Activities of the Wnt-1 Class of Secreted Signaling Factors Are Antagonized by the Wnt-5A Class and by a Dominant Negative Cadherin in Early Xenopus Development

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Abstract. When overexpressed in Xenopus embryos, Xwnt-1, -3A, -8, and -8b define a functional class of Wnts (the Wnt-1 class) that promotes duplication of the embryonic axis, whereas Xwnt-5A, -4, and -11 define a distinct class (the Wnt-5A class) that alters morphogenetic movements (Du, S., S. Purcell, J. Christian, L. McGrew, and R. Moon. 1995. Mol. Cell. Biol. 15:2625-2634). Since some embryonic cells may be exposed to signals from both functional classes of Wnt during vertebrate development, this raises the question of how the signaling pathways of these classes of Wnts might interact. To address this issue, we coexpressed various Xwnts and components of the Wnt-1 class signaling pathway in developing Xenopus embryos. Members of the Xwnt-5A class antagonized the ability of ectopic Wnt-1 class to induce goosecoid expression and a secondary axis. Interestingly, the Wnt-5A class did not block goosecoid expression or axis induction in response to overexpression of cytoplasmic components of the Wnt-1 signaling pathway, β-catenin or a kinase-dead gsk-3, or to the unrelated secreted factor, BVgl. The ability of the Wnt-5A class to block responses to the Wnt-1 class may involve decreases in cell adhesion, since ectopic expression of Xwnt-5A leads to decreased Ca^{2+}-dependent cell adhesion and the activity of Xwnt-5A to block Wnt-1 class signals is mimicked by a dominant negative N-cadherin. These data underscore the importance of cell adhesion in modulating the responses of embryonic cells to signaling molecules and suggest that the Wnt-5A functional class of signaling factors can interact with the Wnt-1 class in an antagonistic manner.

Wnt genes encode a family of secreted glycoproteins that are expressed in restricted temporal and spatial patterns during embryonic development and are involved in intercellular signaling (for review see McMahon, 1992; Nusse and Varmus, 1992; Moon, 1993). Recently, Wnt genes have been grouped into functional classes based on assays performed in Xenopus embryos (Du et al., 1995; Moon et al., 1993) and transformation assays carried out with mammalian cell lines (Wong et al., 1994). Ectopic expression of Xenopus Wnt (Xwnt)1-1, -3A, -8, and 8b (the Wnt-1 class) (for review see Du et al., 1995; Moon et al., 1993b; Cui et al., 1995) induces a secondary axis and rescues Nieuwkoop center activity in embryos ventralized by UV irradiation. In contrast, ectopic expression of members of the Wnt-5A class, including Xwnt-5A, -4, and -11, inhibits morphogenetic movements during gastrulation without overtly altering cell fate (Du et al., 1995; Moon et al., 1993a, b). Mouse Wnt genes have been grouped in a similar manner, with Wnt-1, -3A, and -7A transforming mammary epithelial C57MG cells at a high frequency and Wnt-4 and -5A deficient in transforming activity (Wong et al., 1994). These independent studies support the existence of multiple functional classes of Wnts.

Much progress has been made in identifying downstream components of the signaling pathway stimulated by the products of Wnt-1 class, although no receptor has been reported. Epistasis studies in Drosophila have demonstrated that in response to signaling by wingless, the ortholog of vertebrate of Wnt-1 (Rijsewijk et al., 1987), activation of dishevelled (Klingensmith et al., 1994; Noordermeer et al., 1994; Theisen et al., 1994) leads to the inhibition of zeste-white 3, the ortholog of vertebrate glycogen synthase kinase-3 (Siegfried et al., 1992). This results in activation of armadillo, the ortholog of vertebrate β-catenin.
(Klingensmith et al., 1989; Peifer and Wieschaus, 1990; Riggleman et al., 1990), which is repressed by zeste-white 3 in the absence of a wingless signal. Assays in vertebrates support the involvement of related genes in Wnt-1 class signaling, as injection of RNAs encoding vertebrate dishevelled (Sokol et al., 1995), β-catenin (Funayama et al., 1995; Guger and Gumbiner, 1995; Kelly et al., 1995), and kinase-dead, putative dominant negative forms of gsk-3 (Dominguez et al., 1995; He et al., 1995; Pierce and Kimelman, 1995) into developing embryos mimics the overexpression of members of the Wnt-1 class. Conversely, Heasman et al. (1994) demonstrated that the depletion of maternal β-catenin transcripts leads to the development of Xenopus embryos that lack dorsal structures and are nonresponsive to ectopic Xwnt-8. These results support the involvement of these molecules in a common signaling pathway.

Since β-catenin is both necessary (Heasman et al., 1994) and sufficient (Guger and Gumbiner, 1995; Kelly et al., 1995) for eliciting all embryonic responses previously reported for the Wnt-1 class (Moon et al., 1993b), considerable work has focused on this prospective signaling molecule. Besides its involvement in Wnt signaling, β-catenin is also a cadherin-associated cytoplasmic protein (Nagafuchi and Takeichi, 1989; Ozawa et al., 1989). However, increases in cadherin-dependent cell adhesion without overexpression of β-catenin are not sufficient to induce Wnt-like responses in Xenopus embryos (Detrick et al., 1990; Fujimori et al., 1990; Levine et al., 1994; Guger and Gumbiner, 1995). Thus, β-catenin is likely to have signaling properties independent of its role in cell adhesion. Consistent with modulation of non-membrane-associated β-catenin by components of the Wnt-1 class signaling pathway, we have recently found that endogenous β-catenin in Xenopus embryos is present in nuclei as well as at the plasma membrane, and that the ratio of β-catenin in the nucleus relative to the membrane is increased by a dominant negative Xgsk-3 (Yost, C., M. Torres, J.R. Miller, E. Huang, D. Kimelman, and R.T. Moon, manuscript submitted for publication).

Although increased cell adhesion does not elicit responses that mimic Wnt signaling, there have nevertheless been several indications that both the Wnt-1 class and the Wnt-5A class may lead to changes in cell adhesion. For example, transfection of Wnt-1 into PC12 pheochromocytoma cells elevates levels of E-cadherin, as well as the β-catenin-related protein plakoglobin, while increasing calcium-dependent cell adhesion (Bradley et al., 1993). Similarly, in both AtT20 and C57MG cells transfected with Wnt-1, increases were observed in the accumulation of β-catenin and plakoglobin and in calcium-dependent cell adhesion (Hinck et al., 1994). Finally, Xwn-5A has been reported to reduce cell mixing in gastrula-stage embryos (Moon et al., 1993b), but these experiments do not directly address whether this involved changes in cell adhesion.

Although comparisons of the patterns of expression of

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**Figure 1.** Xwnt-5A antagonizes Wnt-1 class activity. (A) Control embryos possess single axes at stage 25. (B) Embryos injected with prolactin followed by Xwnt-8 RNA have duplicated axes. (C) Embryos injected with Xwnt-5A followed by Xwnt-8 RNA have single axes. (D) Uninjected stage 10 control embryos possess a single site of gsc expression. (E) Embryos injected with prolactin followed by Xwnt-8 RNA display multiple sites of gsc expression. (F) Embryos injected with Xwnt-5A followed by Xwnt-8 RNA have a single site of gsc expression. (Arrows) Axes (A, B, and C) and sites of gsc expression (D, E, and F).
Wnts in both mouse (Parr et al., 1993; Takada et al., 1994) and Xenopus (for review see Moon, 1993) reveal that the two functional classes are generally expressed in distinct regions of the embryo, in situ hybridization data suggest that cells in some embryonic structures receive signals from both classes of Wnts (see Discussion). These overlapping patterns of expression, along with the ability of Wnts to signal in a paracrine manner beyond the cells in which they are expressed, raise the question of how the presence of both the Wnt-1 and Wnt-5A functional classes might affect responding cells.

In the present study we investigated whether there are synergistic or dominant interactions between the Wnt-5A and -1 class signaling pathways by coexpressing members of both classes together and with other signaling factors in embryos of Xenopus laevis. We report that injection of RNAs encoding members of the Wnt-5A class antagonizes the embryonic responses to ectopic expression of the Wnt-1 class, and that this may in part be mediated by reducing cell adhesion.

Materials and Methods

**Expression Constructs**

Xwnt-4 (Du et al., 1995; McGrew et al., 1992), Xwnt-5A (Moon et al., 1993a), Xwnt-8, Xwnt-8myc (Christian et al., 1991), Xwnt-11 (Du et al., 1995), noggin (generated by PCR from published sequence) (Lamb et al., 1993), and bovine prolactin (a gift of P. Walter, University of California at San Francisco) and β-galactosidase (a gift of D. Turner and R. Rupp, Fred Hutchinson Cancer Research Center, Seattle, WA) cDNAs were used in an SP64T expression vector (Krieg and Melton, 1984) as described previously. BVglI was used in the SP64T3 vector (Thomson and Melton, 1993). Xwnt-8b (Cui et al., 1995), a kinase-dead Xgsk-3 (Pierce and Kimelman, 1995), and Xenopus β-catenin cDNA (the latter generated by Yost, C., M. Torres, J.R. Miller, E. Huang, D. Kimelman, and R.T. Moon [manuscript submitted for publication]) by PCR amplification from the published sequence of McCrea et al. (1991) were subcloned into the CS2 ÷ expression vector (a gift of C. Kintner, Salk Institute, La Jolla, CA) (Detrick et al., 1990) was inserted into the pTTS expression vector (a gift of A. Johnson and P. Krieg, University of Texas, Austin), and we generated the clone ΔN-cadherin as described (Kintner, 1992). All cDNAs were transcribed using Message Machine in vitro transcription kits (Ambion Inc., Austin, TX).

**Embryological Methods and RNA Injections**

Eggs and embryos of Xenopus laevis were manipulated, microinjected, and cultured as previously described (Moon and Christian, 1989). Open-faced dorsal lip explants were dissected from stage 10 embryos (Keller, 1991) that had been injected with RNAs as listed in Table V and allowed to elongate in Daniecki's modified medium (Sater et al., 1993) for 6–8 h until sibling embryos reached stages 13 to 15. For the cell adhesion assays, 1–3 ng of Xwnt-5A, Xwnt-8, N-cadherin, or ΔN-cadherin RNA was injected into the marginal zone of both dorsal blastomeres of a four-cell embryo.

To test the effects of Xwnt-5A class members on signaling by other proteins, the marginal zone of both ventral blastomeres of four-cell embryos was first injected with 1–3 ng of RNAs encoding members of the Xwnt-5A class, wild-type N-cadherin, ΔN-cadherin, prolactin, or β-galactosidase.

**Table 1. Effects of the Wnt-5A Class and Dominant Negative N-cadherin on gsc Inducing and Axis Duplicating Activity by the Wnt-1 Class, β-Catenin, Kinase-dead Xgsk-3, noggin, and BVglI**

| RNAs injected*          | Sites of gsc expression | Axis formation | n | Single | Double | Dorsalized | n |
|-------------------------|-------------------------|---------------|---|--------|--------|------------|---|
| Uninjected              | Single: 100 Double: 0 n: 150 | Single: 100 Double: 0 n: 456 |
| Xwnt-8+prolactin        | Single: 17 Double: 83 n: 109 | Single: 11 Double: 55 n: 345 |
| Xwnt-8+Xwnt-5A          | Single: 100 Double: 53 n: 173 |
| Xwnt-8+Xwnt-4           | Single: 88 Double: 12 n: 59 |
| Xwnt-8+Xwnt-11          | Single: 80 Double: 20 n: 45 |
| Xwnt-8b+prolactin       | Single: 65 Double: 34 n: 76 |
| Xwnt-8b+Xwnt-5A         | Single: 83 Double: 8 n: 64 |
| k.d.Xgsk-3+prolactin    | Single: 27 Double: 45 n: 94 |
| k.d.Xgsk-3+Xwnt-5A      | Single: 38 Double: 51 n: 88 |
| β-catenin(1 ng)+prolactin| Single: 39 Double: 50 n: 38 |
| β-catenin+Xwnt-5A       | Single: 17 Double: 61 n: 18 |
| Xwnt-8+ΔNcad            | Single: 100 Double: 0 n: 49 |
| Xwnt-8+Ncad            | Single: 16 Double: 84 n: 61 |
| β-catenin(0.15 ng)+prolactin| Single: 64 Double: 36 n: 67 |
| β-catenin(0.15 ng)+ΔNcad| Single: 92 Double: 8 n: 66 |
| β-catenin(0.5 ng)+prolactin| Single: 60 Double: 40 n: 68 |
| β-catenin(0.5 ng)+ΔNcad | Single: 64 Double: 36 n: 59 |
| noggin+prolactin        | Single: 18 Double: 82 n: 49 |
| noggin+Xwnt-5A          | Single: 96 Double: 4 n: 48 |
| noggin+ΔNcad            | Single: 100 Double: 0 n: 39 |
| BVglI+prolactin         | Single: 25 Double: 75 n: 55 |
| BVglI+Xwnt-11           | Single: 30 Double: 70 n: 56 |
| BVglI+ΔNcad            | Single: 38 Double: 62 n: 52 |

* RNA encoding prolactin (3 ng); Xwnt-5A, Xwnt-4, or Xwnt-11 (1 ng); wild-type N-cadherin (3 ng) or ΔN-cadherin (3 ng) were injected into the marginal zone of both ventral blastomeres of four-cell embryos. Subsequently, RNAs encoding Xwnt-8 (0.1 ng), Xwnt-8b (0.7 ng), β-catenin (0.15 ng, 0.5 ng, or 1 ng), kinase-dead Xgsk-3 (2 ng), noggin (2.5 ng), or BVglI (0.1 ng) were injected into a single ventral marginal cell of 16-cell embryos. These latter doses were determined by arriving at the lowest threshold dose that elicits an axis duplication in the majority of embryos with that specific RNA.

**Stage 25 embryos were scored by visual examination as having a single embryonic axis, duplicated axes, or a dorsalized phenotype, which occurs in response to high levels of Wnt-1 class signaling.

**Numbers refer to percentages; n, number of embryos.
this was followed by injection of 0.1–2 ng of Xwnt-8, Xwnt8-myc, Xwnt-8β, kinase-dead Xgsk-3, β-catenin, noggin, or BVgl RNAs into a single ventral marginal cell of sixteen-cell embryos. We titrated levels of RNAs encoding Xwnt-1 class members, kinase-dead Xgsk-3, β-catenin, noggin, and BVgl to give comparable embryonic responses such as the induction of secondary embryonic axes. Moreover, we used doses of RNA to give responses in under 100% of embryos to insure that the levels of ectopic expression of these proteins were just sufficient to elicit comparable embryonic responses. Identical procedures were carried out in embryos exposed to UV irradiation for 2.5 min to block the formation of endogenous dorsal structures (Du et al., 1995).

We tested whether the block of the Xwnt-1 class by the Xwnt-5A class required their expression in the same cells by injecting 0.1 ng Xwnt-8 RNA into a single ventral marginal blastomere at the 16-cell stage, followed by 1 ng Xwnt-5A or control prolactin RNA at the 32-cell stage into each blastomere adjacent to the Xwnt-8-injected one. We also investigated whether the effect of Xwnt-5A on the elongation of gastrula open face dorsal lip explants required Xwnt-5A to be overexpressed in all cells by injecting rhodamine dextran (>106 M.) (Molecular Probes, Eugene, OR), mixed with either β-galactosidase RNA as a control or with Xwnt-5A RNA, into a single dorsal cell at the four-cell stage. The entire dorsal lip was then explanted as above and analyzed by fluorescence microscopy using a cooled CCD camera (Hamamatsu Corp., Middlesex, NJ) on a microscope (FXA; Nikon Inc., Garden City, NY), with image analysis using Metamorph 2.0 software (Universal Imaging Corp., West Chester, PA).

To examine the interaction of Xwnt-4 and Xwnt-8 expressed after midblastula transition (MBT), 0.2 ng of Xwnt-4 RNA and 0.1 ng of CSKA-Xwnt-8 DNA (Christian and Moon, 1993) were injected into both dorsal blastomeres of a four-cell-stage embryo.

Cell Adhesion Assays

Dorsal lip explants were excised from stage 10 embryos and dissociated to a single cell suspension in 0.1× Ca2+/Mg2+-free Modified Barth's Solution (MBS) (88 mM NaCl, 1 mM KCl, 2.4 mM NaHCO3, 10 mM Hepes, pH 7.5) for 2 h in 35-mm petri dishes coated with 1% agarose in the same buffer. Cells were subsequently reaggregated at room temperature in MBS with 4 mM Ca2+ and horizontal rocking for 15 and 30 min, followed by fixation with 3.7% formaldehyde in 0.1× MBS. Cell reaggregation was analyzed by photographing random cell populations from each treatment and scoring blind for cell clumping. Cell adhesion is operationally defined in this study as the ability of dissociated cells to reaggregate in the presence of Ca2+. We assayed for decreased cell adhesion by analyzing the relative number of large (greater than eight cells) and small (five to eight cells) cell clumps formed in the reaggregation assays relative to control experiments.

In situ Hybridization and Immunocytochemistry

Stage 10 gastrula embryos were processed for in situ hybridization as described (Harland, 1991) using probes for goosecoid (gsc) (Cho et al., 1991), Xbra (Smith et al., 1991), and F-spondin (a gift from A. Klar and T. Jessel, Columbia University, New York). Stage 10 gastrula embryos injected with Xwnt-8-myc RNA and stage 25 tailbud embryos injected with Xwnt-8 RNA and CSKA-Xwnt-8 DNA were processed for whole mount immunostaining to detect the myc and the Tor 70 epitopes, respectively (Christian and Moon, 1993). Whole mount X-gal staining was performed as described in Sanes et al. (1986).

Analysis of RNA by Reverse Transcription (RT) PCR

RNA was extracted from stage 10 gastrula embryos as described in Chomczynski and Sacchi (1987). 5 μg RNA was used to generate cDNA with first strand synthesis kits (Life Sciences, Inc., St. Petersburg, FL). To control for DNA contamination, we omitted reverse transcriptase from the synthesis reactions. RT-PCR was used as described to detect gsc, EFlα (Hemmati-Brivanlou et al., 1994), and histone 4 transcripts. Control EFlα and histone 4 cDNAs were amplified 20 cycles, and exponential amplification was verified by taking an aliquot of the PCR amplification reaction every third cycle (data not shown), gsc was amplified 24 cycles under conditions that generally yield exponential amplification (Hemmati-Brivanlou et al., 1994; unpublished observations). The RT-PCR data presented are qualitative, and any nonexponential amplification would result in reducing any reported differences between samples. All RT-PCR experiments were reproducible in completely independent experiments.

Results

The Wnt-5A Class Antagonizes Embryonic Responses to the Wnt-1 Class

We first injected RNAs encoding members of both classes

| Table II. Ectopic Xwnt-5A Antagonizes Ectopic Wnt-1 Class Activity after Injections into Distinct Cells
| --- |
| RNAs injected* | Single axis | Double axis | Dorsalized |
| --- | --- | --- | --- |
| Uninjected | 99 | 1 | 0 | 205 |
| Xwnt-8 | 5 | 74 | 21 | 170 |
| Xwnt-8 + prolactin | 2 | 91 | 7 | 58 |
| Xwnt-8 + Xwnt-5A | 50 | 47 | 3 | 90 |

*Single blastomeres at the ventral marginal zone of 16-cell-stage embryos were injected with 0.1 ng of Xwnt-8 mRNA, followed by 1 ng of prolactin or Xwnt-5A mRNA into each of the surrounding ventral marginal blastomeres at the 32-cell stage.

Numbers refer to percentages; n, number of embryos.
of Wnts to determine the effect of coexpression in *Xenopus* embryos. As a control, prolactin RNA was injected into the marginal zone of the two ventral blastomeres at the four-cell stage, followed by injection of RNA encoding Xwnt-8 or Xwnt-8b into a single ventral marginal blastomere at the 16-cell stage. As expected, this resulted in the induction of a secondary axis (Fig. 1 B and Table I), since members of the Wnt-1 class mimic the Nieuwkoop center activity (Cui et al., 1995; Smith and Harland, 1991; Sokol et al., 1991). When RNAs encoding Xwnt-5A, -4, or -11 (members of the Wnt-5A class) (Du et al., 1995) were injected instead of the prolactin RNA, induction of a secondary axis by subsequent injection of Xwnt-8 RNA was blocked in most embryos (Fig. 1 C and Table I). These results suggest that the Wnt-5A class antagonizes the ability of the Wnt-1 class to induce a secondary axis in *Xenopus* embryos.

Since ectopic sites of gsc expression are induced by the Wnt-1 class (Christian and Moon, 1993), we next asked whether this response to Wnt-1 class signaling was also blocked by the Wnt-5A class. Whole mount in situ hybridization analysis showed that members of the Wnt-5A class greatly reduced the incidence of ectopic induction of gsc expression in the ventral marginal zone (Fig. 1 F and Table I), whereas injection of prolactin RNA did not interfere with this activity of the Wnt-1 class (Fig. 1 E and Table I). Similarly, the Wnt-5A class inhibited the induction of gsc by the Wnt-1 class in UV-irradiated embryos lacking endogenous gsc expression, as monitored by RT-PCR (Fig. 2 A). Fig. 2, B and C, is described in subsequent sections and addresses questions related to how Xwnt-5A might reduce gsc expression and whether Xwnts can affect gsc expression induced by BVgl.

Additional control experiments support the specificity of the above results. We insured that the Wnt-5A class RNAs injected at the four-cell stage were not competing for translation with Xwnt-8 by injecting RNA encoding Xwnt-11 or prolactin at the four-cell stage, followed by RNA encoding Xwnt-8 with a c-myc epitope tag at the 16-cell stage. After fixing embryos at the gastrula stage and processing them for whole mount immunostaining for the c-myc epitope, we found that overexpression of neither prolactin nor Xwnt-11 had an appreciable effect on Xwnt-8-myc expression (data not shown). To control for mosaic expression of exogenous RNAs, we coinjected Xwnt-5A and β-galactosidase RNAs at the four-cell stage, followed by Xwnt-8-myc at the 16-cell stage. We found that the expression of the proteins encoded by the injected RNAs overlap in 99% of the embryos, as determined by Xgal and anti-myc antibody staining (data not shown). Finally, we coinjected Xwnt-5A and Xwnt-8 RNA into the marginal zone of a single ventral blastomere at the four-cell stage to test whether expression at the same time, as opposed to the ~40-min delay in the above experiments, would still block the Xwnt-8 induction of gsc. We found that at a 1:1 or 10:1 RNA ratio, Xwnt-5A was unable to antagonize the Xwnt-8-mediated induction of ectopic gsc, as scored by in situ hybridization (data not shown). This suggests that for Xwnt-5A to antagonize induction of gsc in response to Xwnt-8, it may need to be expressed before the Xwnt-8 signal, although in assays described below, coinjection of both Xwnt-5A and Xwnt-8 yields an Xwnt-5A-like response.

**Figure 3.** Xwnt-4 antagonizes the activity of Xwnt-8 after MBT. The ability of CSKA-Xwnt-8 to interfere with notochord formation (Christian and Moon, 1993) was scored in stage 25 embryos by anti-Tor 70 whole mount immunocytochemistry to detect the notochord and by F-spondin in situ hybridization to detect the floorplate. (A) Uninjected embryos possess a normal notochord and floorplate. (B) Transcription of Xwnt-8 from the cytoskeletal actin promoter vector after MBT leads to loss of notochord and floorplate staining. (C) Xwnt-4 interferes with the Xwnt-8 activity, restoring the formation of the notochord and the floorplate. (Arrowheads) F-spondin floorplate staining, (Straight arrows) Tor 70 notochord staining, (Curved arrows) anterior limit of the notochord.

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Xwnt-5A Does Not Antagonize Embryonic Responses to β-Catenin or Kinase-dead Xgsk-3

We next investigated whether Xwnt-5A could antagonize the activities of Xgsk-3 and β-catenin, both of which are cytoplasmic elements of the Wnt-1 class pathway (Dominguez et al., 1995; He et al., 1995; Pierce and Kimelman, 1995; Heasman et al., 1994). Ectopic Xwnt-5A did not prevent axis duplication or ectopic gsc induction mediated by injection of RNA encoding β-catenin or a kinase-dead Xgsk-3 (Table I). The kinase-dead version of Xgsk-3 was employed since it mimics the Wnt-1 class response in embryos (Pierce and Kimelman, 1995).

Xwnt-5A Antagonizes Embryonic Responses to Xwnt-8 Even after Ectopic Expression in Distinct Cell Populations

We then tested whether the ability of Xwnt-5A to antagonize embryonic responses to the Wnt-1 class required that both functional classes of Wnts be expressed in the same cells. Xwnt-8 RNA was injected into a single ventral marginal blastomere at the 16-cell stage, followed by the injection at the 32-cell stage of RNAs encoding prolactin, as a control, or Xwnt-5A. The prolactin and Xwnt-5A RNAs were specifically targeted to all ventral marginal blastomeres adjacent to the Xwnt-8-injected blastomere, thus insuring that the two functional classes of Wnts would be expressed in adjacent yet different cell populations (confirmed by lineage tracing experiments, not shown). We found that Xwnt-5A secreted from neighboring cells reduced axis duplication by ectopic Xwnt-8, while prolactin had no effect (Table II). These data demonstrate that the Xwnt-5A antagonism of embryonic responses to the Wnt-1 class can occur even if the two functional classes are expressed in distinct cells.

A Member of the Wnt-5A Class Is Able to Antagonize the Effects of Xwnt-8 Expressed after Midblastula Transition

We also examined the ability of a member of the Wnt-5A class, Xwnt-4 (Du et al., 1995), to block the effects of overexpressing Xwnt-8 later in development. In this experiment, expression of Xwnt-8 was driven by a cytoskeletal actin promoter, which leads to overexpression of Xwnt-8 after MBT when endogenous Xwnt-8 is expressed in ventral-lateral regions (Christian and Moon, 1993). Overexpression of Xwnt-8 in the dorsal marginal zone at this time in development diverts the fate of presumptive notochord cells to more lateral fates, as scored histologically by lineage tracing of Xwnt-8-expressing cells, and by the reduction in staining with the notochord-specific Tor 70 antibody (Christian and Moon, 1993). We hypothesized that overexpression of members of the Wnt-5A class, such as Xwnt-4 that is normally expressed in the floor plate (McGrew et al., 1992), might interfere with the ability of Xwnt-8 to divert the developing notochord to more ventral-lateral cell fates.

Consistent with data reported in Christian and Moon (1993), overexpression of CSKA-Xwnt-8 in both dorsal cells of four-cell stage embryos reduced the formation of the notochord, as scored by staining with the Tor 70 antibody (Fig. 3 B and Table III) relative to control staining (Fig. 3 A). As loss of the notochord would be expected to preclude formation of the floor plate, an in situ hybridization marker for the floorplate, F-spondin (Klar et al., 1992) was also used. We observed a reduction of F-spondin expression in response to CSKA-Xwnt-8 (Fig. 3 B) relative to controls (Fig. 3 A). In contrast, coinjection of Xwnt-4 RNA with the CSKA-Xwnt-8 DNA resulted in normal formation of the notochord and floor plate in a greater percentage of embryos (Fig. 3 C and Table III). Coinjection of Xwnt-4 RNA with CSKA-Xwnt-8 DNA increased the number of embryos staining normally with Tor 70 and F-spondin approximately threefold relative to embryos injected with CSKA-Xwnt-8 DNA alone (Table III). Injection of Xwnt-4 RNA alone did not prevent the formation of the notochord (data not shown) (Ungar et al., 1995). These data demonstrate that the effects of ectopic expression of Xwnt-8 after MBT, diverting the fate of the notochord to a more ventral-lateral fate, can be antagonized by a member of the Wnt-5A class. These data support the hypothesis that the Wnt-5A class reduces embryonic responses to the Wnt-1 class throughout embryonic development, although we have only tested one member of the Xwnt-5A class for its ability to block the activities of CSKA-Xwnt-8 DNA.

Xwnt-5A Decreases Ca2+-dependent Cell Adhesion and Inhibits Morphogenetic Movements In Vitro

Overexpression of Xwnt-5A leads to the inhibition of cell mixing and perturbation of morphogenetic movements in...
Table IV. Cell Aggregation in Calcium-dependent Cell Adhesion Assays

| RNAs injected* | Five to eight cell clumps | Greater than eight cell clumps | n  |
|----------------|---------------------------|-------------------------------|----|
| Pre-Ca²⁺       | 89                        | 11                            | 19 |
| Control        | 42                        | 58                            | 209|
| Xwnt-5A        | 99                        | 1                             | 78 |
| Xwnt-8         | 70                        | 30                            | 71 |
| Xwnt-5A+Xwnt-8 | 98                        | 2                             | 81 |
| Xwnt-5A+N-cadherin | 42                  | 58                            | 106|
| ΔN-cadherin    | 94                        | 6                             | 34 |

*Both dorsal blastomeres of four-cell embryos were injected with a total of 1 ng of RNAs encoding Xwnt-5A or Xwnt-8, or 3 ng of RNAs encoding wild-type N-cadherin and ΔN-cadherin. In experiments with mixed RNAs, 1 ng of each Wnt, or 1 ng of a Wnt with 3 ng of a cadherin were injected. Numbers refer to percentages of clumps of the indicated sizes; n, number of cell clumps counted in five separate samples of each cell adhesion assay.

Figure 5. The inhibition of elongation of the gastrula organizer explants by Xwnt-5A does not require Xwnt-5A to be expressed in all cells of the explant. Embryos were injected into the marginal zone of one dorsal cell at the four-cell stage with either β-galactosidase RNA mixed with rhodamine dextran (A), or with Xwnt-5A RNA mixed with rhodamine dextran (B). Open face explants of the entire dorsal marginal zone were prepared at stage 10 and visualized by fluorescence microscopy after control embryos had developed to stage 13-15. Control explants (A) elongate extensively (6 of 8 explants), while explants expressing Xwnt-5A (B) display no convergence and extension movements (10 of 15 explants), or reduced elongation (3 of 15 explants). In both panels, pseudocolor imaging reveals that the injected dextran and RNAs were restricted to the yellow-red cells.

As the movements of gastrulation have been reported to involve alterations in cadherin-mediated cell adhesion (Briehler and Gumbiner, 1994), we next studied convergence and extension movements in dorsal open face explants after overexpression of various Wnts. Whereas explants from uninjected (Fig. 4 A) and Xwnt-8–injected embryos (Fig. 4 C) elongated normally, overexpression of members of the Wnt-5A class (Fig. 4 B), or a 1:1 ratio of mixed Xwnt-5A and Xwnt-8 RNAs (Fig. 4 D), resulted in the inhibition of explant elongation (Table V). Thus, experimental treatments that resulted in the inability to form large cell aggregates in a reaggregation assay (Fig. 4, F and H; Table IV) also resulted in the inhibition of elongation of dorsal open face explants (Fig. 4, B and D; Table V). Explants derived from embryos injected with N-cadherin or ΔN-cadherin also exhibited blocked elongation (Table V), suggesting that convergence and extension movements are sensitive to either increases or decreases in cell adhesion. Collectively, the reaggregation assay and the open face explant elongation data support the hypothesis that the Wnt-5A class signal decreases Ca²⁺-dependent cell adhesion, thereby affecting morphogenetic movements in embryos. Interestingly, the ability of Xwnt-5A to interfere with convergence and extension movements also occurs when only half of the gastrula explant expresses Xwnt-5A (Fig. 5 B vs control in 5 A). Also, the explants expressing Xwnt-5A often display cells dissociating from the edges of the explant (Fig. 5 B vs control in 5 A), consistent with Xwnt-5A acting to decrease cell adhesion.

Overexpression of Dominant Negative N-cadherin Mimics the Activity of the Wnt-5A Class in Inhibiting Responses to the Wnt-1 Class

As overexpression of Xwnt-5A RNA results in decreasing Ca²⁺-dependent cell adhesion, as well as reducing the induction of gsc and ectopic axes in response to the Wnt-1
class, we hypothesized that a decrease in cadherin-dependent cell adhesion may be sufficient to account for this Wnt-5A class activity. We tested this hypothesis by injecting four-cell embryos with RNAs encoding prolactin as a control (Fig. 6 B) or dominant negative N-cadherin (ΔN-cadherin) (Fig. 6 C) (Kintner, 1992), followed by the injection of Xwnt-8 RNA into a single ventral marginal blastomere at the 16-cell stage. As determined by in situ hybridization, ΔN-cadherin (Fig. 6 C) but not prolactin (Fig. 6 B) blocked the induction of ectopic gsc by Xwnt-8 (Table I). ΔN-cadherin did not alter the expression of a pan-mesodermal gene, Xbra (data not shown). ΔN-cadherin also prevented Xwnt-8 (Fig. 2 B) and Xwnt-8b (data not shown) from rescuing gsc expression in embryos ventralized by UV irradiation, further supporting the hypothesis that one mechanism by which the Wnt-5A class antagonizes the activity of the Wnt-1 class may involve decreases in cell adhesion. Again, the effects of ΔN-cadherin were not an artifact of inhibiting the translation of Xwnt-8, as c-myc epitope-tagged Xwnt-8 was detected by anti-c-myc immunostaining in the presence of ΔN-cadherin (data not shown). Importantly, the overexpression of full-length N-cadherin did not an-

Figure 6. ΔN-cadherin but not N-cadherin inhibits the induction of ectopic gsc expression by Xwnt-8 as assayed by in situ hybridization for gsc in stage 10 gastrula embryos. (A) Uninjected control embryos possess a single site of gsc expression. (B) Embryos injected with prolactin followed by Xwnt-8 RNA possess two sites of gsc expression. (C) Embryos injected with ΔN-cadherin followed by Xwnt-8 RNA possess a single site of gsc expression. (D) Embryos injected with N-cadherin followed by Xwnt-8 RNA possess two sites of gsc expression. (Arrows) Representative sites of gsc expression.
agonize ectopic expression of gsc or axis duplication in response to subsequent injection of Xwnt-8 RNA (Figs. 2 B, lane 5, and 6 D; Table I), indicating that neither changes in cell adhesion, per se, nor increasing the availability of β-catenin binding sites (identical in N-cadherin and ΔN-cadherin) are sufficient to antagonize ectopic gsc induction.

To further rule out β-catenin sequestration as a mechanism by which ΔN-cadherin inhibits responses to the Wnt-1 class signal, we attempted to block ectopic induction of gsc resulting from injection of β-catenin RNA at the 32-cell stage, by prior injection of RNA encoding ΔN-cadherin at the four-cell stage. When low doses of β-catenin RNA (0.15 ng) are injected at the 32-cell stage, prior injection of ΔN-cadherin RNA at the four-cell stage prevents ectopic gsc induction, possibly by sequestering β-catenin polypeptides (Table I). However, ΔN-cadherin RNA is unable to inhibit the gsc-inducing activity of β-catenin at higher doses of β-catenin RNA (0.5 ng) (Table I), even though the dose of β-catenin used in these experiments is below the level required to induce ectopic sites of gsc expression in all injected embryos. As ΔN-cadherin is able to antagonize the activity of members of the Wnt-1 class at doses that induce ectopic gsc expression or axis duplication in nearly all of the embryos when coinjected with control RNAs, but is unable to antagonize functionally equivalent

Figure 7. Members of the Wnt-5A class and ΔN-cadherin do not block the induction of ectopic gsc expression by BVgI, as assayed by in situ hybridization for gsc in stage 10 gastrula embryos. (A) Uninjected control embryos possess a single site of gsc expression. (B) Embryos injected with prolactin followed by BVgI RNA possess two sites of gsc expression. (C) Embryos injected with Xwnt-11 or ΔN-cadherin (D) RNA followed by BVgI RNA also possess multiple sites of gsc expression. (Arrows) Sites of gsc expression.
Members of the Wnt-SA Class and AN-Cadherin

Antagonism Between Functional Classes of Wnts

Noggin but Not BVg1

expression of However, Holt et al. (1994) have demonstrated that over-expression of N-cadherin is not as effective as other cadherins at rescuing the effects of ΔN-cadherin in Xenopus embryos. Thus, it is possible that the N-cadherin in our rescue experiments did not sufficiently reverse the Xwnt-5A-mediated decrease in cell adhesion.

Members of the Wnt-5A Class and ΔN-Cadherin

Inhibit Ectopic Induction of gsc and Embryonic Axes by Noggin but Not BVg1

We next asked whether the ability of the Wnt-5A class and ΔN-cadherin to inhibit embryonic responses to Wnt-1 class signals is Wnt specific, or whether they may also interfere with the dorsalizing activities of other secreted factors. We first tested the ability of Xwnt-5A and ΔN-cadherin to antagonize the reported abilities of ectopic noggin (for review see Lamb et al., 1993) to induce expression of gsc in the ventral marginal zone. Injection of RNAs encoding either Xwnt-5A or ΔN-cadherin blocked the ectopic induction of gsc expression by noggin in both untreated and UV-irradiated embryos, as assayed by gsc in situ hybridization (Table 1) and by RT-PCR (data not shown), respectively.

We also asked whether a member of the Wnt-5A class or ΔN-cadherin would interfere with the induction of gsc by BVg1 (Thomsen and Melton, 1993). In parallel experiments, neither Xwnt-11 (Fig. 7 C) (Ku and Melton, 1993), a member of the Wnt-5A class whose expression pattern overlaps with that of BVg1 (for review see Kessler and Melton, 1995), nor ΔN-cadherin (Fig. 7 D) was able to block induction of gsc or ectopic axes by BVg1 (Table 1). Moreover, neither Xwnt-11 nor ΔN-cadherin blocked the ability of BVg1 to induce gsc expression in UV-irradiated embryos (Fig. 2 C, lanes 4 and 5). Therefore, it appears that embryonic responses to ectopic BVg1 are relatively independent of cadherin-dependent cell adhesion, making an important distinction from the cadherin-dependent induction of gsc by ectopic Wnt-1 class and noggin signals.

Discussion

Antagonism Between Functional Classes of Wnts

Wnts are secreted signaling proteins that can be grouped by functional assays into at least two distinct classes (Wong et al., 1994; Du et al., 1995). We report here that members of the Wnt-5A class antagonize the responses of Xenopus laevis embryos to ectopic expression of members of the Wnt-1 class. Specifically, ectopic expression of the Wnt-1 class in cleavage stage embryos leads to induction of gsc and to the subsequent duplication of the embryonic axes. Both responses are blocked by prior expression of members of the Wnt-5A class. In addition, we observed that ectopic expression of Xwnt-4 (a member of the Wnt-5A class) antagonizes the ability of Xwnt-8 expressed after MBT to divert the differentiation of prospective notochord cells to a more lateral fate (Christian and Moon, 1993). Thus, members of the Wnt-5A class interfere with embryonic responses to ectopic expression of the Wnt-1 class before and after MBT.

We speculate that this antagonism requires secretion of the Wnts, as Xwnt-5A is able to antagonize Wnt-1 class signals without needing to be expressed in the same cells. Interestingly, members of the Wnt-5A class were unable to inhibit gsc induction or axis duplication mediated by a cytoplasmic component of the Wnt-1 class signaling pathway, β-catenin (Heasman et al., 1994; Guger and Gumbiner, 1995), or by cytoplasmic kinase-dead Xgsk-3 (He et al., 1995; Pierce and Kimelman, 1995), a dominant negative protein that mimics activation of this pathway. There are several possible interpretations of this result, one of which is that both functional classes of Wnts impinge on the same signaling pathway, but the antagonism between the two classes occurs upstream of Xgsk-3 and β-catenin. However, it remains possible that the Wnt-5A class activates a distinct signaling pathway that indirectly, perhaps through decreasing cell adhesion (see below), antagonizes the Wnt-1 class.

These data have several implications for Wnt signaling in embryos. While embryos generally express Wnts in unique patterns (for review see McMahon, 1992; Nusse and Varmus, 1992; Moon et al., 1993b), there are areas of overlap between different Wnts and different functional classes of Wnts. First, there is some overlap between the patterns of expression of Wnt-3A (a member of the Wnt-1 class) and Wnt-5A in the late streak–stage egg cylinder of the mouse (Takada et al., 1994). Second, the Xenopus homologs of both of these genes overlap in expression in the posterior regions of the Xenopus neurula and tailbud embryos (Wolda et al., 1993; Moon et al., 1993). Third, Xwnt-1 and Xwnt-3A (Wolda et al., 1993) overlap in expression with Xwnt-4 in the dorsal midline of the mesencephalon until the tail bud stage (McGrew et al., 1992). Moreover, embryonic cells may receive signals from both classes of Wnts since Wnts are secreted and can act in a paracrine manner (for review see Nusse and Varmus, 1992), and our data on gastrula explants demonstrate that cell behavior can be affected even though not all cells are expressing the ectopic Xwnt.

Our data suggest that cells receiving signals from both the Wnt-1 class and the Wnt-5A class may respond in a class-specific manner, although this would likely be modulated by the relative levels of the prospective ligands as well as their relative timing of expression. A class-specific effect in the embryo may help define functional domains and spatial borders within embryonic structures, which is in keeping with observed polarized expression of the two classes in some structures. For example, the anterior vertebrate neural tube expresses Wnt-1 and Wnt-3A (members of the Wnt-1 class) in the dorsal midline, while the floorplate expresses Wnt-4 (a member of the Wnt-5A class) (Parr et al., 1993; McGrew et al., 1994; Moon et al., 1993b; Unger and Moon, 1995). Similarly, the dorsal otic vesicle expresses Xwnt-3A, while the ventral otic vesicle expresses Xwnt-4 (for review see Moon, 1993).

However, our data do not support the dominant effect of the Wnt-5A class over the Wnt-1 class in all circumstances, as sequential expression of Xwnt-5A then Xwnt-8...
blocks gsc induction by Xwnt-8, but coexpression does not.
Similarly, it is worth noting that in the developing chick limb bud, ectopic Wnt-7A (which belongs to the Wnt-1 class) (Wong et al., 1994) is capable of inducing the Lim homeobox gene Lmx1 (Riddle et al., 1995) in a manner that may be dependent on the presence of Wnt-5A (Dealy et al., 1993), raising the question of synergy rather than antagonism. Finally, Xwnt-5C, tentatively a member of the Wnt-5A class, acts indistinguishably from Xwnt-1 in inducing engrailed-1 (Koster et al., 1996), despite the Wnt-1 and Wnt-5A classes having no similar activities in other assays (Du et al., 1995). Despite such questions, which arise due to the fact that the functional classes of Wnts are only now being recognized, the current data suggest that studies of Wnts should take into account that some Wnts may have indistinguishable activities, whereas Wnts belonging to another functional class may modulate or oppose that activity. This is of some interest in considering Wnts in human breast cancer (e.g., Huguet et al., 1994, 1995).

**Wnts and Cell Adhesion**

As overexpression of members of the Wnt-5A class leads to the inhibition of morphogenetic movements during gastrulation (Moon et al., 1993a), we investigated whether this effect may be due to changes in cell adhesion. We found that Xwnt-5A decreased Ca^{2+}-dependent cell adhesion in a manner similar to ΔN-cadherin, which lacks its extracellular domain and inhibits cadherin-mediated cell adhesion (Kintner, 1992). A possible mechanism by which members of the Wnt-5A class might decrease cadherin-mediated cell adhesion is via stimulation of an src-like tyrosine kinase, since src-mediated tyrosine phosphorylation of cadherin-associated β-catenin can result in decreased cadherin-dependent cell adhesion (Behrens et al., 1993; Hamaguchi et al., 1993; Matsuyoshi et al., 1992). In support of this hypothesis, we have observed subtle increases in tyrosine phosphorylation of β-catenin in response to the injection of RNA encoding Xwnt-5A but not Xwnt-8 (data not shown).

Although cultured cells overexpressing Wnt-1 accumulate β-catenin and exhibit increased cadherin-dependent cell adhesion (Bradley et al., 1993; Hinck et al., 1994), we did not observe increases in Ca^{2+}-dependent cell reaggregation of dissociated dorsal lip explant cells overexpressing Xwnt-8, a member of the Wnt-1 functional class (Du et al., 1995). Nevertheless, our data reproducibly demonstrate that the Wnt-5A class, which can antagonize the Wnt-1 class, decreases cell adhesion.

**Antagonism of Wnt-1 Class and Noggin Activity by the Wnt-5A Class Is Mimicked by Decreasing Cadherin-mediated Cell Adhesion**

Since overexpression of Xwnt-5A inhibits cadherin-dependent cell adhesion, we asked whether decreased cell adhesion alone might be capable of antagonizing the induction of ectopic gsc and axis duplication by the Wnt-1 class, noggin, or BVgl. Our results show that a dominant negative cadherin, ΔN-cadherin, was able to act like the Wnt-5A class and thus antagonize the induction of gsc and axis duplication by members of the Wnt-1 class as well as by noggin, but not by BVgl. In contrast, overexpression of N-cadherin had no effect on Wnt-1 class activity in similar assays. The fact that overexpression of ΔN-cadherin, but not N-cadherin, mimics the Wnt-5A block of embryonic responses to the Wnt-1 class and noggin further suggests that decreased cadherin-mediated cell adhesion is involved in the embryonic responses to the Wnt-5A class. Evidence that factors that regulate cell adhesion may be important during embryogenesis is supported by data showing that the maintenance of cell adhesion in Xenopus embryos is required for proper expression of MyoD (Holt et al., 1994).

How might decreased cell adhesion in response to either ΔN-cadherin or the Wnt-5A class interfere with cellular responses to several putative signaling factors? With respect to the effects of ΔN-cadherin, at least two mechanisms need to be considered. The first mechanism could involve the artifactual sequestration of β-catenin, which is required for Wnt signaling (Heasman et al., 1994), simply through the overexpression of a cadherin domain that contains available β-catenin binding sites. Thus, sequestering β-catenin on the cytoplasmic domains of ectopic cadherins could conceivably interfere with its signaling role. Our data are inconsistent with this β-catenin sequestration model because ΔN-cadherin can block Wnt-1 class signals at doses of Xwnt-8 RNA that induce ectopic gsc expression or axis duplication in nearly all injected embryos, but the ΔN-cadherin is unable to block the activity of functionally equivalent doses of ectopic β-catenin. Furthermore, ectopic N-cadherin, which possesses identical intracellular catenin binding domains as ΔN-cadherin (Kintner, 1992), did not affect embryonic responses to the Wnt-1 class.

The second mechanism addressing the ability of ΔN-cadherin and Wnt-5A class members to antagonize embryonic responses to Wnt-1 class members involves decreasing cadherin-dependent cell adhesion. It is conceivable that the Wnt-5A class leads to decreased cell adhesion, which interferes with the responses of embryos to a Wnt-1 class ligand independently of whether the complete Wnt-1 signaling pathway has been activated. If this were the case, one would predict that the Wnt-5A class would be able to prevent induction of secondary axes in response to a cytoplasmic component of the Wnt-1 signaling pathway, β-catenin, or a cytoplasmic activator of this pathway, a kinase-dead gsk-3. Importantly, we observed that the duplication of the embryonic axis in response to ectopic expression of these downstream elements was not blocked. A working hypothesis based on these observations is that a threshold level of cell adhesion is required for, and perhaps involved in, receptor-mediated signal transduction by the Wnt-1 class, but once intracellular components of the Wnt signal transduction pathway have been activated (or bypassed by injection of RNAs), cellular and embryonic responses are less sensitive to decreases in cell adhesion in response to the Wnt-5A class.

What then of the observation that members of the Wnt-5A class and ΔN-cadherin block embryonic responses to noggin but not to BVgl? Is it possible that the Wnt-1 class and noggin can operate in a shared pathway, which would explain how interference with this pathway blocks responses to both types of signaling molecules. Alternatively, noggin may act in an independent pathway which is itself sensitive...
at some level to decreases in cell adhesion. The observation that responses to Bvgl are not antagonized by the Wnt-5A class supports the hypothesis that Bvgl activates a distinct pathway. The observation that ΔN-cadherin does not interfere with embryonic responses to Bvgl suggests that the pathway stimulated by this factor is less sensitive than the Wnt-1 class pathway to decreases in cell adhesion.

Additional experimentation will be required to rigorously determine how the Wnt-5A class and ΔN-cadherin block embryonic responses to the Wnt-1 class and to noggin. However, previous studies and recent work suggest further consideration of the phosphatidylinositol (PI) cycle as a target for Wnt action by both functional classes. Previously it has been shown that lithium, a potent inhibitor of PI turnover, yields a phenotype indistinguishable from that obtained from the overexpression of Xwnt-1 class RNAs (for review see Christian and Moon, 1993). However, recent work suggests that the Wnt-5A activities may be a consequence of activating, not inhibiting, the PI cycle. First, stimulating the PI cycle and calcium release during early Xenopus development, by activation of ectopic serotonin receptors, leads to a phenotype similar to that of the Wnt-5A class (Ault, K.T., G. D Turnowicz, A. Gionale, P.L. Harger, and W.B. Buse, manuscript submitted for publication). Second, expression of Xwnt-5A leads to increases in the release of calcium from intracellular stores (Yang-Snyder, J., D.C. Slusarski, R.T. Moon, and W.B. Buse, manuscript submitted for publication), as also reported for the ectopic serotonin receptor (Ault, K.T., G. D Turnowicz, A. Gionale, P.L. Harger, and W.B. Buse, manuscript submitted for publication). Third, activation of the PI cycle by ectopic expression of serotonin receptors antagonizes the induction of gsc and secondary axes by Xwnt-8 (Yang-Snyder, J., D.C. Slusarski, R.T. Moon, and W.B. Buse, manuscript submitted for publication), indistinguishable from the antagonism of Xwnt-8 by Xwnt-5A in the present study. Therefore, we hypothesize that the Wnt-5A class antagonism of the Wnt-1 class may occur at the level of the PI cycle, or through the PI cycle, modulating cell adhesion.

In conclusion, we have demonstrated that members of the Wnt-5A class decrease cadherin-dependent cell adhesion and antagonize the embryonic responses to Wnt-1 class members expressed ectopically before and after midblastula transition. These activities of the Wnt-5A class are mimicked by a dominant negative cadherin that decreases cell adhesion. Therefore, Wnt-5A class antagonism of the Wnt-1 class may be mediated in part by decreasing cell adhesion below a threshold level necessary for embryonic responses to signaling by the Wnt-1 class.

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