKIα and *FGF8* may be regulated by similar mechanisms.

Recently, it was reported that *N-cadherin* is expressed on the right side of the node and left side of the primitive streak (Garcia-Castro et al., 2000). This asymmetric expression is seen between stages 3+ and 5 at the primitive streak, and between stages 4+ and 5 at the node. Blockade of N-cadherin function by a monoclonal antibody resulted in the altered expression of *Snail* and *Pitx2*, but had no effect on *Nodal*. Based on these data, it has been suggested that *N-cadherin* is a component of a pathway parallel to *Nodal*. As PKI α appears upstream of *Nodal*, it is unlikely that *N-cadherin* and PKI α are in the same pathway.

Our results also indicate that PKIα is not downstream of *Shh*. Nonetheless, the two molecules that we found were regulated by PKIα are *Nodal* and *Pitx2*, and these genes are downstream of *Shh*. It has been noted that *hh* signaling pathways interact with PKA activity. In *Drosophila*, loss of the C subunit of PKA leads to an increase in the level of full-length Cubitus interruptus, as well as the induction of the *hh* target genes *dpp*, *wg* and *ptc* (Chen et al., 1998). Paradoxically, transgenic PKA potentiates the activation of these genes, suggesting that PKA could regulate *hh* signaling pathways both positively and negatively (Ohlmeyer and Kalderon, 1997; Chen et al., 1998). Furthermore, it has been suggested that the regulation of the *hh* pathway by PKA in *Drosophila* is not mediated by cAMP (Li et al., 1995; Jiang and Struhl, 1995; Ohlmeyer and Kalderon, 1997). Our finding that PKIα regulates *Nodal* and *Pitx2* raises the possibility that a putative *Drosophila* homolog of PKI may mediate the interaction of PKA and *hh* pathways.

One might predict that the treatment with Rp-cAMPS would result in the suppression of *Nodal* in both sides of embryos. However, we did not observe such events at a significant frequency. It is possible that the inhibition of PKA activity is not sufficient to suppress *Nodal* expression. It is also possible that Rp-cAMPS does not mimic PKIα functions, owing to the difference in mechanisms by which these two molecules inhibit PKA. Rp-cAMPS is an analog of cAMP, and inhibits PKA activity by inactivating the R subunit. However, PKI inactivates the C subunit by binding it as a pseudosubstrate. It has been suggested that there may be a greater number of C subunits relative to R subunits in some cells (Walsh et al., 1990). The PKA activity carried out by these C subunits can be suppressed by PKI, but not by Rp-cAMPs. Such PKA activity could constitute the basal activity, which may play a role in the establishment of L-R axis.

Several asymmetric genes along the L-R axis are also involved in the formation of the dorsoventral and anteroposterior axes (Danos and Yost, 1995; Danos and Yost, 1996; Yost, 1995). PKIα is expressed in the dorsal region of somites around stage 10 (M. K., unpublished). Inhibition of PKA activity by a transgenic dominant-negative R subunit led to abnormal patterning in the mesoderm including somitic myotomes (Hammerschmidt et al., 1996; Concordet et al., 1996). Hence, PKIα may be a component of signal transduction pathways repeatedly employed in pattern formation.

Features of PKI-mediated PKA regulation

Our findings suggest that an extracellular signal(s) could regulate PKA activity by controlling the expression of PKI. What are the distinct features of PKA modulation governed by PKI when compared with cAMP-mediated regulation? First, PKI directly interacts with the PKA-C subunit, and could thus inhibit the PKA previously activated by cAMP. Second, unlike cAMP (which is a small diffusible molecule), the intracellular distribution of PKI could be localized to certain structures or organelles. This would allow a spatially specific activation/inactivation of PKA within a single cell. Finally, the kinetics of PKA regulation based on the modulation of PKI gene expression are likely be drastically different from those mediated by cAMP. Turnover of cAMP is rapid, while transcriptional regulation of PKI would occur with much slower kinetics. Regulation of the PKI gene may thus modify basal PKA activity for lengthy periods during events such as pattern formation.

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