somes to the membrane (*5, 6, 16*). To determine whether prevention of the SRP recycling reaction would interfere with subsequent translocation cycles, we first incubated K-RM with the guanine ribonucleotides and saturating quantities of the SRP relative to the membrane content of the SRP receptor. The treated membranes were then tested for activity in a nascent polypeptide insertion assay (*5*). Cosedimentation of a portion of the nascent preprolactin polypeptide with the membranes indicated that the treated membranes remained competent for ribosome targeting and nascent chain insertion (Fig. 4, A and C). Prior incubation of K-RM with Gpp(NH)p and the SRP reduced the amount of nascent preprolactin associated with membranes (Fig. 4B). Prior incubation of K-RM with Gpp(NH)p in the absence of the SRP did not inhibit subsequent insertion of nascent preprolactin in the presence of GTP (*14*).

The GTP hydrolysis cycle of the SRP–SRP receptor complex may function in regulating protein translocation across the endoplasmic reticulum. The 54-kD subunit of the SRP (SRP54) can be cross-linked to nascent signal sequences (*17, 18*) and has been shown to contain the signal sequence recognition domain of the SRP (*19*). Upon binding ribonucleotide, GTP binding proteins display an increased affinity for a downstream effector protein (*20, 21*). We propose that a GTP-induced increase in association between SRP and its receptor is directly coupled to release of the nascent polypeptide from the signal sequence binding site of SRP54. Displacement of SRP54 allows insertion of the signal sequence into a translocation competent site in the RER membrane (*22–26*). The translocation site contains a 35- to 39-kD glycoprotein that has been termed the signal sequence receptor or mp39 (*24–26*). Vectorial insertion of the nascent chain into the RER would be ensured by the inherent delay in GTP hydrolysis which is a characteristic of GTP binding proteins, so that SRP54 is unable to rebind the signal sequence prior to membrane insertion of the polypeptide. The subsequent GTP hydrolysis reaction would convert the receptor to a GDP-bound form with a reduced affinity for SRP, thereby allowing the return of SRP to the cytoplasm for participation in subsequent cycles of ribosome targeting (*27*).

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Effect of Wnt-1 and Related Proteins on Gap Junctional Communication in *Xenopus* Embryos

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The proto-oncogene *wnt*-1 (previously referred to as *int*-1) is thought to be important in embryonic pattern formation although its mechanisms of action are unknown. Premature and increased expression of the Wnt-1 protein, achieved by injection of synthetic *wnt*-1 RNA into fertilized *Xenopus* eggs, enhanced gap junctional communication between ventral cells of the developing embryo. This result is consistent with the hypothesis that Wnt proteins activate a receptor-mediated signal transduction pathway and that gap junctional communication can be a target of this pathway. The effects of two Wnt-1–related proteins on gap junctional communication were also investigated: overexpression of Xwnt-8 increased gap junctional coupling in a manner similar to Wnt-1, whereas Xwnt-5A did not. These findings are consistent with the existence of multiple receptors for Wnt proteins.

THE PROTO-ONCOGENE *INT*-1 encodes a 44-kD product that associates with the cell surface or extracellular matrix (*1*) after its secretion. Isolation of genes related to *int*-1 led to the reclassification of these genes as members of the *wnt* family (*2*). On the basis of its transient and spatially restricted expression in the neural tube of mouse embryos (*3*) and its homology with the *Drosophila* segment polarity gene *wingless* (*4*), it has been proposed that *wnt*-1 (*int*-1) is important in pattern formation in vertebrate embryos (*2, 5, 6*). This view is supported by the demonstration that microinjection of synthetic *wnt*-1 RNA into fertilized *Xenopus* eggs leads to overexpression of Wnt-1 and a bifurcation of the embryonic axis (*6*) and by the observation that deletion of this gene in mouse by homologous recombination results in embryos that lack the midbrain and some parts of the rostral metencephalon (*7*).

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We have attempted to elucidate potential cellular mechanisms by which the products of the *wnt* gene family may affect embryonic development. Microinjection into fertilized *Xenopus* eggs of synthetic *wnt*-1 and *Xwnt*-8 RNA, but not *Xwnt*-5A RNA, resulted in increased gap junctional coupling between blastomeres in the ventral region of 32-cell embryos. Gap junctional communication has been implicated in pattern formation (*8*) and is thought to be modulated by receptor-mediated signaling pathways (*9*). Therefore, our data are consistent with the hypothesis that premature and increased expression of Wnt-1 and Xwnt-8 leads to activation of receptor-mediated signal transduction pathways, which have the potential for modulating gap junctional communication and thus influencing pattern formation. The inability of Xwnt-5A to affect gap junctional communication suggests that it may act at a distinct receptor.

Gap junctional communication in *Xenopus* embryos has been measured by microinjection of Lucifer yellow (*10*). With the use of this dye, and fluorescein isothiocyanate (FITC)–conjugated dextran as a negative

control, it has been established that there is a strong dorsal-ventral polarity in gap junctional communication in the 32-cell embryo; that is, blastomeres in the dorsal region of the animal hemisphere transfer dye to nonsister cells in ~60% of embryos, whereas blastomeres in the ventral region transfer dye in only ~10% of embryos (*10, 11*). It has been proposed that this asymmetry in gap junctional communication reflects early developmental events that eventually determine dorsal-ventral polarity of the embryo (*12*).

We first tested whether deregulation of endogenous expression of *wnt*-1 in *Xenopus* embryos—accomplished by microinjection of synthetic RNA into fertilized eggs—affects cell coupling between either ventral or dorsal blastomeres at the 32-cell stage. Fertilized *Xenopus* eggs were microinjected with *wnt*-1 RNA before the first cleavage (*13*). As a control, half of the embryos were injected with 323 RNA, which encodes a form of Wnt-1 in which a single cysteine has been mutated, rendering the protein inactive with regard to effects on the embryonic axis (*6*). Approximately 4 hours after injection of the synthetic mRNA, at the 32-cell stage, Lucifer yellow was microinjected into single tier-1 blastomeres. The embryos were then fixed 10 or 30 min after dye injection and viewed by epifluorescence microscopy (*13*). In embryos injected with *wnt*-1 RNA, there was a large effect on gap junctional communication between ventral cells: specifically, 44% of embryos injected with wild-type *wnt*-1 RNA showed transfer of Lucifer yellow to nonsister ventral blastomeres, whereas only 17% of embryos injected with 323 RNA demonstrated transfer of Lucifer yellow (Table 1 and Fig. 1, A and B). In contrast, Wnt-1 had little effect on gap junctional coupling between dorsal cells: 50% of embryos injected with 323 RNA and 52% of embryos injected with *wnt*-1 RNA showed transfer of Lucifer yellow (*11*). These results suggest that microinjection of *wnt*-1 RNA and the subsequent translation and secretion of Wnt-1 polypeptides initiate events that open gap junctions in the ventral region of the developing embryo, so that cell coupling in the ventral region is similar to that normally found in the dorsal region.

Histological examination confirmed that microinjection of *wnt*-1 RNA into fertilized eggs led to increased transfer of Lucifer yellow to nonsister ventral cells of the 32-cell embryo. After the number of cells that contained Lucifer yellow in intact embryos had been counted with the use of an epifluorescence microscope, selected embryos were embedded, sectioned, and examined by fluorescent and light microscopy (Fig. 1, C and D). In all cases, the number of cells that transferred dye in whole embryos matched the number determined histologically. The histological examination of serial sections also demonstrated that there was no obvious discontinuity in the plasma membranes of embryonic cells, thus minimizing the possi-

Table 1. Ventral cell gap junctional communication in 32-cell *Xenopus* embryos that had been injected with wild-type *wnt*-1 or mutant *wnt*-1 (323) RNA before the first cleavage. Embryos were injected with RNA, cultured for 4 hours to the 32-cell stage, and then assayed for their ability to transfer Lucifer yellow or FITC-dextran between tier-1 ventral blastomeres (*13*). Data are pooled from 12 different experiments and presented according to the method chosen to determine dorsal-ventral polarity (*15*). The observed incidence of Lucifer yellow transfer between nonsister ventral cells in embryos injected with *wnt*-1 RNA was significantly different ($P < 0.001$) than that observed in embryos injected with 323 mutant RNA, as analyzed by the χ^2 test. Transfer of Lucifer yellow or FITC-dextran to nonsister cells was defined as detection of dye in more than two cells (*13*). The percentage of total embryos found to transfer dye between nonsister ventral animal pole cells is shown, together with, in parentheses, the number of embryos used for each experiment.

Dorsal-ventral axis determination method	Time after dye injection (min)	Embryos showing transfer of Lucifer yellow (%)		Embryos showing transfer of FITC-dextran (%)	
		wnt-1	323	*wnt*-1	Uninjected
Pigmentation	10	46 (174)	27 (117)	4 (25)	8 (30)
Sperm entry point marked*	10	47 (90)	21 (60)		
Ventral side of the first cleavage furrow marked†	10	49 (29)	5 (43)		
Dorsal side of the first cleavage furrow marked†	10	32 (88)	8 (71)		
	Summary	44 (381)	17 (291)	4 (25)	8 (30)
Dorsal side of the first cleavage furrow marked†	30	91 (13)	13 (7)		

*With 1% Nile blue. †With 1% Nile blue at the eight-cell stage.

Table 2. Ventral cell gap junctional communication in 32-cell *Xenopus* embryos that had been injected with *Xwnt*-8 or *Xwnt*-5A RNA before the first cleavage. Ventral cell dye transfer in uninjected embryos was compared to that in embryos injected before the first cleavage with *Xwnt*-8 or *Xwnt*-5A RNA (*13*). After culture for 4 hours to the 32-cell stage, Lucifer yellow or FITC-dextran was injected into a single, tier-1, animal pole ventral blastomere. After 10 min, embryos were fixed, and dye transfer was determined (*13*). Data are pooled from six different experiments and presented according to the method chosen to determine dorsal-ventral polarity (*15*). χ^2 analysis of the data demonstrated that the incidence of ventral cell dye transfer in embryos injected with *Xwnt*-8 RNA was significantly different from that observed in control embryos ($P < 0.001$). In contrast, the difference in ventral cell dye transfer between embryos injected with *Xwnt*-5A RNA and control embryos was not statistically significant ($P < 0.5$). The percentage of total embryos found to transfer dye between nonsister ventral animal pore cells is shown, together with, in parentheses, the number of embryos used for each experiment.

Dorsal-ventral axis determination method	Time after dye injection (min)	Embryos showing transfer of Lucifer yellow (%)			Embryos showing transfer of FITC-dextran (%)		
		Xwnt-8	*Xwnt*-5A	Uninjected	*Xwnt*-8	*Xwnt*-5A	Uninjected
Pigmentation	10	61 (31)	21 (28)	17 (12)			8 (29)
Dorsal side of first cleavage furrow marked with 1% Nile blue at eight-cell stage	10	43 (69)	24 (46)	16 (50)	4 (28)	0 (10)	
	Summary	48 (100)	23 (74)	16 (62)	4 (28)	0 (10)	8 (29)

bility that injection of *wnt*-1 RNA led to incomplete mitosis or the formation of cytoplasmic bridges.

We further demonstrated that FITC-dextran, which cannot pass through gap junctions (*10, 14*), was transferred between ventral cells in only 4% of *wnt*-1 RNA–injected embryos (Table 1). To confirm that the sites of microinjection of Lucifer yellow had been accurately designated as either dorsal or ventral, we identified these regions of the embryo by several methods (*15*). Finally, as a control to establish that the increased transfer of Lucifer yellow in embryos injected with *wnt*-1 RNA did not subsequently decrease later in the cell cycle, embryos were scored for dye transfer at 30 min rather than 10 min after dye injection. The 30-min time point was just prior to the cleavage that gives rise to the 64-cell embryo. Again, embryos injected with *wnt*-1 RNA, but not 323 RNA, displayed increased transfer of Lucifer yellow to nonsister ventral cells (Table 1), with the dye transfer often extending across the vegetal hemisphere to the dorsal region (Fig. 1, E and F). Thus, at two distinct periods during the 32-cell stage, enhanced gap junctional communication between ventral cells was observed in embryos injected with *wnt*-1 RNA.

We then investigated whether premature and increased expression of two recently discovered Wnt-1–related proteins would also affect gap junctional communication in early *Xenopus* embryos. We chose *Xwnt*-8 and *Xwnt*-5A for this experiment because transcripts encoding these members of the Wnt family are expressed earlier than the neurula stage, when transcripts for *Xwnt*-1, *Xwnt*-3, and *Xwnt*-4 are first detected (*16, 17*). Microinjection of *Xwnt*-8 RNA into fertilized eggs led to Lucifer yellow transfer to nonsister ventral cells in 48% of 32-cell embryos (Table 2), similar to the enhanced dye transfer obtained with *wnt*-1 RNA (Table 1). In contrast, 16% of embryos not injected with synthetic RNA and 23% of embryos injected with *Xwnt*-5A RNA (*18*) transferred Lucifer yellow into nonsister ventral cells of 32-cell embryos (Table 2). As with *wnt*-1, microinjection of FITC-dextran instead of Lucifer yellow into ventral cells did not lead to increased transfer of fluorescent material in *Xwnt*-8 RNA–injected embryos (Table 2), supporting the hypothesis that the transfer of Lucifer yellow in *Xenopus* embryos occurs by means of gap junctions (*10*).

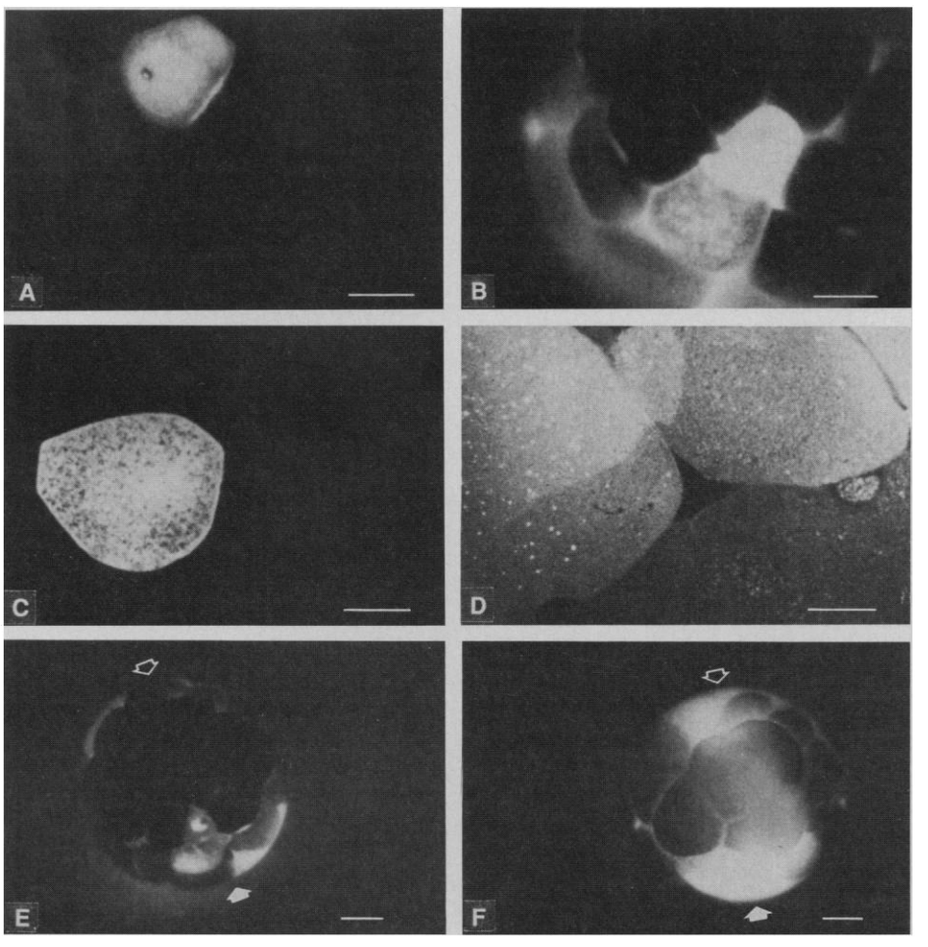

Fig. 1. Photomicrographs of 32-cell *Xenopus* embryos. All embryos were injected before the first cleavage with the indicated synthetic RNA. When embryos had reached the 32-cell stage, Lucifer yellow was injected into a single, tier-1 ventral blastomere (*13*). (**A**) Whole embryo injected with 323 mutant *wnt*-1 RNA. After 10 min, dye transfer is confined to the site of injection. Bar, ~150 μm. (**B**) Whole embryo injected with wild-type *wnt*-1 RNA. Dye transfer is apparent through several cells. Bar, ~150 μm. (**C**) Plastic section through an embryo injected with 323 RNA. Dye is apparent only in the injected cell. Bar, 90 μm. (**D**) Plastic section through an embryo injected with wild-type *wnt*-1 RNA. Dye has transferred to several neighboring cells. Bar, 70 μm. (**E**) Whole embryo injected with wild-type *wnt*-1 RNA as in (A) but viewed 30 min after dye injection. Bar, 250 μm. (**F**) Same embryo as in (E) but positioned with the vegetal hemisphere toward viewer. Dye has extended vegetally from the ventral injection site to dorsal cells in the form of a band across two opposing tier-4 blastomeres. Open arrows, dorsal; closed arrows, ventral.

The mechanisms by which Wnt proteins transduce signals in receptive cells is unknown, but, by analogy with other peptide signaling molecules, an interaction with specific cell surface receptors is probably required (*1, 2, 4*). However, Wnt receptors have yet to be identified in any organism. Although *Xwnt*-1 and *Xwnt*-8 are not normally expressed until the neurula and midblastula stages, respectively (*16, 17*), Wnt receptors may be present much earlier. We propose that the similar effects of Wnt-1 and Xwnt-8 on gap junctional communication result from their ability to activate the same receptor pathway in the early embryo. Conversely, the failure of Xwnt-5A to affect gap junctional communication may be due to its acting at a distinct receptor; this receptor may not be present before the 32-cell stage or may not activate signal transduction pathways that modulate gap junctions.

Although it has been suggested that Wnt-1 and Wnt-3A may be functionally redundant (*19*), the lack of effect of Xwnt-5A on gap junctional communication suggests that not all Wnt proteins are functionally equivalent. We further propose that the observed increase in gap junctional communication between ventral blastomeres of 32-cell embryos expressing Wnt-1 or Xwnt-8 is one of several potential effects resulting from the activation of a specific receptor-mediated signal transduction pathway. The functional outcome of this receptor activation at the 32-cell stage may include increased gap junctional coupling between cells on the ventral side of the embryo, whereas activation of this pathway later in development may have additional or distinct consequences.

A close correlation may exist between ventral cell gap junctional coupling and later axis duplication or enhancement of dorsal-anterior structures. In support of this rela-

tion, injection of either *wnt*-1 or *Xwnt*-8 RNA into fertilized eggs leads both to the development of embryos that display enhanced gap junctional communication in ventral cells at the 32-cell stage (Tables 1 and 2) and to duplication of the embryonic axis and an enhanced dorsal-anterior phenotype that is apparent by the neurula stage (*6, 16*). Furthermore, microinjection of *Xwnt*-5A RNA into fertilized eggs has no apparent effect on gap junctional communication at the 32-cell stage (Table 2) and, rather than affecting the embryonic axis, causes head and tail defects in tadpoles (*18*).

To test whether disruption of the dorsal-ventral polarity in gap junctional communication at the 32-cell stage is an absolute requirement for formation of embryos with duplicated axes and enhanced dorsal-anterior structures, we microinjected *wnt*-1 or *Xwnt*-8 RNA into single dorsal or ventral blastomeres of 32- to 64-cell embryos (*11, 16*). This still affects the embryonic axis and dorsal-anterior structures, although the effect of Wnt expression on gap junctional communication in these embryos is unknown. One interesting possibility is that injection of *wnt*-1 or *Xwnt*-8 RNA at any period tested leads to ventral cells assuming some or all characteristics of dorsal cells, with increased gap junctional communication being one aspect of this dorsal character.

Ventral blastomeres in 32-cell embryos treated with LiCl also display enhanced gap junctional communication (*20*). Similar to the phenotype produced by ectopic expression of *wnt*-1 (*6*) or *Xwnt*-8 (*16*), both LiCl (*21*) and overexpression of activin B (*22*) cause embryos to develop with exaggerated or duplicated dorsal-anterior structures, though it is not known if these phenotypes arise by similar mechanisms. In comparing the effects of Wnt proteins and LiCl on gap junctional communication, it should be noted that Wnt-1 and Xwnt-8 are endogenous proteins and are likely to modulate gap junctions through their action on a highly specific signaling pathway, whereas LiCl is an exogenous agent and has pleiotropic effects on cells that result from nonspecific disruption of multiple pathways (*23*).

Our results have shown that deregulation of normal expression of *wnt*-1 and *Xwnt*-8, but not *Xwnt*-5A, enhances ventral cell gap junctional communication in early embryos of *Xenopus laevis*. Future studies are required both to address whether expression of specific members of the *wnt* gene family in spatially distinct regions of uninjected embryos is correlated with alterations in gap junctional communication and to elucidate the signal transduction pathways by which gap junctions are modulated by Wnt-1 and Xwnt-8. The potential correlation between gap junctional coupling and the patterns of expression of *wnt*-1 or *Xwnt*-8 in vertebrates or *wnt*-1 (*wingless*) in *Drosophila* has not been elucidated. However, recent studies in *Drosophila*, together with our data, suggest that such research merits consideration. Specifically, the *armadillo* gene product, thought to be a component in the *wingless* signaling pathway (*24*), has been shown to be the homolog of vertebrate plakoglobin (*25*). Plakoglobin can be found between adhering cells in regions that are adjacent to or that contain gap junctions (*26*), as well as in regions that do not contain gap junctions. Wnt-1 (and its related proteins) remains "a signal in search of a receptor" (*25*), but our assay of gap junctional coupling in 32-cell *Xenopus* embryos should facilitate dissection of the Wnt signal transduction pathway.

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13. Adult *Xenopus laevis* were induced to spawn, and the eggs were fertilized in vitro. After removal of jelly, but before the first cleavage, the fertilized eggs were microinjected with 0.5 to 1.0 ng of in vitro–transcribed RNA [R. T. Moon and J. L. Christian, *Technique* **1**, 76 (1989)]. The injection sites were random relative to the future dorsal-ventral axis, and we have shown that β-galactosidase (*11*) and *wnt*-1 (*6*) RNA injected in this manner become widely distributed in the embryo. It has been demonstrated by whole-mount immunocytochemistry that *wnt*-1 RNA injected in this manner is translated to yield detectable amounts of polypeptide (*6*). Therefore, the injection sites did not bias the localization of expression of Wnt proteins. After determination of dorsal-ventral polarity (*15*), symmetrically cleaving embryos were maintained in 5% Ficoll in 0.1× modified Barth solution at 17°C. At the 32-cell stage, ~1 nl of 4% Lucifer yellow was injected with a new needle into single, tier-1 animal pole blastomeres on either the dorsal [previously referred to as the "a" or "b" blastomeres (*10*)] or ventral [previously referred to as the "d" or "e" blastomeres (*10*)] side of the embryo. Ten or 30 min after dye injection, the embryos were transferred to fixative consisting of 0.25% glutaraldehyde and 3% paraformaldehyde in 0.05 M phosphate-buffered saline (PBS) (pH 7.4) for 1 to 2 hours at 4°C; the embryos were then maintained in 0.05 M PBS at 4°C until examination. Lucifer yellow or FITC-dextran (10,000 kD) was localized with the use of a Leitz Dialux 20 epifluorescence microscope. Embryos were scored as transferring fluorescent material only when fluorescence extended beyond the injected cell and its sister cell because cytoplasmic bridges may remain between sister cells generated from the previous cleavage. Selected fixed embryos that had been scored for dye transfer were dehydrated, embedded in plastic, and sectioned at 7 μm [D. H. Giebelhaus, D. W. Eib, R. T. Moon, *Cell* **53**, 601 (1988)]. Some embryos were cultured to stage 20 (*27*) and examined for the characteristic phenotype obtained by injection of *wnt* RNA (*6, 16, 18*).
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