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Series Introduction: Invasive growth: from development to metastasis

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Perspective

Invasive growth is a complex morphogenetic program in which proliferative responses are integrated by apparently independent events such as migration, survival, matrix degradation, and induction of cell polarity. In the first step of this sequence (Figure 1), a cell within a colony or solid tissue is instructed to disrupt cadherin-based intercellular junctions and acquire a fibroblastoid, motile phenotype, initiating detachment from the primary site of accretion. This dramatic reshaping is accompanied by cytoskeletal rearrangements and enhanced production of matrix proteases, which digest basal lamina components and facilitate cell movement through the surrounding environment. During this phase, invading cells must induce a constant and dynamic remodeling of integrin-mediated adhesive contacts with the ECM, which provides a mechanical support for cell migration and prevents the induction of apoptosis. Cell depolarization and invasion are followed by stimulation of cell growth, which allows new regions of the extracellular environment to become populated with cells, setting the stage for the restoration of normal tissue complexity. Ultimately, these cells stop dividing, repolarize, and start terminal differentiation, arranging themselves into three-dimensional structures that are usually organized as branching tubules (1). Invasive growth in normal development Not surprisingly, the physiological conditions for invasive growth are found in embryonic and fetal development. For instance, during epithelial morphogenesis, cells form ramified tubules and papillary outgrowths that compose the parenchymal architecture […]

SERIES INTRODUCTION

Invasive growth: from development to metastasis

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Invasive growth is a complex morphogenetic program in which proliferative responses are integrated by apparently independent events such as migration, survival, matrix degradation, and induction of cell polarity. In the first step of this sequence (Figure 1), a cell within a colony or solid tissue is instructed to disrupt cadherin-based intercellular junctions and acquire a fibroblastoid, motile phenotype, initiating detachment from the primary site of accretion. This dramatic reshaping is accompanied by cytoskeletal rearrangements and enhanced production of matrix proteases, which digest basal lamina components and facilitate cell movement through the surrounding environment. During this phase, invading cells must induce a constant and dynamic remodeling of integrin-mediated adhesive contacts with the ECM, which provides a mechanical support for cell migration and prevents the induction of apoptosis. Cell depolarization and invasion are followed by stimulation of cell growth, which allows new regions of the extracellular environment to become populated with cells, setting the stage for the restoration of normal tissue complexity. Ultimately, these cells stop dividing, repolarize, and start terminal differentiation, arranging themselves into three-dimensional structures that are usually organized as branching tubules (1).

Invasive growth in normal development

Not surprisingly, the physiological conditions for invasive growth are found in embryonic and fetal development. For instance, during epithelial morphogenesis, cells form ramified tubules and papillary outgrowths that compose the parenchymal architecture of several organs, including exocrine glands, gut, liver, and kidney. Similarly, the clustering and reorganization of angioblasts to form capillary-like structures during vasculogenesis, and the formation of new capillaries by sprouting or splitting from pre-existing vessels during angiogenesis, represent two other examples of invasive growth. A specialized aspect of this phenomenon is observed in the wiring of the nervous system, when axons must elongate and emit ramifications to attain targets that are often significant distances away.

Although best documented in cells of epithelial, endothelial, and neural origin, invasive growth also takes place in other tissues. In the bone marrow, it occurs when hemopoietic precursors dissociate from their niches, migrate, cross blood vessel walls, and even-

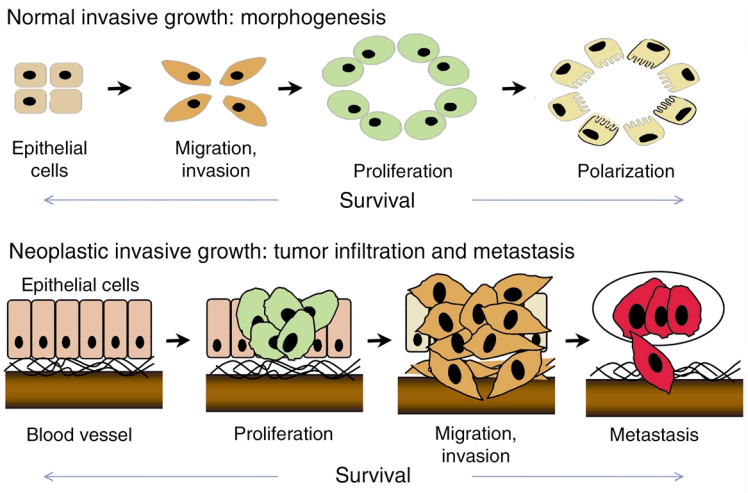

Figure 1
The invasive growth program under physiological and pathological conditions. In both settings, invasive growth results from analogous biological processes – cell-cell dissociation and migration, cell multiplication, and survival – but the endpoints are different. Normal cells exploit invasive growth to colonize new territories and build polarized three-dimensional structures, thus forming the parenchymal architecture of several organs. Cancer cells implement this program aberrantly to infiltrate the adjacent surroundings and form metastases.

tually reach the circulation. In the bone, it comes about when osteoclasts proliferate and invade the mineralized matrix, dynamically erode it, and recruit osteoblasts to generate osteogenic centers. Finally, the morphogenesis of some peripheral muscles depends on the migration of myoblast precursors from myotomes and shares many of the features of invasive growth.

It has long been known that many cytokines and growth factors can produce many of the signal features of invasive growth: proliferation, differentiation, chemotaxis, and protection from apoptosis. Perhaps surprisingly, full execution of the various steps that make invasive growth possible does not arise from the concerted action of many such factors. Rather, the temporal and spatial control of this process can be largely attributed to two molecules that are structurally related to plasminogen (the plasminogen-related growth factor-1 [PRGF-1] and PRGF-2; ref. 2), and, possibly, to a phylogenetically related family of ligand-receptor pairs represented by the semaphorins and plexins. In this Perspective series, we will discuss the role of PRGFs, integrins, the cadherin-catenin system, and the semaphorins in the control of cell growth, migration, and survival during invasive growth, and we will consider the signaling mechanisms responsible for converting the biochemical activity of all these molecules into biological effects.

PRGFs as specific mediators of invasive growth

PRGF-1, also known as HGF or scatter factor (SF), derives from a single-chain, biologically inert glycoprotein precursor, which is secreted and then sequestered by cell surface and matrix proteoglycans. Under appropriate conditions, this pro-protein is converted into its bioactive form by limited intramolecular proteolysis between two positively charged amino acids. Several proteases are reported to activate PRGF-1 in vitro, including urokinase-type and tissue-type plasminogen activators, a serine protease isolated from serum and homologous to coagulation factor XII, and coagulation factor XII itself. Mature PRGF-1 is a heterodimer consisting of a 62-kDa α and a 32-kDa β chain held together by a disulfide bond. The α chain contains an N-terminal hairpin loop followed by four peculiar motifs known as kringles (80–amino acid double-looped structures formed by three internal disulfide bridges), whereas the β chain is homologous to serine proteases of the blood-clotting cascade. The α chain is responsible for receptor binding, and the β chain is required for full receptor activation and execution of the biological responses (3).

PRGF-1 was discovered independently as a strong growth-promoting agent in hepatocytes (hence "HGF") and as a mesenchymal-derived effector of dissociation and cell motility (the scattering referred to by the name "scatter factor") in polarized epithelial cells (4, 5). After biochemical purification and cDNA cloning, the two proteins were shown to be the same molecule (6). Following severe tissue damage in various epithelia, PRGF-1 is a potent survival and regeneration factor. Its role in organ reconstruction depends on both potentiation of cell growth and modulation of complex architectural events that are instrumental for the re-establishment of normal tissue patterning. Indeed, PRGF-1 promotes remodeling of epithelial cells cultured in three-dimensional collagen gels (7), induces the formation of branching tubular structures in mammary gland (8) and metanephric organ cultures (9), and contributes to lung (10), tooth (11), and hair follicle (12) maturation.

More generally, PRGF-1 stimulates the several facets of invasive growth in virtually every tissue of the body. It acts as a potent angiogenic factor (13); it controls bone formation and resorption (14) as well as cartilage remodeling (15); it promotes amplification and differentiation of multipotent and erythroid precursors, their motility through the bone marrow stroma, and their dismission into the bloodstream (16); and it supports survival and neurite outgrowth of sensory and sympathetic neurons (17). The pivotal role of this cytokine in invasive growth is highlighted by the phenotype of PRGF-1–deficient (*Hgf*$^{-/-}$) mice. These mutants die in utero because of severe impairment of the placental trophoblast, which appears undersized and unable to colonize the maternal tissues to expand the placenta. Moreover, *Hgf*$^{-/-}$ mice display extensive loss of liver parenchymal cells and lack muscles of the forelimbs, diaphragm, and tip of the tongue — all tissues that derive from long-range migration of precursor cells (18, 19).

The second member of the family, PRGF-2, was initially named macrophage-stimulating protein (MSP) due its ability to make resident peritoneal macrophages responsive to chemoattractants (20). The synthesis and modular structure of MSP are remarkably similar to those of PRGF-1, in that the molecule is secreted as an inactive single-chain precursor, which is then converted into the active heterodimer by an endoproteolytic cleavage that generates a 53-kDa α chain and a 25-kDa β chain. Interestingly, even though the full-length MSP is necessary to evoke cellular responses, the receptor-binding site of PRGF-2 is located in the serine protease–like β chain (21). Like PRGF-1, PRGF-2 stimulates growth, motility, and branching morphogenesis of liver progenitor cells as well as proliferation and scattering of keratinocytes (22). In addition, PRGF-2 can participate in the development of liver, lung, gut, kidney, and specific parts of the nervous system, including spinal ganglia and the nucleus of the hypoglossus nerve (23). In osteoclasts, an MSP-dependent ligand-receptor autocrine loop facilitates bone resorption (24). In the hematopoietic system, MSP not only activates the phagocytic and chemotactic capability of macrophages but also promotes maturation and differentiation of megakaryocytes (25).

PRGF receptors, the initiators of the invasive growth response

The receptors for PRGF-1 and -2 are encoded, respectively, by the protooncogenes *MET* (26, 27) and *RON* (28, 29). The protein products of these protooncogenes are single-pass, disulfide-linked α/β heterodimers arising by proteolytic processing of a common precursor in the post-Golgi compartment (30). In both receptors, the α chains are extracellular glycoproteins while the

β chains are transmembrane subunits harboring an intrinsic tyrosine kinase activity in their cytoplasmic portion. The intracellular domains of Met and Ron include well-conserved catalytic sites flanked by regulatory juxtamembrane and carboxy-terminal sequences. Two tyrosines (Y^{1234} and Y^{1235}), located in the activation loop within the kinase domain, are essential for full enzymatic activity of the receptor, and their phosphorylation results in strong upregulation of the kinase in an autocatalytic fashion (31). By contrast, phosphorylation of a serine residue in the juxtamembrane domain by either protein kinase C or Ca^{2+}/calmodulin–dependent kinases results in a strong inhibitory effect on receptor activity (32).

The Met carboxy-terminal domain is unique among receptor tyrosine kinases because it contains a single, multifunctional docking site, made of the tandemly arranged degenerate sequence Y*VH/NV, which is responsible for the bulk of receptor signaling activity (33). Substitution of the critical tyrosines with phenylalanines does not interfere with the receptors' autocatalytic activity or their ability to phosphorylate exogenous substrates, but it can impede direct interaction with a vast array of signaling molecules and completely abolishes Met-dependent biological responses. This multifunctional docking site is essential to transduce the PRGF-1 signal in vivo, during mouse embryonic development. Indeed, knock-in mice bearing phenylalanine substitutions in place of the docking-site tyrosines display a severe loss-of-function phenotype remarkably similar to that of mice entirely lacking PRGF-1 or Met (34). Conversely, insertion of this docking site into the tail of other tyrosine kinase receptors rescues the defect in invasive growth, indicating that this sequence is not only necessary, but also sufficient, to elicit PRGF-1–dependent responses (35).

Downstream pathway specificity for PRGF receptor signaling

An intriguing aspect of signal transduction is how activation of a given growth factor receptor can evoke a particular set of cellular responses when the biochemical signaling pathways it stimulates appear nonspecific. In the case of PRGF receptors, this issue is all the more striking, since the invasive growth program that occurs following PRGF stimulation is unique and cannot be recapitulated by other growth factors, but the signaling events activated by these receptors are not obviously different from those involved in other, generic transduction pathways. One possible explanation is that the quantitative aspects of downstream signaling, differences in signal intensity and duration, affect the nature of the biological outcome. Indeed, PRGF-1 can induce a prolonged activation of both Ras-dependent and phosphatidylinositol 3-kinase–dependent (PI3K-dependent) pathways, with significantly elevated activity even 6 hours after stimulation; in contrast, EGF, which is incapable of eliciting the invasive growth response, stimulates these two pathways only transiently (36). These data are consistent with observations made in PC12 cells, where EGF promotes proliferation through rapid activation of mitogen-activated protein kinases, whereas nerve growth factor sustains differentiation through long-lasting activation of these effectors (37).

However, quantitative, threshold effects — producing qualitatively different biological responses from a single, generic signal — may not fully account for the association between Met and invasive growth. Evidence for qualitative specificity in signals downstream of Met derives from a number of in vitro experiments suggesting that the different steps of invasive growth can be experimentally separated from each other and can be ascribed, at least in part, to the action of distinct signaling effectors. Autophosphorylation of the Met docking site results in recruitment and activation of PI3K, the Grb2-Sos complex, Src, and the transcriptional factor STAT3 (33, 38). In addition, the phosphorylated receptor binds Shc and Gab-1, two adaptor molecules that amplify the Met signaling platform by providing additional docking sites for the many of these signaling molecules, as well as dedicated sites for phospholipase C–γ (PLC-γ). Using mutants of Met that can activate the Ras- or PI3K-dependent pathways selectively, we and others have demonstrated that Ras stimulation alone is both necessary and sufficient for proliferation, whereas specific targeting of PI3K is sufficient to promote motility (39, 40). Complementary peptide inhibition and dominant negative approaches have shown that activation of STAT and PLC-γ is required for cell polarization and formation of branched tubular structures (38, 41). Thus, it is possible to dissociate the multifaceted aspects of invasive growth experimentally and to identify committed effectors for each of them. The simultaneous activation of the Ras cascade, which promotes growth, and the PI3K pathway, which promotes motility and suppresses apoptosis, leads to efficient cell dissociation, invasion of the ECM, and metastasis, whereas isolated activation of either pathway does not. The synchrony and cooperation between the various pathways are critical for the correct execution of the program as a whole, and it is this coordination that appears to depend on Met or its relatives.

Qualitative determination of cell fates through activation of specific signaling pathways has recently found an experimental in vivo validation. Maina et al. (42) generated *Met* mutant mice in which the specificity of the multifunctional docking tyrosines responsible for signaling is skewed toward interactions with PI3K, Src, or Grb2. Mice in which both of the docking tyrosines are changed to phenylalanine show defects in placental and myoblast proliferation, as well as in axon growth. Interestingly, mutants carrying optimal PI3K or Src binding motifs complement a specific subset of these developmental defects; Src interactions are sufficient to rescue the placental and myoblast defects, whereas axon growth appears to rely specifically on PI3K binding to Met. Hence, cell-specific developmental events involving invasive growth are regulated, at least in part, by activation of nonredundant pathways.

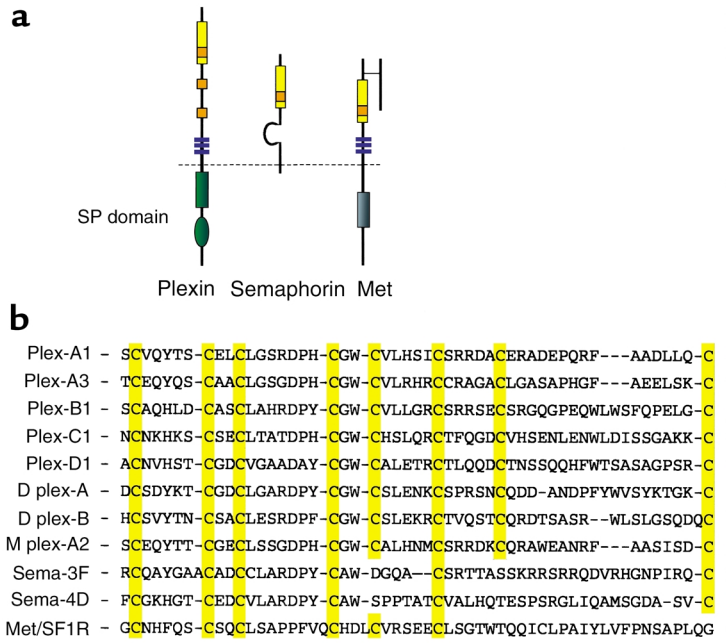

Figure 2
(**a**) Structural features shared by plexins, semaphorins, and PRGF receptors. Plexins are divided into four subfamilies (A–D) based on sequence similarity, structural features, and tissue distribution. All plexins include a highly conserved cytoplasmic domain (SP domain, green box and oval) and, in the extracellular portion, a *sema* domain (yellow) and one or more MRS motifs (orange), which are also present in semaphorins and PRGF receptors. Almost 20 semaphorins are known in humans, falling into five subclasses (3 to 7). As an example, a class 4 transmembrane semaphorin is depicted. (**b**) Protein alignment of an MRS conserved domain. Two or three MRS repeats are found in plexins, whereas only one is present in PRGF receptors and semaphorins. The alignment shows the first MRS motif of human plexin-A1, -A3, -B1, -C1, and -D1, as well as the *Drosophila* plexin-A and -B, the mouse plexin-A2, and the MRSs from Sema-3F, Sema-4D, and Met. The number and the spacing of cysteine residues (yellow) are conserved.

Structural and functional analogies between PRGF receptors, semaphorins, and plexins

The extracellular region of the PRGF receptors contains the so-called *sema* domain, a conserved sequence encompassing about 500 amino acids. The *sema* domain was originally found in the extracellular domains of two independently identified protein families, semaphorins and plexins. Furthermore, the extracellular portions of each of these proteins include a so-called Met-related sequence (MRS) peptide module, containing eight cysteines whose relative positions are well conserved, and three glycine-proline-rich (G-P) repeats (Figure 2). The striking structural similarities between PRGF receptors, semaphorins, and plexins suggest that all these proteins are encoded by cognate genes derived from a common ancestor and forming a new superfamily. From an evolutionary viewpoint, plexins may be envisioned as large transmembrane semaphorins, endowed with a distinctive cytoplasmic domain likely to be involved in signal transduction. On the other hand, PRGF receptors, which have not been identified in invertebrates, probably appeared later in evolution by combining extracellular modules of plexins and semaphorins (such as MRS and *sema* domains) with a cytoplasmic tyrosine kinase domain.

PRGF receptors, semaphorins, and plexins not only are structurally related but also, much more importantly, share intriguing analogies in their biological function and are likely to cooperate in the control of efficient execution of the invasive growth program. In particular, the Met-driven process by which myogenic cells migrate to reach their target tissues is reminiscent of the guidance of long neuritic processes, which is mediated by semaphorins (34). Semaphorins (also named collapsins) were initially identified for their ability to induce steering or collapse of axon growth cones in vitro. In vertebrates, almost 20 semaphorins are known and — according to a newly introduced nomenclature — they fall into seven subfamilies, depending on whether they are soluble, membrane-bound, or glycosylphosphatidylinositol-linked (43). By acting along the paths of growth cone navigation and inhibiting the access to inappropriate zones during axon guidance, semaphorins induce cell dissociation and mutual repulsion, two aspects typical of the invasive growth program. Intriguingly, neurite outgrowth and axon guidance are also controlled by PRGFs and their receptors. Thus, limb buds expressing PRGF-1 attract the axons of a subpopulation of motor neurons that are more abundant in the sections of the spinal cord innervating the limbs (17). Semaphorins do not act exclusively in the developing nervous system but are expressed in a variety of embryonic and adult tissues, where they exert multiple functions (44). For example, semaphorins have also been implicated in endothelial cell motility, capillary sprouting, and in vitro microvessel formation, as well as in the control of T cell proliferation and B cell aggregation, survival, and differentiation in the germinal center. Not surprisingly, all these activities are also critically modulated by PRGFs.

Plexins alone serve as receptors for membrane-bound semaphorins; secreted semaphorins bind to a hetero-oligomeric complex formed by plexins and another class of receptor, the neuropilins (45, 46). Intriguingly, overexpression of plexin-A3 in epithelial cells results in dramatic repulsion of adjacent fibroblasts in mixed cultures in vitro, suggesting that plexins serve a crucial function not only in axon guidance, but more widely in the generation of cell-repelling cues in epithelial morphogenesis and tissue remodeling. The cell-cell communication code controlled by the semaphorin-plexin pairs may represent a general process exploitable by a variety of cells in different biological settings.

The malignant counterpart of invasive growth

Under normal conditions, invasive growth is based upon a finely tuned interplay between related phenomena

including cell proliferation, motility, ECM degradation, and survival. In transformed tissues, aberrant implementation of this interplay is responsible for cancer progression and metastasis, a process by which neoplastic cells weaken tissue constraints and invade foreign compartments, where they may migrate, proliferate, and survive. Hence, the PRGF-dependent xenophilic tendency of malignant tumors is fostered by the very same events that, physiologically, account for the generation and maintenance of organ complexity and architecture.

The oncogenic potential of Met has been documented in a variety of human cancers. In hereditary and sporadic cases of papillary renal neoplasms and in sporadic hepatocellular carcinomas, mutations of *MET* have been found (47) that affect the catalytic behavior of the kinase and lead to constitutive activation of a transforming and invasive signal (48). Interestingly, this kind of tumorigenesis is strictly dependent on ligand stimulation and can be inhibited by PRGF-1 antagonists (49). This suggests that the onset of papillary renal carcinomas is contingent on both the presence of inherited or acquired alterations in Met and the local availability of its normal ligand. In addition, neoplastic cells harboring activating mutations of the *MET* gene undergo clonal expansion during the metastatic spread of head-and-neck squamous cell carcinomas (50). Met overexpression, in the absence of missense mutations, has been found in a variety of aggressive tumors, including thyroid and colorectal carcinomas, and strictly correlates with higher metastatic potential and poor prognosis. In the case of colorectal tumors, such protein overexpression is accompanied by gene amplification and confers a selective advantage for the capability of metastasizing to the liver (51). Finally, while absent in normal adult skeletal muscles, Met is expressed in a significant fraction of human rhabdomyosarcomas and is associated with endogenous secretion of HGF/SF by the same cells, thus generating an autocrine loop that sustains cell invasiveness and tumor malignancy (52).

In light of the evidence that PRGFs, semaphorins, and plexins control common biological functions, it is striking that semaphorin overexpression has also been associated with the invasive and metastatic progression of tumors. For instance, Sema-3C is overexpressed in cancer cells resistant to radiation and cytostatic drugs, in recurrent squamous carcinomas, and in metastatic lung adenocarcinomas (53, 54). Another secreted semaphorin, Sema-3E, is overexpressed in metastatic cell lines in comparison with the nonmetastatic parental population (55). Finally, a recent report has identified a natural soluble form of neuropilin-1 exerting antitumor activity in vivo (56). Currently, the available data implicating these proteins in tumor progress remain sparse compared with those obtained for Met and PRGF-1.

This series

In their accompanying article in this issue, Danilkovitch-Miagkova and Zbar discuss the invasive growth seen in human tumors, particularly papillary renal carcinomas, where *MET* is a well-defined and common oncogene (57). They consider a variety of molecular mechanisms underlying Met deregulation, including heritable point mutations in c-*MET* itself, which render the receptor more active as a tyrosine kinase and favor tumor progression. They show that gene duplication affecting the *MET* locus and activation of the kinase by ligand overexpression or receptor misexpression represent alternative routes to a similar endpoint.

The remaining articles concern the adhesive interactions that are critical for invasive growth. Brakebusch et al. consider the dynamic regulation of integrin affinity for extracellular ligands, as occurs during cell migration and invasion, and they discuss the effects of integrin signaling on the cell cycle and apoptosis (58). Conacci-Sorrell et al. take up related questions with regard to cadherin-mediated adhesion in the adherens junctions of normal and malignant cells (59). These authors emphasize the dual role in cadherin function of β-catenin, which participates both in the mechanical coupling of cadherins to the cytoskeleton and in the transcriptional regulation of genes that are important for cancer cell survival and tumor progression. Finally, Goshima et al. review the roles of semaphorins and their receptors in axon guidance and axoplasmic transport, both of which involve cytoskeletal rearrangements (60). They conclude with a discussion of class 3 semaphorins in the formation of the normal branched architecture of the pulmonary epithelium, a clear example of the profound effects of this signaling system on a complex morphogenic pathway.

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