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# *Wnt* Genes

Review

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## Proto-Oncogenes and Development

During the past decade an avalanche of evidence has implicated proto-oncogenes in the control of cell proliferation. Products of proto-oncogenes include many of the secreted growth factors and cell surface receptors that mediate intercellular signaling events (Aaronson, 1991), the components of cytoplasmic pathways that interpret those signals (Cantley et al., 1991), and the nuclear factors that execute their commands through transcriptional regulation (Lewin, 1991). We are now witnessing a similar outpouring of support for the parallel notion that genes important in carcinogenesis are central to pattern formation in the embryo or differentiation in cell lineages. Preexisting or man-made mutations of mouse proto-oncogenes affect a variety of developmental processes (Forrester et al., 1992). Well-established growth factors and oncogenes can induce differentiation under appropriate conditions (e.g., Alema et al., 1985). The homologs of proto-oncogenes in flies, worms, and yeast are essential components of developmental mechanisms in those organisms (Hoffmann et al., 1992), and a few genes isolated as determinants of development, such as homeobox-encoding genes, have later been shown to have oncogenic potential (Perkins et al., 1990; Rabbitts, 1991).

Among the most striking connections between oncogenesis and development are provided by *Wnt* genes, the subject of this review. (The term *Wnt* is an amalgam of *wingless* [*wg*] and *int* [see below and Nusse et al., 1991].) The first *Wnt* gene was cloned from the mouse genome as a relatively obscure proto-oncogene ten years ago (Nusse and Varmus, 1982). But the numerous *Wnt* genes isolated from diverse species in the past few years have now attracted unusual attention because of the dramatic developmental phenomena attributed to them. Ectopic expression of *Wnt* genes induces axis duplication in frog embryos (McMahon and Moon, 1989), as well as mammary cancer in mice; *Wnt* gene deficiencies prevent normal development of mammalian brains (McMahon and Bradley, 1990; Thomas and Capecchi, 1990) and normal segmentation of insect embryos (Rijsewijk et al., 1987a).

Any effort to understand these remarkable effects must begin with the fact that *Wnt* genes encode secretory glycoproteins and apparently cause cells to proliferate, to differentiate, or perhaps simply to survive by signaling through autocrine and paracrine routes. In this sense, Wnt proteins are multipotent factors, capable of inducing different biological responses in different cellular contexts, a phenomenon also described for better-known secretory proteins such as the fibroblast growth factors (FGFs), the transforming growth factors β (TGFβ), and nerve growth factor (NGF). Establishing a more detailed picture of the actions of *Wnt* genes, however, presents considerable challenges. The *Wnt* gene family is large, suggesting functional redundancy, the patterns of expression are complex, proteins encoded by *Wnt* genes have resisted isolation in biologically active form, and receptors for Wnt proteins, presumably essential components in cell–cell signaling, remain to be identified.

## The *Wnt* Gene Family

With the benefit of hindsight, we now recognize that phenomena studied for several decades are the consequences of *Wnt* gene mutations. Viral insertion mutations regularly promote mammary tumors in laboratory mice (Bittner, 1936; Korteweg, 1936), a spontaneous frameshift mutation of mice (*swaying*) impairs cerebellar structure and function (Lane, 1967; Thomas et al., 1991), and *wg* mutations in Drosophila can transform a wing to a notum or disrupt segment polarity (Sharma, 1973; Nüsslein-Volhard and Wieschaus, 1980).

*Wnt* genes were initially cloned as candidate proto-oncogenes (mouse *Wnt-1* and *Wnt-3* [Nusse and Varmus, 1982; Roelink et al., 1990]), as a gene near the cystic fibrosis locus (human *Wnt-2*; Wainwright et al., 1988), and as human, Drosophila, and Xenopus homologs of mouse *Wnt-1* (Van't Veer et al., 1984; Rijsewijk et al., 1987a; Noordermeer et al., 1989). Once these few *Wnt* genes were in hand to identify conserved sequences for primers, the polymerase chain reaction (PCR) produced an outpouring of *Wnt* gene family members. Up to ten or more *Wnt* coding sequences have been isolated from the widely studied species listed in Table 1, as well as from zebrafish (Molven et al., 1991), several other vertebrates (including birds, reptiles, and jawless fishes), sea urchins, starfish (Sidow, 1992), Axolotl (Busse et al., 1990), and leech (Kostriken and Weisblat, 1992). It is doubtful whether the full *Wnt* repertoire is in hand for any of these species. Even in the mouse, which has yielded the largest number of published sequences, most were recovered from cDNA libraries of midgestational embryonic RNA with a single set of PCR primers (Gavin et al., 1990). Other libraries and other primers may well produce more genes.

Each of the fully sequenced *Wnt* open reading frames encodes a protein that appears destined for secretion, harbors one or more sites for N-linked glycosylation, and conserves up to 23 or 24 cysteines in nearly parallel positions (Figure 1). Most of the deduced proteins are about 350 to 380 aa in length, with over 100 conserved residues fairly evenly distributed across the entire sequence. Some Wnt proteins have additional internal, amino-terminal, or carboxy-terminal domains; for example, the wg protein has 85 aa inserted just before the region encoded by its final exon, the DWnt-3 protein has a 155 aa insertion and

Table 1. *Wnt* Genes Identified in Commonly Studied Organisms, with Chromosomal Assignments

| Species | Genes | Chromosome Assignments | References |
|---|---|---|---|
| Homo sapiens | *Wnt-1* | 12q13 | Van't Veer et al., 1984; van Ooyen et al., 1985 |
| | *Wnt-2* | 7q3.1 | Wainwright et al., 1988 |
| | *Wnt-3* | 17q21 | R. N., unpublished data |
| Mus musculus | *Wnt-1* | 15 | Nusse and Varmus, 1982; Nusse et al., 1984; van Ooyen and Nusse, 1984; Fung et al., 1985 |
| | *Wnt-2* | 6 | McMahon and McMahon, 1989 |
| | *Wnt-3* | 11 | Roelink et al., 1990 |
| | *Wnt-3A* | 11 | Roelink and Nusse, 1991 |
| | *Wnt-4* | 4 | Gavin et al., 1990 |
| | *Wnt-5A* | 14 | Gavin et al., 1990 |
| | *Wnt-5B* | 6 | Gavin et al., 1990 |
| | *Wnt-6* | 1 | Gavin et al., 1990 |
| | *Wnt-7A* | 6 | Gavin et al., 1990 |
| | *Wnt-7B* | 15 | Gavin et al., 1990 |
| Xenopus laevis | X-*Wnt-1* | ? | Noordermeer et al., 1989 |
| | X-*Wnt-3* | ? | Christian et al., 1991a, 1991b |
| | X-*Wnt-4* | ? | Christian et al., 1991a, 1991b |
| | X-*Wnt-5A* | ? | Christian et al., 1991a, 1991b |
| | X-*Wnt-8* | ? | Christian et al., 1991a, 1991b |
| Drosophila melanogaster | wg | 28A | Rijsewijk et al., 1987a |
| | D-*Wnt-2* | 45E | Russell et al., 1992 |
| | D-*Wnt-3* | 17B | Russell et al., 1992 |
| C. elegans | Ce-*Wnt-1* | II | Kamb et al., 1989 |
| | Ce-*Wnt-2* | IV | R. Waterston, personal communication |

a long amino-terminal extension, and the mouse Wnt-2 and Xenopus Wnt-8 proteins have short carboxy-terminal extensions. In most cases, Wnt proteins display 30%–60% amino acid identity when two proteins within a single species or two from widely divergent species are compared. In fact, *Wnt* genes are sufficiently well conserved to permit recognition of the homologs of individual mouse genes in different orders of vertebrates (e.g., Christian et al., 1991a) and even in insects or echinoderms (Sidow, 1992). For example, as pointed out by Gavin et al. (1990), mouse Wnt-1 protein and its orthologs in Xenopus, zebrafish, and Drosophila share one cysteine residue in the amino-terminal region that is not present in other family members; conversely, other Wnt proteins contain two nearby cysteine residues not found in Wnt-1 proteins (Figure 1).

Based upon such arguments it appears that at least four and perhaps as many as seven *Wnt* genes must have existed before the divergence of arthropods and chordates (Sidow, 1992). In addition, amino acid substitutions in Wnt protein sequences have accumulated at an especially slow rate during vertebrate evolution (Sidow, 1992), consistent with the central inductive roles proposed for Wnt proteins during embryogenesis. Even though some pairs of proteins (e.g., mouse Wnt-3 and -3A, Wnt-5A and -5B, or Wnt-7A and -7B) are very highly related, the appearance of these pairs in cartilagenous fish implies that the duplications producing them must still have occurred at least 400 million years ago. As might be expected under these circumstances, there are very few differences between the Wnt proteins of mouse and human that have been compared (van Ooyen et al., 1985; McMahon and McMahon, 1989).

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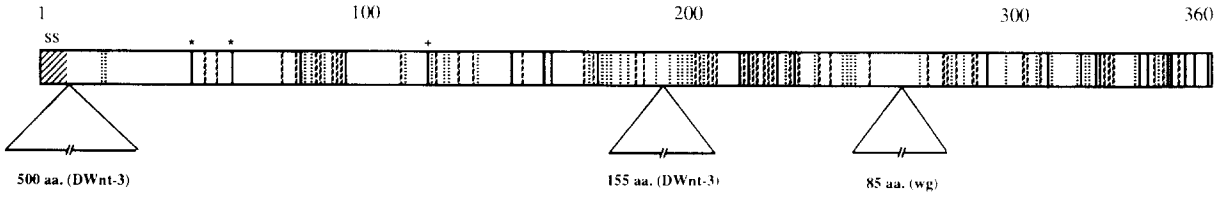


Figure 1. Schematic Representation of Conserved Amino Acid Residues in Wnt Proteins

Conserved cysteine residues are represented by continuous bars; a cysteine unique for Wnt-1 and wg is marked by a plus sign; and two cysteines found in all other Wnt proteins by asterisks. Other absolutely conserved residues are shown as waved bars, and residues conserved in at least 75% of Wnt proteins by striped bars. A signal sequence (ss) is present at the amino terminus. Inserts in Wg and DWnt-3 proteins are shown below. The figure is based on an alignment provided by A. Sidow.

Table 2. Expression Patterns of *Wnt* Genes in the Mouse

| Gene | Expression in Embryos | Expression in Adults | Expression in Mammary Gland |
|---|---|---|---|
| *Wnt-1* | Brain; ring around midbrain–hindbrain junction; tip headfolds; dorsal wall diencephalon; roofplate spinal cord | Testes (postmeiotic spermatids) | Not detectable |
| *Wnt-2* | Developing allantois; pericardium heart; ventral–lateral mesoderm | Lung; brain; heart; placenta | Virgins |
| *Wnt-3* | D2 neuromer diencephalon (dorsal thalamus); ventral horns spinal cord; developing cerebellum; ectoderm overlying mandible, genital tubercle, and limbs | Thalamus; Purkinje cells in cerebellum; pons; Shaft hair root in skin | Not detectable |
| *Wnt-3A* | Ectoderm and mesoderm primitive streak posterior hindbrain; anterior midbrain; roofplate spinal cord; archicortex of the telencephalon | Lung | Not detectable |
| *Wnt-4* | Embryo; fetus (sites not determined) | Brain; lung | Virgins; early pregnancy |
| *Wnt-5A* | Face; brain (ventral area midbrain) and spinal cord; gradient in developing limbs | Heart; lung | Early pregnancy |
| *Wnt-5B* | Embryo; fetus, not restricted | Heart; liver; brain; lung; testes; kidney | Midpregnancy |
| *Wnt-6* | Embryo; fetus (sites not determined) | Testes | Midpregnancy, early lactation |
| *Wnt-7A* | Embryo; fetus (sites not determined) | Brain; lung | Not detectable |
| *Wnt-7B* | Embryo; fetus (sites not determined) | Brain | Virgins; early pregnancy |

of *Wnt* genes and the structural determinants of their expression. Of the several genes that have been sequenced, most have three introns in corresponding positions; however, a Caenorhabditis elegans *Wnt* gene has a radically different distribution of exons and introns (G. Shackleford, L. Shiue, H. E. V., unpublished data), and a Drosophila gene, D*Wnt-3*, has no introns at all (Russell et al., 1992). There is fragmentary evidence for alternative modes of expression: multiple sites for initiation of transcription (Nusse et al., 1990) and for polyadenylation (Gavin et al., 1990) and at least one example of alternative splicing to produce two slightly different proteins from mouse Wnt-4 (Gavin et al., 1990). However, the important determinants of the complex transcriptional programs described below (Tables 2 and 3) have not been defined.

## Wnt Proteins and Their Signaling Mechanism

The predicted primary protein products of *Wnt* genes display many of the characteristics of secreted growth factors: a hydrophobic signal peptide, followed by a recognition site for signal peptidase, the absence of additional transmembrane domains, abundant and strongly conserved cysteine residues with potential for disulfide cross-linking, and prospective sites for N-linked glycosylation (Figure 1).

Despite these strong clues to the nature of Wnt proteins, it has been difficult to document that they are secreted and thus far impossible to prepare them in a useful cell-free form. Most of the available information about Wnt proteins has been obtained from the study of the mouse *Wnt-1*, human *Wnt-2*, and the Drosophila *wg* genes, largely because antisera have been prepared to detect their products. Since tissues that express endogenous *Wnt-1* are not readily cultured and since nearly all cultured cell lines (excepting embryonal carcinoma lines such as P19 [St. Arnaud et al., 1989; Schuuring et al., 1989]) fail to express the gene, most work on Wnt-1 proteins has been performed with cells programmed to express exogenous cDNA under the control of a heterologous promoter.

In these ectopic settings, a large portion of Wnt-1 and Wnt-2 protein remains associated with internal membranous components of cells, in a series of glycosylated forms, ranging from 36 to 42 kd in size (Brown et al., 1987; Papkoff et al., 1987; Blasband et al., 1992). It is likely that most of the Wnt protein is in the endoplasmic reticulum (ER) and cis-Golgi apparatus; intracellular Wnt-1 proteins are associated with the 78 kd, ER-based chaperonin called BiP (Kitajewski et al., 1992), and the carbohydrate modifications are immature (susceptible to endoglycosidase H [Papkoff, 1989]).

Table 3. *Wnt* Genes in Xenopus

| Gene | Expression | Duplication Axis | Effects on Gap Junctions |
|---|---|---|---|
| X*Wnt-1* | Neurula | Anterior | Ventral opening |
| X*Wnt-3A* | Neurula | Partial anterior | |
| X*Wnt-4* | Neurula (dorsal in brain) and floor plate spinal cord | | |
| X*Wnt-5A* | Oocyte/tadpole (maternal) | Posterior | No effect |
| X*Wnt-8* | Midblastula; band in marginal zone (excluding organizer) | Anterior | Ventral opening |

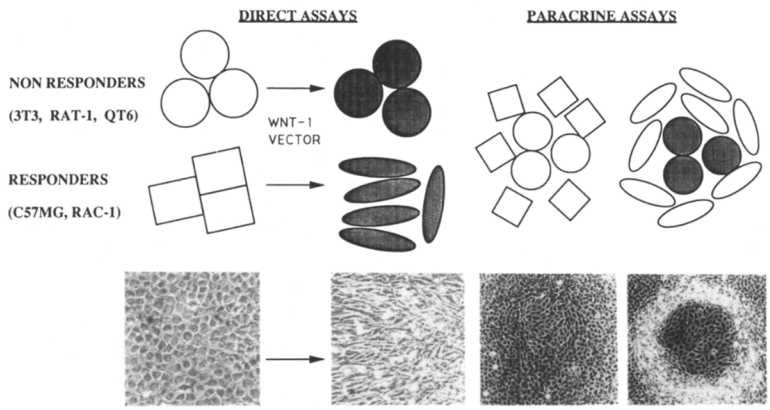


Figure 2. Direct and Paracrine Cell Culture Assays for *Wnt* Genes

In a direct assay for *Wnt* genes, nonresponsive cells (circles) or mammary epithelial cells (squares) that respond to *Wnt* genes by morphological and proliferative changes (oblongs) produce Wnt proteins (as indicated by stippling) after introduction of a *Wnt* expression vector. In one version of a paracrine assay, nonexpressing or *Wnt*-expressing colonies of nonresponsive cells are surrounded by untreated responders. Photographs at the bottom show, on the left, C57MG cells before and after introduction of a retrovirus vector carrying *Wnt-1*, and, on the right at lower magnification, nonexpressing C57MG cells surrounding colonies of rat-1 cells that are or are not infected with the virus vector bearing *Wnt-1*. The photographs are from Jue et al. (1992) and J. Kitajewski and H. E. V.

Under normal conditions, Wnt proteins have not been detected by either immunological or biological assays in culture medium from cells expressing exogenous *Wnt* genes. But immunoreactive forms can be recovered by treating the cultures with the polyanion, suramin (Papkoff, 1989; Papkoff and Shryver, 1990; Blasband et al., 1992), by solubilizing the extracellular matrix after removing the cells, or by growing the cultures in the presence of soluble heparin, to which Wnt proteins bind at physiological salt concentrations (Bradley and Brown, 1990). Wnt proteins identified in these ways are apparently secreted, although at different efficiencies in different cell lines, and their mobilities in gel electrophoresis suggest varying degrees of further modification. Based upon antibody binding and iodination experiments with intact cells (Papkoff and Shryver, 1990), Wnt proteins appear to be associated with the cell surface, as well as with the extracellular matrix. However, it is not known whether these associations are specific, whether certain forms of the protein (e.g., oligomers or heterologous complexes) are biologically active, or whether components of the extracellular matrix, such as glycosaminoglycans, can act as cofactors to promote the response to Wnt proteins, in the manner recently demonstrated for FGFs (Klagsbrun and Baird, 1991).

Despite the failures to obtain Wnt-1 protein in a biologically active, cell-free form, the secreted protein appears to have biological activity, as measured in cell transformation assays that depend upon paracrine effects (Figure 2; Jue et al., 1992). In these tests, transforming activity can be supplied by any of several cell lines that expresses an introduced *Wnt-1* gene without detectably responding to it (e.g., mouse 3T3, rat-1, quail sarcoma, or HeLa cells). When *Wnt*-responsive C57MG cells, derived from a normal mouse mammary gland, are mixed with or surround the donor cells, they undergo morphological changes indistinguishable from those produced when a *Wnt-1* vector is expressed directly in C57MG cells (see below and Figure 2).

Although available antibodies against Wnt-1 proteins do not block the paracrine effect, it seems very likely to be attributable to Wnt-1 proteins: many cells can serve as donors, yet the only responders are the mammary cells that are also susceptible to direct transformation by *Wnt-1* and related genes. In addition, the effect is blocked by heparin (Jue et al., 1992), and it is not observed with a *Wnt-1* mutant whose product lacks the signal peptide (Mason et al., 1992). This mutant also fails to transform C57MG cells directly (Mason et al., 1992) or to cause axis duplication in Xenopus embryos (McMahon and Moon, 1989); thus, entry into the secretory pathway appears to be essential for biological activity of Wnt proteins. In Drosophila, where genetic evidence exists for the paracrine activity of *wg* (see below), immunostaining of embryos has been used to show that Wg protein arrives at the surface of cells containing *wg* RNA and can be found in adjacent cells (van den Heuvel et al., 1989).

The implied importance of the conserved cysteine residues in Wnt proteins has been further documented by the study of *Wnt-1* mutants. When the conserved cysteine codon at the penultimate position in mouse *Wnt-1* is changed to a tryptophan codon (Cys369Trp), the gene loses its ability to transform C57MG cells directly or by a paracrine mechanism (Mason et al., 1992) and to induce axis duplication in Xenopus embryos (McMahon and Moon, 1989). Mutation of other cysteine residues also impairs transforming activity; in one case (Cys151Ser) transformation of C57MG cells is temperature sensitive (Mason et al., 1992). Curiously, the same change at a nonhomologous cysteine residue in wg protein (Cys107Ser) is responsible for a spontaneous temperature-sensitive mutant of *wg* (M. van den Heuvel, C. Harryman, and R. N., unpublished data). With one exception, glycosylation sites, in contrast to cysteine residues, are not well conserved, and none of the four sites in Wnt-1 protein is required for transforming activity; indeed, a mutant protein lacking all four sites is still transformation competent (Mason et al., 1992). However, one glycosylation site mutant (Asn359Gln) is also temperature sensitive for transformation.

### *Wnt* Genes in Mammary Carcinogenesis

*Wnt* genes are sources of differentiation-inducing signals during normal developmental events, but they also have

the potential to promote carcinogenesis through local effects on cell proliferation, particularly in the mammary gland. Indeed, the first *Wnt* gene to be molecularly cloned, mouse *Wnt-1*, was isolated during a search for cellular genes insertionally activated by proviral DNA in mammary carcinomas induced by the mouse mammary tumor virus (MMTV) (Nusse and Varmus, 1982). (Until recently [Nusse et al., 1991], it was called *int-1*, for MMTV integration site.) Although transcription of the c-*myc* proto-oncogene, the progenitor of the retroviral oncogene, v-*myc*, had earlier been shown to be stimulated by adjacent avian leukosis virus DNA in chicken B-cell lymphomas (Hayward et al., 1981), *Wnt-1* was the first of many proto-oncogenes to be discovered originally as a target for proviral insertion mutations (van Lohuizen and Berns, 1990). The proviral tagging method has subsequently identified at least four additional proto-oncogenes that are transcriptionally activated by MMTV proviruses in mouse mammary carcinomas: two members of the FGF gene family, *int-2* (also known as *FGF-3*; Dickson et al., 1984; Dickson and Peters, 1987) and *hst-1* (also known as K-fgf or *fgf-4*; Peters et al., 1989), another member of the *Wnt* gene family (*Wnt-3*; Roelink et al., 1990), and *int-3*, a gene encoding a presumptive transmembrane receptor with regions homologous to the products of *Notch*, *lin-12*, and *Tan-1* (Gallahan and Callahan, 1987; Robbins et al., 1992). In a provocatively high percentage of tumors, insertions have occurred in two of these genes, usually *Wnt-1* and *int-2* (Peters et al., 1986; Mester et al., 1987), suggesting cooperative effects in carcinogenesis (see below). In addition, a third *Wnt* gene, *Wnt-2*, has been implicated in mammary tumorigenesis because it is amplified and overexpressed in subclones of two transplanted, virus-induced tumors (Roelink et al., 1992).

Notably, none of the MMTV-activated genes has been encountered as a naturally occurring retroviral oncogene; only *hst* has been implicated as a proto-oncogene in other contexts (Taira et al., 1987), and all five appear to affect extracellular signaling events—four by production of secretory proteins and one by production of a presumptive cell surface receptor. These observations are surprising, since genes such as c-*myc* and c-*Ha-ras*—which have been repeatedly transduced by retroviruses, are involved in many kinds of tumors, and encode intracellular regulators—do have the potential to induce mammary carcinomas when engineered to form a transgene under the control of an MMTV long terminal repeat (Stewart et al., 1984; reviewed by Adams and Cory, 1991).

Initially, a causative role for *Wnt* genes in mammary carcinogenesis was based upon three circumstantial arguments: the repeated occurrence of proviral insertion mutations, very commonly in the *Wnt-1* locus, occasionally in *Wnt-3*; the consequent activation of expression of these genes, which are normally silent in the mammary gland, most often by provision of a viral enhancer that acts upon the normal *Wnt* promoter (Nusse et al., 1984, 1990; Roelink et al., 1990; Table 2); and the retention of a complete open reading frame, even when the insertions occur within exons (van Ooyen and Nusse, 1984; Roelink et al., 1990). Since *Wnt* loci are not known to be favored sites for integration, it is presumed that the transcription-activating insertions in the *Wnt-1* and *Wnt-3* loci confer a strong growth advantage upon individual cells, accounting for the clonal character of the tumors (Cohen et al., 1979).

These indirect arguments for the oncogenic effects of *Wnt* insertion mutations have been made more persuasive by gene transfer experiments in cell culture and animals. Although the mouse *Wnt-1* gene has no evident phenotypic effects upon primary embryo cells or most established cell lines, it can induce morphological changes and enhanced growth properties when expressed in at least two mouse mammary epithelial cell lines, C57MG (Brown et al., 1986) and RAC (Rijsewijk et al., 1987b). Similar findings have been obtained with human *Wnt-2* (Blasband et al., 1992), mouse *Wnt-3A* (N. Parkin and H. E. V., unpublished data), and *wg* (A. M. C. Brown, personal communication). (Indeed, only one other type of cultured cell, the rat pheochromocytoma line, PC12, is known to respond to *Wnt* genes [G. Shackleford and H. E. V., unpublished data].) The conversion of cuboidal, mammary epithelial cells to elongated, highly refractile cells that continue to replicate in a dense culture provides a useful assay for *Wnt* genes (Figure 2). The phenomena induced in mammary cell lines, however, do not necessarily simulate the changes anticipated for a mammary oncogene. Transformation of one of the lines (C57MG) is not accompanied by an ability to form tumors in animals (Brown et al., 1986), and cells taken directly from naturally occurring mouse mammary tumors more closely resemble the original C57MG and RAC cell lines than the *Wnt*-transformed cells (e.g., Sonnenberg et al., 1987).

Transgenic mice have provided conclusive evidence for the oncogenic potential of *Wnt-1*. Mice transmitting a *Wnt-1* transgene that mimics activated alleles in virus-induced tumors exhibit extensive hormone-independent hyperplasia of mammary epithelium, so that glands in virgin female and male animals resemble those of pregnant animals (Tsukamoto et al., 1988). By 1 year of age, mammary carcinomas indistinguishable from virus-induced tumors have appeared in most females, in one or two of the ten glands, and in about 15% of males. Occasional salivary gland carcinomas are also observed.

As in several other transgenic models for oncogenesis (Adams and Cory, 1991), the stochastic appearance of mammary carcinomas in *Wnt-1* transgenic animals suggests that additional events, presumably mutations affecting proto-oncogenes or tumor suppressor genes, are required for full oncogenic conversion of cells proliferating under the influence of the *Wnt-1* transgene. Genes capable of collaborating with an activated *Wnt-1* gene during mammary tumorigenesis have been sought by crossing lines of transgenic mice and by infecting transgenic mice with MMTV. Both approaches support earlier indications from studies of nontransgenic animals that the *Wnt-1* and *int-2* genes can act cooperatively to promote tumors. When *Wnt-1* and *int-2* transgenic animals are mated to produce bitransgenic animals, mammary tumors appear earlier and, in males, at a much higher frequency than in either type of parental animal (Kwan et al., 1992). Infection with MMTV also accelerates the onset of tumors in virgin

and breeding female mice carrying the *Wnt-1* transgene. Many of the tumors are derived from infected cells, and they exhibit proviral insertion mutations of *int-2* (most frequently), *hst-1*, or (occasionally) both genes (G. Shackleford, C. MacArthur, H. Kwan, and H. E. V., unpublished data). Other proviruses may have caused contributory mutations at additional, and perhaps novel, loci that have yet to be characterized. The frequent cooperation of *Wnt* and *FGF* gene family members in MMTV-induced mammary carcinogenesis is especially intriguing. Wnt-1 and int-2 proteins, although unrelated by sequence, are secretory glycoproteins with surprisingly similar biochemical properties (Kiefer et al., 1991), and Wnt and FGF proteins can also collaborate during the induction of Xenopus mesoderm in vitro (Christian et al., 1992; see below).

The pathogenesis of mammary tumors under the influence of an activated *Wnt* gene remains poorly understood. The appearance of mammary hyperplasia in *Wnt-1* transgenic mice indicates that Wnt-1 protein can act as a growth stimulus in early phases of the carcinogenic process, but roles for *Wnt* genes in later stages—malignant conversion, escape from hormonal (e.g., pregnancy) dependence, or acquisition of metastatic potential—have not been defined. The *Wnt* genes most convincingly implicated in mammary tumorigenesis (*Wnt-1* and *Wnt-3*) are not normally expressed at detectable levels in the mammary gland; hence, receptors for their products might not be expected to be present on mammary cells. However, several other members of the *Wnt* gene family are expressed during maturation of the mammary gland (see below and Table 2). Thus, it is likely that the surfaces of mammary cells are equipped with either a general receptor for Wnt proteins or receptors for the normally produced Wnt proteins that can be usurped by products of the activated genes. It has not been established whether those *Wnt* genes that are normally active in the mammary gland can act as oncogenes if expressed at yet higher levels. Nor has an alternative proposal been formally excluded: that oncogenic Wnt proteins interfere with growth-restraining signals supplied by Wnt proteins normally found in the mammary gland.

Local effects upon cell growth by Wnt and other secretory proteins may occur by either an autocrine or a paracrine mechanism, but no evidence decisively distinguishes between these mechanisms in mammary tumorigenesis. Because mouse mammary tumors are composed mostly of clonal populations of cells bearing an insertionally activated *Wnt* or *FGF* locus, or both, the autocrine mode appears to be preferred. Nevertheless, in a few tumors with multiple mutations, transplantation has ultimately separated tumor cells with different mutations (Mester et al., 1987; Roelink et al., 1992), implying that the original tumors were oligoclonal and perhaps sustained in part by paracrine mechanisms.

Tumors induced by secretory factors, such as Wnt proteins or FGFs, may be useful models for human cancers in which growth factors or their receptors have been implicated in pathogenesis. To date, however, mutations of *Wnt* genes have not been reported in tumors other than mammary or salivary gland carcinomas in MMTV-infected or transgenic mice. In particular, such mutations have not been observed in human breast cancer (Van de Vijver et al., 1989; Van de Vijver and Nusse, 1991), although the number of *Wnt* genes studied and the extent of the surveys have been limited. Two other targets for MMTV insertion mutations, *int-2* and *hst*, are sometimes amplified in human cancer, including breast cancer (e.g., Ali et al., 1989), but the amplified *FGF* genes are not expressed, and coamplified genes (such as *bcl-1/PRAD1* [Lammie et al., 1991]) are also candidate oncogenes.

### Expression of the *Wnt* Genes in the Mouse and the *Wnt-1* Phenotype

The highly restricted pattern of expression of *Wnt-1* in adult and embryonic mice (Jakobovits et al., 1986; Shackleford and Varmus, 1987; Wilkinson et al., 1987) was the first indication that *Wnt* genes might regulate major events in mammalian development. Since then, many *Wnt* family members have been shown to be expressed in equally provocative patterns during mouse embryogenesis (Gavin et al., 1990; McMahon and McMahon, 1989; Roelink and Nusse, 1991; Table 2). Although the lack of suitable antisera has prevented definition of Wnt protein distribution within tissues, the sharp boundaries observed by in situ hybridization to *Wnt* RNA suggest that the genes are involved in the establishment of specific cell fates in a regional manner.

This proposal has received its most resounding support from the phenotype of *Wnt-1* mutants in mice. Presumptive null mutations have been obtained in two ways: *Wnt-1*$^{neo}$ alleles result from homologous targeting events that insert *neo* into the second exon of *Wnt-1* in embryonic stem cells (Thomas and Capecchi, 1990; McMahon and Bradley, 1990), whereas *Wnt-1*$^{sw}$ is a naturally occurring single nucleotide deletion mutation that prematurely terminates translation in the middle of the *Wnt-1* open reading frame in *swaying* mice (Lane, 1967; Thomas et al., 1991). Mice homozygous for either *Wnt-1*$^{neo}$ or *Wnt-1*$^{sw}$ and double heterozygotes display a range of phenotypes. At one extreme, animals die around the time of birth, lacking the entire cerebellum and a significant portion of the midbrain (McMahon and Bradley, 1990; McMahon et al., 1992). The loss of these structures can be detected in embryos as early as day 9.5, although the remaining parts of the central nervous system (CNS), at that age and later, are remarkably intact. At the other extreme, some homozygous mutant animals survive into adulthood, suffering from ataxia and lacking the anterior half of the cerebellum (Thomas and Capecchi, 1990; Thomas et al., 1991). Thus, the region affected in *Wnt-1* mutants minimally includes the anterior half of the cerebellum, but often extends significantly into the midbrain or the posterior cerebellum. The explanation for the variable penetrance of the *Wnt-1* mutations is unknown, but may be due to several factors, including the genetic background.

The *Wnt-1* expression pattern in the developing CNS between days 8 and 14 of embryogenesis partially coincides with the regions affected in *Wnt-1* mutants (Figure 3 and Table 2; Wilkinson et al., 1987; McMahon et al., 1992). Around day E9.5, *Wnt-1* RNA is detected in a circle

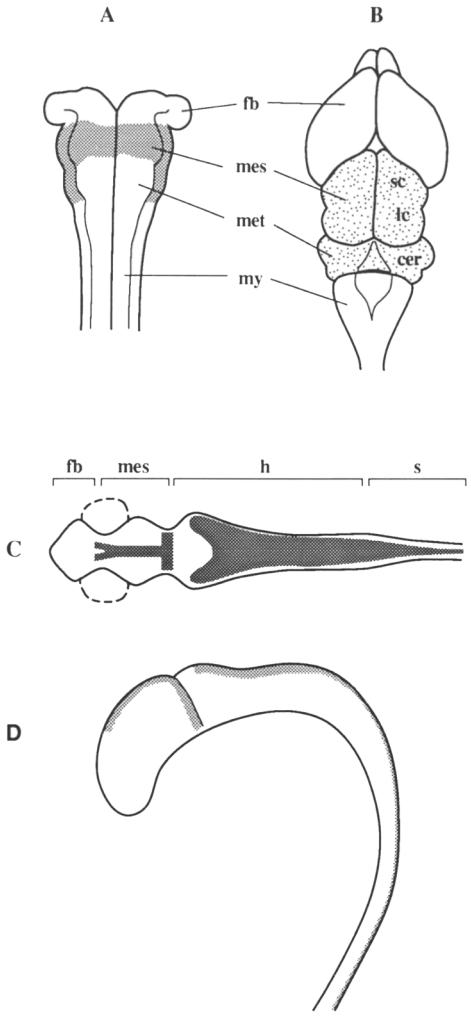


Figure 3. Expression of *Wnt-1* in Mouse and Zebrafish Embryos and the *Wnt-1* Phenotype

The expression pattern of *Wnt-1* in the brain of an 8.5 day embryo (stippled in [A]) is compared with the area deleted in a newborn *Wnt-1* mutant (stippled area in [B]). The superior and inferior colliculi (sc, ic), derived from the midbrain (mes), and the cerebellum (cer), derived from the anterior hindbrain (metencephalon [met]), fail to develop in the example shown; but in some embryos, the midbrain and posterior half of the cerebellum remain intact, as discussed in the text. The forebrain (fb) and myelencephalon (my) seem to be unaffected. (C) and (D) show the pattern of expression (stippled area) of *Wnt-1* in the CNS of a 22 hr zebrafish embryo. Dorsal view (C); side view (D). Expression is seen in dorsal structures of the mesencephalon (mes), the hindbrain (h), and spinal cord (s), and in a ring anterior to the midbrain–hindbrain junction. As in the mouse, *Wnt-1* is not expressed in the anterior hindbrain and the forebrain (fb). (Reproduced with permission from Nature *347*, p. 335, 1990, and from EMBO J. *10*, p. 805, 1991.)

of cells in the most posterior portion of the midbrain, just anterior to the junction with the hindbrain (Wilkinson et al., 1987), in the region that contributes to the anterior half of the cerebellum (Hallonet et al., 1990). In addition, the gene is expressed at early times in the region that probably becomes the midbrain (or tectum) in the mature animal. A strikingly similar pattern of *Wnt-1* RNA in the zebrafish embryo, including the ring of cells in the midbrain, underscores the functional relevance of these expression sites (Molven et al., 1991; Figure 3). *Wnt-1* is not expressed, however, in the anterior hindbrain, the region from which the posterior half of the cerebellum is derived (Hallonet et al., 1990). It has been postulated that the absence of the entire cerebellum is an indirect effect of *Wnt-1*, mediated through the loss of expression of the mouse *engrailed* (*en*) genes (McMahon et al., 1992). In this scheme, *Wnt-1* would normally maintain *en* expression through an inductive event in adjacent tissue, in much the same way as found in Drosophila embryos (see below). Expression of *en* would then be essential for the correct outgrowth of the posterior half of the cerebellum.

*Wnt-1* mutants appear to have a normal spinal cord and hindbrain, even though *Wnt-1* is normally expressed in the caudal part of the hindbrain and in the dorsal midline of the spinal cord from the earliest times that those structures are recognizable (Figure 3; Wilkinson et al., 1987; Molven et al., 1991; McMahon et al., 1992). The absence of a mutant phenotype at other *Wnt-1* expression sites in the CNS is probably due to complementing genes, particularly other *Wnt* genes. The *Wnt-3A* gene, which is expressed in the caudal hindbrain and the roofplate in the spinal cord, but not the posterior midbrain, is a leading candidate to complement the *Wnt-1* deficiency (Roelink and Nusse, 1991; McMahon et al., 1992).

In adult mice, *Wnt-1* is normally expressed only in the male germline, in the round spermatid stage of spermatogenesis (Shackleford and Varmus, 1987). Since at least some *swaying* males are fertile (P. Neumann, personal communication), *Wnt-1* is not absolutely required for male gametogenesis.

The sites of expression of most additional members of the *Wnt* family are still incompletely catalogued. Transcripts of many *Wnt* genes have been found in different, spatially restricted patterns in embryos, in most organs of adult animals, with the possible exception of lymphoid tissues, and in cells derived from all three germ layers (see Table 2; Gavin et al., 1990; McMahon and McMahon, 1989; Roelink and Nusse, 1991). Frequent expression of other *Wnt* genes during development of the CNS suggests functions similar to those provided by *Wnt-1*, at other times and locations (Table 2). *Wnt* genes may also be involved in normal development of the limbs. For instance, *Wnt-3* is expressed in the ectoderm overlying the limb buds (Roelink and Nusse, 1991), and *Wnt-5A* is expressed initially in the ectoderm and later in a gradient in the limb mesenchyme, with the highest levels at the distal end (Gavin et al., 1990). These patterns suggest a role in directing the growth and polarity of the limbs, possibly in conjunction with similar gradients of homeobox gene products (Dollé et al., 1989; Oliver et al., 1988). Pairs of *Wnt* genes highly related to each other (*3* and *3A*; *5A* and *5B*; *7A* and *7B*) usually have distinct, nonoverlapping expression sites (Gavin et al., 1990); for example, in contrast to *Wnt-5A*, *Wnt-5B* shows no gradients in limb buds, implying that very similar proteins act at different sites, perhaps through a common biochemical mechanism.

Although the role of *Wnt* genes in postnatal differentiation has yet to be carefully studied, special attention has

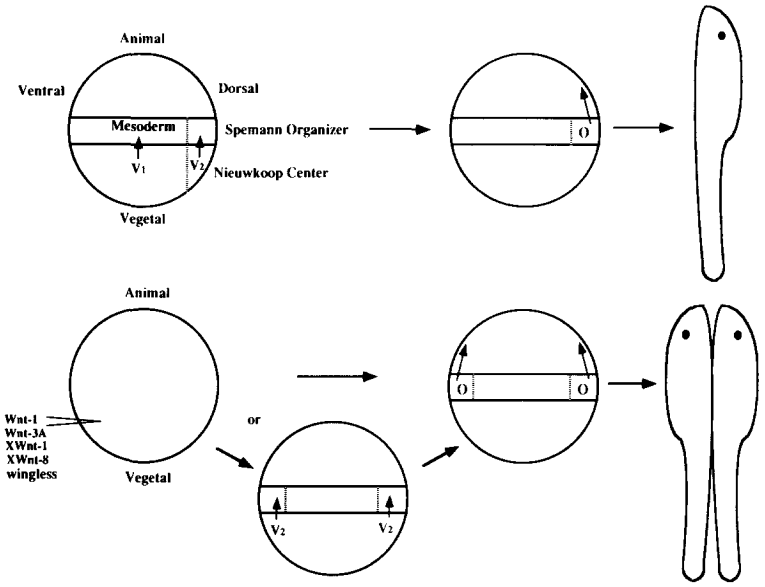


Figure 4. The Role of *Wnt* Genes in Early Frog Development

The upper half shows a current model of mesoderm induction in Xenopus embryos. A signal from the vegetal half (V1) induces mesoderm in the overlying animal cap of the blastocyst. At the dorsal side of the embryo, a special signal (V2) from the Nieuwkoop center induces a Spemann organizer (O) in the mesoderm. This organizer determines the axis of the embryo. The lower half shows that the injection of any of several *Wnt* RNAs results in the formation of a second axis through the generation of a second Spemann organizer. This could result from the injected cells acting as a second organizer (upper arrow; Sokol et al., 1991) or as a second Nieuwkoop center, which in turn induces a second organizer (lower pathway). In the latter case, the *Wnt*-injected cells would not be present in the induced second axis, consistent with the findings of Smith and Harland (1991).

been directed to the normal mammary gland, in view of the oncogenic effects of *Wnt* genes discussed earlier. At least six *Wnt* genes are expressed in the mammary gland, in a variety of temporal patterns, in virgin, pregnant, and lactating animals; none of these seems to be frequently activated in mouse mammary cancer (Gavin and McMahon, 1992; J. Rosen, personal communication; Table 2). Although difficulties with in situ hybridization techniques have impeded description of the spatial patterns, it seems likely that *Wnt* genes are important regulators of development in the mammary gland—and perhaps in other adult organs as well.

## Inductive Properties of *Wnt* Genes in Xenopus Embryos

The formation of mesoderm in Xenopus embryos is one of the earliest and most intensely studied inductive events in developmental biology (reviewed in Slack, 1991). The finding that *Wnt* genes can contribute to the experimental induction of Xenopus mesoderm has therefore attracted considerable attention and provided new insights into the biological functions of the genes.

After the polarity of frog embryos has been established by the site of sperm entry and subsequent cortical rotation, cells in the vegetal hemisphere induce mesoderm in the marginal equatorial zone. Mesodermal cells on the ventral side differentiate into muscle and blood cells, while dorsal mesoderm induces neural ectoderm, thereby determining the anterior–posterior axis of the embryo. Transplantation experiments have shown that these dorsal mesodermal cells, which constitute the so-called Spemann organizer, can induce an additional axis at an ectopic site. In the simplest model (Figure 4), dorsal–vegetal cells secrete a substance that induces equatorial mesoderm to form a Spemann organizer, which in turn produces neural inductive factors. UV treatment, which blocks cortical rotation, prevents the formation of dorsal mesoderm and subsequent axis formation, and therefore results in a ventralized embryo.

Mesoderm induction can be mimicked in vitro by addition of factors to isolated ectodermal caps; the induced mesoderm can then itself induce the appearance of neural structures, instead of epidermis, in the ectodermal pieces. Alternatively, the injection of appropriate mRNAs into individual blastomeres can provoke formation of an additional axis or rescue UV-treated embryos. Through such experiments, several growth factors from the FGF and the TGFβ family, activin in particular, have been implicated as mesoderm-inducing growth factors (Kimelman and Kirschner, 1987; Smith et al., 1990; Thomson et al., 1990; Slack et al., 1989). But activin has been reported to induce only a partial dorsal axis, lacking anterior structures, and activin RNA appears unable to rescue UV-treated embryos (Sokol et al., 1991).

Various members of the *Wnt* gene family, injected as mRNA, have more potent effects. For instance, injection of mouse *Wnt-1* RNA into fertilized eggs causes the primary axis to split anteriorly, producing two-headed embryos (McMahon and Moon, 1989). More impressive, injections of *Wnt* RNA into individual ventral blastomeres in 16- to 32-cell embryos induce a complete secondary axis (Figure 4), including the most anterior structures and sometimes a tertiary axis when an additional blastomere is injected (Sokol et al., 1991). Moreover, UV-treated embryos can be rescued almost completely by *Wnt-1* RNA, just as though functional organizer tissue had been implanted. In line with the supposition that Wnt-1 protein has organizing activity, the *Wnt*-injected cells can sometimes be detected in the induced axis itself when injected into certain cells (Sokol et al., 1991). Injection into other blastomeres, however, can still induce a dorsal axis consisting of uninjected cells, indicating that *Wnt* acts as the dorsal–vegetal inducer or the so-called Nieuwkoop center (Smith and Harland, 1991; Figure 4).

Table 4. Drosophila Segment Polarity Genes

| Phenotype | Genes | Structure of Protein/Homology to: | Interaction with *wg* | Maternal Effect? | Autonomous? |
|---|---|---|---|---|---|
| *naked*-like | *naked* | Not determined | Phenotype of *naked* and *zeste-white*-3 similar to HS-*wg*; inhibit expression of *en* | ? | ? |
| | *zeste-white-3* | Serine/threonine kinase | | Yes | Yes |
| *patched*-like | *patched* | Multiple transmembrane | Inhibits *wg* expression | No | No |
| | *costal-2* | Not determined | In *patched* pathway | Yes | No |
| *wg*-like | *wg* | Secreted factor | | No | No |
| | *arm* | β-Catenin/plakoglobin | Required for normal function of *wg*; protein localization controlled by *wg* | Yes | Yes |
| | *porcupine* | Not determined | Required for normal function of *wg* | Yes | No |
| | *dsh* | Sequence known, novel structure | Required for normal function of *wg* | Yes | Yes |
| | *fused* | Serine/threonine kinase | In *patched* pathway | Yes | ? |
| | *gooseberry* | Homeobox; paired box | Maintains late *wg* expression | No | No |
| | *hedgehog* | Not determined | May relieve *patched* inhibition of *wg* | No | No |
| | *CiD/Cell* | Zinc finger | | No | No |
| | *smooth* | Not determined | | ? | ? |
| *en*-like | *en* | Homeobox | Expression transiently dependent on *wg*; and vice versa | No | Yes |

Of the several other members of the *Wnt* gene family tested in these biological assays, Xenopus *Wnt-1* and mouse *Wnt-3A* (R. Moon, personal communication), *wg* (Chakrabarti et al., 1992), and the Xenopus *Wnt-8* RNA can induce a dorsal axis (Table 3). In fact, an X*Wnt-8* clone has also been isolated by screening cDNAs from a Xenopus expression library for competence to rescue UV-treated embryos (Smith and Harland, 1991). The fact that five *Wnt* genes give the same phenotype suggests that their products are all capable of acting on similar endogenous receptors. Xenopus *Wnt-5A* RNA has different effects: it induces head defects and a posterior duplication of the axis after injection of early embryos (R. Moon, personal communication). This may mean that Wnt-5A protein acts through a different Wnt receptor.

While these results strongly suggest that *Wnt* genes are involved in the induction of dorsal mesoderm and the subsequent establishment of the body axis, it is not clear how they do so. None of the several Xenopus *Wnt* genes tested is naturally expressed during the determination of the organizer. Some show patterns similar to those found in the mouse: X*Wnt-1*, X*Wnt-3A*, and X*Wnt-4* RNAs, for example, are present in the developing CNS at the neurula stage, and X*Wnt-8* RNA is found in the marginal zone of the midblastula, but not at the right place (dorsal–vegetal cells) or during the right time to act as an endogenous inducer (Christian et al., 1991a, 1991b; Smith and Harland, 1991; Table 3). X*Wnt-5A* is the only one to be provided maternally, but it does not induce axes. Possibly an as yet unidentified member of the Xenopus *Wnt* family is the real mesoderm inducer, acting through a receptor that can cross-react with the products of injected *Wnt* RNA. Alternatively, the biochemical effects of Wnt protein might intersect with the signal transduction pathway normally stimulated by an endogenous but different type of inducer.

The ultimate specificity of *Wnt* action as a mesoderm modifier may depend on cooperation with other secreted factors, in the way that some differentiation factors achieve specificity through combinatorial action (Sporn and Roberts, 1988). When animal caps isolated from Xenopus embryos are treated in culture with bFGF, they produce mesoderm of ventral character; but a similar experiment with animal caps from embryos injected with X*Wnt-8* results in mesoderm formation with a distinctly more dorsal nature (Christian et al., 1992). Those explants are also able to produce neural structures not seen after bFGF treatment alone. Since bFGF is present at the right time and place to act as a natural mesoderm inducer (Kimelman et al., 1988), but cannot induce dorsal mesoderm on its own, it has been proposed that *Wnt* modifies the response to bFGF. This observation may be relevant as well to the previously mentioned cooperation between the FGF-related *int-2* gene and *Wnt-1* in virus-induced mammary tumors and in transgenic mice.

Part of the mechanism of action of the *Wnt* genes in Xenopus embryogenesis may involve the regulation of gap junctional activity and hence cellular communication. Injection of *Wnt* RNA opens gap junctions at the ventral side of the embryos, where junctions are normally closed (Olson et al., 1991; Olson and Moon, 1992). There is a good correlation between the effects on the anterior dorsal axis and the ventral gap junctions: *Wnt* genes that induce double-headed embryos open junctions, whereas the double tail–inducing X*Wnt-5A* fails to do so.

### The Drosophila *wg*/*Wnt* Gene Family

*wg*, the Drosophila *Wnt-1* ortholog (Rijsewijk et al., 1987a), plays a key role in the establishment of segment polarity in fly embryos. Because of the extensive genetic analysis of Drosophila segmentation, insects provide a system particularly amenable to deciphering the machinery of *Wnt* action.

During the first few hours of development, the Drosophila embryo is rapidly organized into compartments called parasegments. This process is under the control of a hierarchy of segmentation genes, most of which encode transcription factors (Ingham, 1988). After cellularization, the pattern within each parasegmental unit is further refined

through a diverse group of genes called segment polarity genes. Mutations in those genes lead to deletions and mirror image duplications of the cuticle, usually scored as alterations in the denticle pattern (Nüsslein-Volhard and Wieschaus, 1980). In genetic screens approaching saturation for zygotically required genes, a dozen or so segment polarity genes have been identified and classified according to the region of the cuticle deleted in mutants (Nüsslein-Volhard and Wieschaus, 1980; reviewed in Klingensmith and Perrimon, 1991; Hooper and Scott, 1992 [Table 4]).

Embryonic lethal alleles of *wg* have a very strong segment polarity phenotype and all of the known null mutations map within the protein-encoding domain of *wg* (M. van den Heuvel and R. N., unpublished data). In the absence of the gene, the naked zone of the cuticle is replaced by a continuous lawn of denticles, and parasegmental and segmental boundaries are absent. *wg* null mutations also cause subtle defects in the CNS: the respecification of one neuron present in each segment (Patel et al., 1989). The gene is also required for later development. Several alleles, all of which are rearranged outside the coding domain, are embryonic viable. For example, the first identified allele, $wg^1$, derails the outgrowth of several imaginal discs, in particular the wing disc (Sharma, 1973; Babu, 1977; Baker, 1988), and gave the gene its name.

Early on, it was conjectured that wg could be a signaling molecule. Clonal analysis of *wg* mutant cells suggested that the phenotype is nonautonomous in mosaics, i.e., mutant cells can be rescued by surrounding wild-type cells (Morata and Lawrence, 1977; Baker, 1988). The broad zone of deleted cuticle in *wg* mutants relative to the *wg*-expressing area also indicated that *wg* is required for the differentiation of adjacent cells (Baker, 1987). Secretion of the *wg* gene product has subsequently been observed directly (van den Heuvel et al., 1989; González et al., 1991), consistent with its predicted sequence (Rijsewijk et al., 1987a; Cabrera et al., 1987; Uzvölgyi et al., 1988) and the genetic behavior of *wg*.

In the trunk of the early embryo, *wg* is expressed in 1–2 rows of cells, just anterior to the parasegment boundary (Baker, 1987). This pattern of expression is initiated by earlier acting segmentation genes (such as the pair-rule genes *fushi tarazu* and *even-skipped* [Ingham et al., 1988]), but later becomes dependent on the other segment polarity genes, which interact with each other in a complicated network. Remarkably, the staining patterns for *wg* RNA and protein in whole mount embryos are nearly identical. The protein adheres to the surface of producing cells or associates with the extracellular matrix (van den Heuvel et al., 1989), behaving similarly to its mammalian counterpart and also to a leech *Wnt* gene product (Kostriken and Weisblat, 1992) and to wg protein made in Xenopus oocytes injected with *wg* RNA (Chakrabarti et al., 1992). But upon close inspection in the electron microscope, some wg protein can be detected, presumably endocytosed, in cells adjacent to those that make the RNA (van den Heuvel et al., 1989; González et al., 1991), indicating cell-to-cell spread.

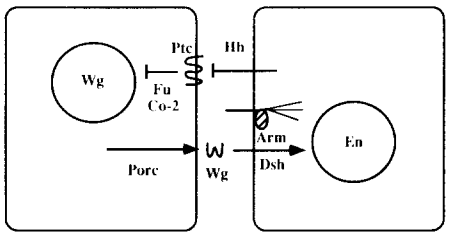


Figure 5. Simplified Scheme of Interactions between the Products of *wg* and Other Segment Polarity Genes

wg positively regulates expression of the *en* (En) gene in adjacent cells. The product of *porcupine* (Porc), a nonautonomous segment polarity gene, is required for wg function. The wg receptor is unknown. *armadillo* (Arm) and *dishevelled* (Dsh) are required in the pathway between wg and en. The arm protein is homologous to β-catenin and therefore likely present in a complex with cytoskeletal and transmembrane proteins. Transcription of *wg* is negatively regulated by the patched (Ptc) protein, a multiple transmembrane protein, through the actions of *fused* (Fu), a serine/threonine kinase, and *costal-2* (Co-2). Inhibition of patched is relieved by *hedgehog* (Hh) activity. Not shown here are many additional regulatory interactions between segment polarity genes.

### The *wg* Signal Transduction Pathway

*wg* influences the expression of many other genes in the Drosophila embryo, including *Distal-less*, a homeobox-encoding gene essential for proximal–distal patterning in the limbs (Cohen, 1990), and *labial*, a homeotic gene expressed in the midgut cells adjacent to wg-producing visceral mesoderm (Immerglück et al., 1990). The best-known example of such regulatory interactions is the effect of *wg* upon *en* in the epidermis of extended germband embryos (Figure 5). *en* encodes a homeodomain transcription factor that is expressed within the posterior part of each segment, in a row of cells just posterior to those expressing *wg*. Levels of *en* RNA and protein decline prematurely in *wg* mutants (DiNardo et al., 1988; Martinez-Arias et al., 1988; Bejsovec and Martinez-Arias, 1991; Heemskerk et al., 1991); conversely, *en* is necessary for maintenance of *wg* expression. The intercellular signal mediating the control of *wg* by *en* has been postulated to be the product of the *hedgehog* gene, which may interact with the cell surface protein encoded by *patched* (Ingham et al., 1991). Genetic tests indicate that *hedgehog* activates *wg* by preventing the patched cell surface protein from inhibiting expression of *wg* (Figure 5).

*wg* also controls the localization of the *armadillo* (*arm*) gene product in both an autocrine and a paracrine fashion. *arm* is a segment polarity gene in the *wg* class (Table 4), and it encodes a protein highly similar to the vertebrate proteins plakoglobin and β-catenin, found associated with cadherins in desmosomes and adherens junctions (Peifer and Wieschaus, 1990; McCrea et al., 1991). *arm* RNA and protein are present in all embryonic cells (Riggleman et al., 1989), and the protein colocalizes at the inner cell surface with F-actin, like the junctional proteins to which it is related. In wild type but not in *wg* mutant embryos, the arm protein seems to accumulate to especially high levels in cells making *wg* RNA and in immediately adjacent cells (Riggleman et al., 1990).

When *wg* is expressed ectopically under the control of a heat shock–inducible promoter, the expression of many *wg*-dependent genes changes. In particular, the areas of cells expressing *en* and *Distal-less* broaden, and arm protein is detected at elevated levels in all cells in the embryo (J. Noordermeer, R. N., and P. Lawrence, unpublished data). This implies that wg receptors are widespread.

In spite of the extensive analysis of *wg* and other segmentation genes, one of the most sought-after components in this signaling system has not been identified: the wg receptor. *arm* and *dishevelled* (*dsh*) both fulfil some criteria for a Wg receptor gene: mutations behave in a cell-autonomous fashion (Table 4; J. Klingensmith and N. Perrimon, personal communication); *arm* and *dsh* deficiencies appear very similar, if not identical, to *wg* deficiencies; and, being maternally acting genes, they are probably expressed ubiquitously. Moreover, in embryos mutant for *arm* and *dsh*, expression of *en* decays in the same pattern as in *wg* mutants, further indicating that their gene products may indeed be in the same pathway (M. van den Heuvel and R. N., unpublished data). Neither arm nor dsh protein, however, exhibits the hallmarks of cell surface receptor molecules, such as extracellular domains or catalytic functions (J. Klingensmith and N. Perrimon, personal communication). These proteins might be present in a receptor complex, but most likely do not make direct contact with the wg ligand.

It is possible that genetic screens have failed to identify the wg receptor because of functional redundancy (the presence of multiple receptor genes) or because of pleiotropic effects through interactions with multiple *Wnt* genes. If pleiotropism is responsible, it could be due to the presence of at least two *wg*-related genes D*Wnt-2* and D*Wnt-3* (Russell et al., 1992; A. McMahon and A. M. C. Brown, personal communication). Transcripts of both genes are detected in early embryos and in limb primordia. D*Wnt-2* is expressed in a predominantly segmented pattern and in the presumptive gonads, whereas D*Wnt-3* RNA is found in mesodermal and neurogenic regions. If the D*Wnt-2* and D*Wnt-3* proteins interact with the same receptor as wg protein, receptor mutants would probably not resemble *wg* mutants and the receptor gene might not be classified as a segment polarity gene. A confounding factor may also be that the mutational analysis of Drosophila segmentation genes has not been exhausted for genes with a maternal contribution (Perrimon et al., 1989).

## Common Mechanisms of *Wnt* Gene Action in Diverse Organisms?

The conservation of Wnt protein sequences during a billion years of evolution (see Figure 1) argues that the proteins retain common functional properties, a notion confirmed by the activities of *Wnt* genes transplanted to distantly related organisms. Thus, *Wnt* genes from flies and mice can induce axis duplication in frog embryos, and *wg* can transform mouse epithelial cells.

Such interchangeability of *Wnt* genes is dramatic, but is it instructive? One potentially useful strategy is to ask whether genes that are known to interact in one organism exhibit homologous relationships in others. For example, the sites of expression of two *en* genes in the mouse overlap those of *Wnt-1* in the early embryonic midbrain (Davis and Joyner, 1988; McMahon et al., 1992), and there is evidence from both mice and flies for the notion that *Wnt* genes are required to maintain, but not to initiate, expression of *en* genes. Unlike the situation in the Drosophila embryo, however, where adjacent rows of cells express either *wg* or *en*, some cells in the developing mouse brain must express both genes. Furthermore, although *en* RNA-positive cells are virtually absent in the developing brain of *Wnt-1*-deficient mice, this may be due to the loss of relevant cells rather than to the loss of a positive regulator of *en*. Thus, it remains uncertain whether *en* and *Wnt* genes cross-regulate each other in vertebrate species as they do in the fly embryo.

The pursuit of *Wnt* genes in many organisms offers the hope of perceiving an important universal effect of Wnt proteins in some experimentally favorable setting. The induction of gap junctions by *Wnt* RNA in ventral cells of Xenopus blastomeres has raised the possibility that a general function of Wnt proteins might be to promote cell–cell communication, perhaps by stimulating adhesion of cells as a prelude to formation of gap junctions. Such ideas have been nurtured by the evidence that Drosophila *wg* regulates arm protein, which is, in turn, closely related to proteins present in adherens junctions of vertebrate cells. Since the arm homologs, plakoglobin and β-catenin, associate with transmembrane proteins, such as cadherins, it is tempting to consider the possibility that such transmembrane proteins might be Wnt receptors. In this scheme, Wnt proteins would promote cell–cell adhesion by altering cadherin-like receptors, redistributing arm and its relatives, and ultimately forming gap junctions.

There are, however, problems with this hypothesis. Intuitively, abundant and widespread proteins, such as cadherins, might seem poor candidates for Wnt receptors, given the paucity of cultured cell lines that respond to *Wnt* genes. It is also unlikely that *Wnt*'s role in carcinogenesis would be mediated in this way since transformed mammalian cells tend to be deficient in gap junctions, not overendowed with them (e.g., Eghbali et al., 1991). Furthermore, it is not known whether redistribution of arm protein actually accompanies changes in cell adhesion in the fly embryo, as predicted by the model. Finally, gap junctional communication in Drosophila imaginal discs does not coincide with sites of *wg* action (Fraser and Bryant, 1985).

## Prospects

*Wnt* genes are now recognized as mediators of cell–cell signaling events that are important during pattern formation and experimental carcinogenesis, but our understanding of these genes is still in its early stages. The full genetic repertoire has probably not been isolated from any organism, the patterns of expression are incompletely defined for any single gene except *wg*, and the consequences of null mutations are known only for one *Wnt* gene in the mouse and one in the fly.

Most importantly, receptors for Wnt proteins, central in-

gredients in the pathways for signaling, are not known. Many of the proposed organismal effects of Wnt proteins in neural development, tumorigenesis, axis formation, and segmentation need to be related to changes observed in individual cells, such as changes in shape, growth, adhesion, and gap junctions. Any hope of understanding the biochemical events that mediate such changes depends upon identifying and characterizing the molecules that interact directly and specifically with Wnt proteins. Unfortunately, the immediate prospects for isolating Wnt receptors are dimmed by the experimentally unfavorable nature of Wnt proteins, by uncertainties about the distribution, abundance, diversity, and biochemical properties of the receptors, and by the possibility that the numerous Wnt proteins might each be acting through a multisubunit complex formed with components of the extracellular matrix. To overcome these difficulties, it may be essential to learn to prepare some biologically active Wnt protein in a soluble, cell-free form. This is likely to require studies of a variety of Wnt family members, generation of more *Wnt* mutants, and better mapping of domains involved in secretion, heparin binding, and signaling. If Wnt receptors can be identified, permitting analysis of biochemical responses to Wnt proteins under simple conditions in cultured cells, it should be possible to define with precision the signaling mechanisms that allow Wnt proteins to promote cell growth, differentiation, or survival in the many biological settings reviewed here.

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