INTRODUCTION

There is increasing evidence for a positive role of Wnt signaling molecules in the activation of myogenesis in amniote embryos (Münsterberg et al., 1995; Stern et al., 1995; Capdevila et al., 1998; Tajbakhsh et al., 1998), but the mechanism of this process remains elusive. At least another molecule, Sonic hedgehog, is also required to initiate myogenesis in the presomitic mesoderm (Borycki et al., 1998). Moreover, Noggin, a target of Wnt signaling, seems to contribute to the process mainly by neutralizing the inhibitory actions of BMPs (Hirsinger et al., 1997; Marcelle et al., 1997; Pourquie et al., 1996).

It has been proposed that Shh and Wnts may initiate myogenesis by activating the myogenic regulatory factor Myf5 in newly formed somites (Münsterberg et al., 1995); in the mouse, activation of Myf5 in the dorsomedial lip of the dermomyotome leads to epaxial myogenesis. Studies with explant cultures have shown that other signals from the surface ectoderm, which can be replaced by Wnt7a, appear to preferentially activate MyoD in the hypaxial (ventral) domain of somites (Cossu et al., 1996; Tajbakhsh et al., 1998).

Interestingly, Shh, which is expressed medially, is indispensable for epaxial but not for hypaxial myogenesis (Borycki et al., 1999). Therefore, Wnts together with Shh may contribute to the activation of myogenesis through the activation of Myf5 or MyoD (Tajbakhsh et al., 1998), although, an additional role for Wnts in the maintenance/expansion of the myogenic cell population is also possible.

Recently, putative receptors of Wnts have been identified (Bhanot et al., 1996), both as classic transmembrane proteins, termed Frizzled, and as soluble, presumably secreted...
antagonists, differently named by various laboratories (reviewed in Wodarz and Nusse, 1998). The latter molecules are of particular interest for their obvious potential to bind Wnt molecules in the extracellular space and thus prevent their interaction with the target cell.

Attempts to understand ligand-receptor specificity are complicated by lack of solubility of Wnt proteins; much information has been accumulated recently by studying the activation of downstream genes or through biological assays, such as axis duplication, which are induced by certain but not all Wnts and can be enhanced or inhibited by various membrane or soluble receptors. In *Xenopus*, Frzb1 (also known as sFRP3) inhibits axis duplication induced by Xwnt8 and also muscle development (Leyns et al., 1997; Wang et al., 1997; Mayr et al., 1997). In mammalian cells, expression of *SARP1* (also known as *sFRP2*) renders cells resistant to apoptosis while expression of *SARP2* (*sFRP1*) renders cells more susceptible (Mekkonyan et al., 1997). The effect of the mammalian equivalent of Frzb1 (*SARP3/sFRP3*) has not yet been reported. From the current picture, it emerges that in general soluble Wnt binding antagonists inhibit Wnt activity, thus acting as natural dominant negative molecules.

To address the possible role of soluble frizzled-related proteins in regulating myogenesis, we first examined their expression in postimplantation mouse embryos and then focused our study on *Frzb1*, which is expressed in presomitic mesoderm, limb buds and branchial arches at the onset of myogenesis. Here we show that, in vitro, murine *Frzb1* inhibits myogenesis in organ cultures of presomitic mesoderm and newly formed somites. When overexpressed in vivo, using a transplacental delivery system, *Frzb1* interferes with the development of caudal paraxial mesoderm and, in less severely affected embryos, an effect on myogenesis can be distinguished.

**MATERIALS AND METHODS**

**Whole-mount in situ hybridization**

Embryos were prepared for whole-mount in situ hybridization as previously described (Tajbakhsh et al., 1997). β-galactosidase staining, before the whole-mount in situ hybridization of the *Myl5* embryonic embryos, in which the *Myl5* locus is targeted with nLacZ, was performed as described (Houzelstein and Tajbakhsh, 1998). The following probes were used: *Enl* (Acampora et al., 1998), *Pax3* (Goulding et al., 1991), *Mox1* (Cundia et al., 1992), *BMP4* (Jones et al., 1991) and *Noggin* (McMahon et al., 1998). Photographs of whole-mount stained embryos were taken with a Leica MZ8 stereomicroscope using color reversal film Ektachrome 64T. Stained embryos were embedded in 7% gelatin/15% sucrose and 30 μm cryostat sections were cut. An average number of 10 embryos were analyzed for each marker.

**Embryonic explant cultures**

*Myl5* embryonic embryos at E9.5 (20-24 somites) were used for these experiments (Tajbakhsh et al., 1996). The explant culture method was as previously described (Cossu et al., 1996; Tajbakhsh et al., 1998). C3H10T1/2 cells, used as a feeder layer, were transiently transfected with the pcDNA3/Frzb1 vector (a derivative of the in vivo gene pcDNA vector containing the full cDNA of the murine *Frbz1* gene; Leyns et al., 1997). 2×10² cells were transfected with 10 μg of DNA using the PEI (polyethylenimine) method (Boussif et al., 1995).

**Placenta injection**

WOP cells (Without Origin of Polyoma DNA replication, a 3T3 derivative transformed by a replication-defective polyomavirus: Dailey and Basilo, 1985) were transiently transfected (1.8×10⁵ cells with 10 μg of DNA, using the pEII protocol) with the pcDNA3/Frzb1 expression vector, with control empty vector, with a pcDNA3/αcZ vector or with the pcDNA3/mShiA5/UTR vector expressing full-length murine Sonic hedgehog cDNA (a gift from A. McMahon). A day after the transfection, 2×10⁴ cells in 5 μl of PBS were injected into the maternal side of the placenta through the uterine wall, using a syringe with a 30 GA/2 needle. Mice had been anesthetized by intramuscular injection of a 3:1 mixture of Ketamine/Xilazine (Parke-Davis/Bayer) in a volume of 1 μl/g of body weight. Usually one uterine horn of the embryos (at approximate age of E8.5, E8.75: 10-14 somites) was injected. We noticed that injection in both horns requires extensive manipulation of the uterus and this frequently causes abortion. For the same reason, only 5 or 6 placentas were injected in mice carrying many embryos in one horn, thus avoiding injection into the placentas closer to the ovary. The mice survived surgery, and, under these conditions, abortion occurred in about 20% of operated animals. Mice were killed after 2 additional days and embryos were collected and processed for morphological and biochemical analysis.

**Immunocytochemistry**

Immunocytochemistry on tissue sections and cultured cells was carried out as described (Tajbakhsh et al., 1994) using the following antibodies: anti-MyoD (Koishi et al., 1995), anti-myosin heavy chains (Salvadori et al., 1995), anti-nestin (Tajbakhsh et al., 1994) and anti-PECAM antibody (Santa Cruz Biotechnology).

**Immunoprecipitation**

To study the transfer of secreted proteins from cells injected into the placenta to the embryo, WOP cells were metabolically labeled for 2 hours with 1 mCi/ml of [35S]methionine (NEN, specific activity 1 mCi/mmol) in complete medium. Labeled cells were injected into the placenta as described above and after 24 hours isolated embryos were collected and the protein extracted as described below. The cleared supernatant was separated on a Sephadex G-50 fine gel filtration column. Radioactivity was eluted in fractions corresponding to molecular mass ranging from 60 to 5 kDa. The supernatant of labeled cells was also separated on the same column, and, in this case, radioactivity was eluted in all fractions, from void volume to low molecular mass. In order to detect Frzb1 in embryos following placental injection, an HA-tagged construct was generated and the protein detected by immunoprecipitation. WOP cells were transfected with the pcRS2mFrzbHA vector, containing the HA epitope tag between the alanine 159 and the aspartic acid 160, or with the pcRS2 vector alone. Pools of eight Frzb1-1HA placentally injected or control embryos were collected after 24 or 48 hours and homogenized by passage through a G25 needle in Modified RIPA Lysis buffer (50 mM Tris-HCl pH 7.4; 300 mM NaCl, 10 mM KCl, 1 mM EDTA, 5 mM DTT, 0.25% sodium deoxycholate, 1% Nonidet P40, 5 μg/ml aprotinin, 5 μg/ml leupeptin, 5 μg/ml pepstatin, 1 mM PMSF). The homogenate was centrifuged at 2000 g and the supernatant was diluted to 150 mM NaCl (final concentration). Protein G-agarose (Santa Cruz Biotechnology) was used with Modified RIPA buffer and incubated with the anti-HA, 11 monoclonal antibody (BABBICO; diluted at 1:150) for 1 hour at 4°C. The mixture was washed in Modified RIPA buffer...
Inhibition of myogenesis by a soluble Wnt antagonist and incubated for 1 hour at 4°C with the experimental samples. The immunoprecipitate was washed 5 times in RIPA buffer, resuspended in SDS loading buffer and run on a 10% SDS-PAGE gel. The Frzb1 protein band was visualized by western blot using the anti-HA.11 monoclonal antibody diluted at 1:500.

RESULTS

Expression of Frzb1 during somitogenesis

As a first approach to investigate the possible role of soluble frizzled-related proteins in mouse myogenesis, we analyzed their expression pattern in postimplantation mouse embryos, ranging in age from E8.5 (10-12 somites) to E10.5 (35-40 somites). Fig. 1A shows that at E8.5, Frzb1 is expressed at high levels in the presomitic mesoderm and in the neural folds; at E9.5 Frzb1 is highly expressed in the presomitic mesoderm and in the cranial half of newly formed somites (Fig. 1B). At E10.5 Frzb1 is expressed in the dorsal intersomitic space between more mature somites, in the forming limb buds, in the brain and in the branchial arches (Fig. 1,C,D). In embryos in which one allele of the Myf5 locus has been targeted with nLacZ (Myf5<sup>a2</sup> embryos; Tajbakhsh et al., 1996), at E10.5, Frzb1 is expressed in mesenchymal cells in an area adjacent (dorsomedially) to newly formed myotomes (revealed by β-galactosidase staining, Fig. 1E) and this can be seen in sections (Fig. 1F,G). Of the soluble Frizzled-related soluble molecules studied so far (sFRP1-4; Hoang et al., 1998; Leimeister et al., 1998; our unpublished results) only Frzb1 appears to be expressed in premyogenic regions of the mouse embryo. We therefore investigated its possible role in myogenesis.

Frzb1 inhibits myogenesis in explants from mouse somites

A full-length cDNA encoding murine Frzb1 was cloned in a retroviral vector and recombinant virus was produced in BOSC-23 cells. Initial attempts to infect directly explants of presomitic mesoderm yielded inefficient transduction (≤1% of the population as evaluated by expression of an HA tag). C3H10T1/2 fibroblasts were therefore transfected with a Frzb1-expressing vector and used as a feeder layer for the somite cultures.

Fig. 1. Frzb1 is expressed, although not exclusively, in the myogenic areas of postimplantation embryos. Whole-mount in situ hybridization reveals expression (A) in the presomitic mesoderm and neural folds of E8.5 embryos, (B) in presomitic mesoderm and cranial halves of newly formed somites and lateral mesoderm of E9.5 embryos, (C) at mesencephalon/metencephalon and diencephalon/telencephalon junctions, in the limbs and in the mandibular arches of E10.5 embryos and (D) in the mesenchyme adjacent to the neural tube. (E) Analysis of Frzb1 expression in similarly aged, β-galactosidase stained Myf5<sup>a2</sup> embryos revealed contiguous but non-overlapping areas of expression of Frzb1 and Myf5. This is also illustrated in (F) coronal and (G) transverse sections of the same embryo where purple labeling is Frzb1 and blue is Myf5. nt, neural tube; mt, myotome.

Fig. 2. Frzb1 inhibits expression of both Myf5 and MyoD in vitro. Explants of presomitic mesoderm from E9.5 Myf5<sup>a2</sup> embryos were divided in half along the axis and co-cultured for 3 days (with their half neural tube and surface ectoderm but without lateral mesoderm) (A,C) on control C3H10T1/2 cells or (B,D) on C3H10T1/2 cells expressing Frzb1; the cultures were then stained (A,B) for β-galactosidase activity and (C,D) with anti-MyoD polyclonal antibody. (C,D) The large tissue mass shows a background (non nuclear) staining.

and incubated for 1 hour at 4°C with the experimental samples. The immunoprecipitate was washed 5 times in RIPA buffer, resuspended in SDS loading buffer and run on a 10% SDS-PAGE gel. The Frzb1 protein band was visualized by western blot using the anti-HA.11 monoclonal antibody diluted at 1:500.

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MyoD antibodies. Fig. 2 shows that many Myf5- and MyoDexpressing cells were detected in cultures grown on control C3H10T1/2 fibroblasts. In contrast, only a few Myf5- or MyoD-positive cells (less than 1% of controls) were detected in cultures of presomitic mesoderm grown on C3H10T1/2 fibroblasts expressing Frzb1. In cultures of the most recently formed (I-III) somites, myogenesis was also inhibited by Frzb1 but less dramatically. The number of positive cells ranged from 30 to 50% of controls (data not shown). In cultures from older (V-VIII) more anterior somites, inhibition by Frzb1 could no longer be detected (data not shown). In parallel cultures, the total number of cells was measured at the end of the culture period and, in all cases, was found to be similar in explants grown on control C3H10T1/2 fibroblasts and in explants grown on C3H10T1/2 fibroblasts expressing Frzb1. In addition, analysis of the extent of apoptosis by acridine orange staining did not reveal significant differences between these cultures (data not shown). In order to test whether differentiation was inhibited and not simply delayed several cultures were grown for 5 or 7 days; also in these cases the number of Myf5- or MyoD-expressing cells did not increase significantly in explants grown on C3H10T1/2 fibroblasts expressing Frzb1 (data not shown).

The in vivo effect of Frzb1 on myogenesis and mesoderm development

In order to investigate a possible role for Frzb1 in regulating myogenesis in vivo, we developed a gain-of-function approach based on transplacental delivery of molecules secreted by cells transfected with the appropriate expression vector and injected into the maternal side of the placenta. For this purpose, WOP cells were transiently transfected with a vector expressing the full-length murine Frzb1. Under our conditions, tranfection efficiency ranged from 70 to 90% of the cell population, rendering selection unnecessary. 2 days after transfection 2×10^4 WOP cells were injected through the uterine wall into the maternal side of the placenta of embryos at E8.5-E8.75 (Fig. 3A). About 20% of injected cells (see Methods) could be recovered from the placenta 2 days after the injection, although there was variability among different placentas injected. Cryostat sections of placentas injected with lacZ-expressing WOP cells, revealed numerous β-galactosidase-positive cells predominantly localized to one site (probably the injection site) and a few positive cells at a moderate distance (Fig. 3B). We never observed β-galactosidase cells in the amniotic fluid or in the embryo, suggesting that, under these conditions, WOP cells do not cross the placental barrier. When transfected with a Frzb1-HA expression vector, WOP cells secreted high levels of Frzb1-HA protein which could be immunoprecipitated from the corresponding embryos (Fig. 3C). Indeed, gel filtration analysis of radioactive proteins recovered from the embryos, injected with metabolically labeled WOP cells (cultured with [35S]methionine), revealed that proteins smaller than 60 kDa crossed the placental barrier (data not shown).

Embryos that had developed in the presence of excess amounts of Frzb1 protein exhibited growth retardation and severe malformations in the caudal region of the body (Fig. 4). The extent of this phenomenon was roughly proportional to the number of injected WOP cells, ranging from apparently normally developed embryos (less than 10^4 cells) to complete resorption (more than 5×10^4 cells). Injection of cells transfected with the empty vector or with a vector expressing full-length murine Sonic hedgehog did not appear to alter embryonic development (data not shown).

Morphological analysis of Frzb1-treated embryos revealed a retarded development with marked reduction of the caudal
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Frzb1 inhibits myogenesis in vitro

The results reported in this paper show that the Wnt antagonist Frzb1 inhibits the activation of mammalian myogenesis both in vitro and in vivo. We had previously shown that the activation of epaxial and hypaxial myogenesis could be mimicked by Wnt1 and Wnt7a (and to a minor extent also by other Wnts such as Wnt4 and Wnt5a), which can replace the neural tube and the surface ectoderm in activating preferentially Myf5 or MyoD, respectively (Tajbakhsh et al., 1998).

Frzb1, unlike the related genes sFRP1 (Hoang et al., 1998), sFRP2 and sFRP4 (Leimeister et al., 1998) is expressed at high levels in virtually all the premyogenic areas of the embryo, such as presomitic mesoderm, newly formed somites, limb buds and branchial arches (this manuscript and Hoang et al., 1998).

Frzb1 inhibits myogenesis in vivo

In order to monitor whether overexpression of Frzb1 would specifically interfere with Wnt signaling, we examined injected embryos for the expression of genes whose expression is known to be activated by Wnts. Fig. 6A shows that Myf5 expression is apparently normal in the cranial somites but cannot be detected in all the somites that had formed caudally to the forelimb in Frzb1-treated embryos. In contrast, Pax3 (Fig. 6B) or Mox1 (Fig. 6G) expression levels appear normal in the paraxial mesoderm even of severely malformed embryos. In embryos labeled for both Pax3 and Myf5 expression (Fig. 6C), sections immediately caudal to the forelimb revealed expression of both genes (Fig. 6D) while more caudal sections (at an approximate distance of three somites) revealed expression of Pax3 but not of Myf5 (Fig. 6E). En1 is expressed normally in the isthmus and in cranial somites but, much like Myf5, is not detectable in the more caudal somites of injected embryos (Fig. 6F).

It has been shown that Noggin is one of the target genes of Wnt signaling in presomitic mesoderm and somites (Hirsinger et al., 1997; Marcelle et al., 1997). Much as in amphibians (Zimmerman et al., 1996), Noggin is thought to bind BMP4 and thus neutralize its inhibitory action on dorsal structures of postimplantation mouse embryos. Therefore Frzb1-treated embryos were analyzed for the expression of Noggin and BMP4. Fig. 7A shows that, in control embryos, Noggin is expressed axially and in the dorsal somites; in contrast, expression of Noggin is maintained axially but abolished in somites of Frzb1-injected embryos (Fig. 7B). The expression of BMP4 appeared to be unaffected (data not shown).

Frzb1 causes severe malformations of the caudal region of injected embryos. Morphology of control (C) and treated (T) embryos, revealing different severity of malformations in different embryos.

Immunofluorescence analysis of sections just caudal to the forelimb level revealed that, in moderately affected embryos, the expression of nestin (Fig. 5E), which marks both neurons and the myotome was only slightly affected (Fig. 5F); skeletal muscle markers such as myosin heavy chains were modestly affected in myotomes of moderately affected embryos (Fig. 5H) and lacking in severely affected ones (Fig. 5I). In contrast, the expression of the endothelial marker, PECAM, was unaffected even in the most severely perturbed embryos (Fig. 5L) where it labeled the vessels that appeared markedly dilated, possibly due to the reduced cellularity of the mesoderm. The epithelial gut and mesonephros appeared less affected or unaffected (data not shown).

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These findings suggest a possible role for Frzb1 in regulating skeletal myogenesis. When explants of presomitic mesoderm (with neural tube and surface ectoderm) were cultured on Frzb1-producing fibroblasts, the activation of myogenesis was dramatically (over 90%) inhibited and expression of both Myf5 and MyoD was severely reduced. Under the conditions employed, explants grown on Frzb1-producing cells and on control fibroblasts were a similar size and neither proliferation nor cell death appeared to be significantly altered by Frzb1. The effect observed was less dramatic in explants of newly formed somites and there was virtually no effect in more mature somites. Indeed, overexpression of Frzb1 does not inhibit differentiation of the established myogenic cell line C2C12 (U. B., G. C., unpublished observations). Together these results suggest that sequestering Wnts mainly results in blocking the consequent activation of myogenic regulatory genes but does not interfere significantly with terminal differentiation.

A novel method allows the study of the effect of Frzb1 in vivo

Information on the phenotype of Frzb1 null embryos is not yet available. Furthermore, transgenic manipulation is currently complicated by the lack of a suitable promoter expressed at the right time and place to direct overexpression of Frzb1. In order to study the possible action of Frzb1 on myogenesis, in vivo, we therefore adapted a method of transplacental delivery described 20 years ago (Fleischman and Mintz, 1979). WOP cells, transfected with a Frzb1 expression vector, were injected into the maternal side of the placenta where they could be detected up to 2 days after injection. At variance with hemopoietic cells employed in the original work, injected WOP cells remain in the placenta and do not invade the embryo proper or appear in the amniotic fluid. Frzb1 was secreted by WOP cells and transferred to the embryo where it caused dose-dependent malformations, ranging from slight alterations of somitogenesis and neural tube closure to complete shrinking of the caudal mesoderm. This method allows delivery to the embryo of biologically active extracellular molecules, which are thought to play a role in a given morphogenetic or histogenetic event, by both overexpression of the active molecule or expression of modified molecules with a dominant negative function. The temporal window but not the area of expression can be selected and, in this respect, the method described here differs from a recently reported method that allows direct intraembryonic injection of biologically active molecules (Liu et al., 1998). It should be noted, however, that intraplacental injection is straightforward and does not require instrument-assisted manipulation thus allowing the testing of a given molecule before generating a specific mouse mutant strain.

Given the novelty of the method employed, we determined whether the effect observed was specifically due to overexpression of Frzb1. This conclusion is supported by several lines of evidence: first, the absence of any effect when
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Frzb1 overexpression affects predominantly presomitic mesoderm and inhibits myogenesis in vivo

The effect of Frzb1 appears mainly directed towards mesoderm development, although the neural tube closure appears to be delayed. Indeed, the modest effect on the cranial structures may be due to the fact that, by the time placenta-derived Frzb1 accumulates in the embryo, critical morphogenetic events in brain and face development have already occurred whereas caudal structures are still undergoing critical events such as somitogenesis. The effects of Frzb1 described here on mouse somitogenesis are congruent with the proposed role of Wnts in *Xenopus* muscle development. Microinjection of Wnt8 cDNA expressed at gastrula and later stages promotes ectopic muscle differentiation (Christian and Moon, 1993). Conversely, inhibition of Wnt signaling by microinjection of a dominant-negative Wnt8 construct (Hoppler et al., 1996), dominant-negative dishevelled mRNA (Sokol, 1996) and of Frzb1 mRNA (Leyns et al., 1997) lead to inhibition of muscle development in the trunk region.

There is variability among Frzb1-treated mouse embryos, ranging from modest morphological alterations and slightly delayed development, to severe malformations of caudal structures. This probably correlates with the number of Frzb1-producing cells that are retained in the placenta, although this is difficult to test. However, there is a correlation between number of cells injected and the severity of the resulting phenotype.

Differentiation is only partly affected in the least severe phenotypes, in which muscle and neural markers are retained, albeit at a reduced level. In the most severe phenotypes, myosin heavy chain and nestin expression is greatly reduced or absent, probably reflecting developmental retardation. Remarkably, blood vessels appear to be unaffected as shown by maintained expression of the endothelial marker PECAM, even in the most severe phenotypes.

A preferential effect on mesoderm suggests that Frzb1 acts, mainly on this tissue, in the strict temporal window of the placenta injection. However, it is possible that redundancy of different Wnts (some of which may not be bound by Frzb1) may explain a less severe effect, much as is observed in the single Wnt1 or Wnt3a knock-out when compared to the double mutation (Ikeya et al., 1997). Gene inactivation of Wnt5a (Yamaguchi et al., 1999) results in reduced extension of the A-P axis due to reduced divisions of mesenchymal progenitors; Wnt3a knock-out embryos (Takada et al., 1994) have defects in the somites and in tailbud formation. Frzb1-injected embryos show a stronger phenotype than Wnt3a null embryos but they still express Mox1 suggesting that Frzb1 affects already determined mesodermal cells and not their progenitors.

The expression of Noggin offers a clear example of the differential effect of Frzb1 on different structures since axial expression is preserved whereas expression in somites is greatly reduced cranially and abolished caudally. In contrast, BMP4 expression is unchanged in the lateral mesoderm (data not shown). This may result in several consequences: if the balance between Noggin and BMP4 is perturbed, an increased concentration of the latter may contribute to the inhibition of myogenesis (Hirsinger et al., 1997; Marcelle et al., 1997). The persistence of BMP4 compared to a reduced concentration of Wnts, due to the antagonism of Frzb1, may also contribute to increased apoptosis (Schmidt et al., 1998). Indeed preliminary experiments, using TUNEL analysis, have shown that apoptosis is detected in most severely affected embryos (unpublished observations). It is becoming evident that the effect of most signaling molecules on the differentiation of a given tissue is the combined result of different activities on cells expressing the empty vector were injected into the placenta (thus ruling out a possible toxicity of the surgical procedure); second, the absence of a teratogenic effect when cells engineered to express *Sonic hedgehog* were similarly injected into the placenta. This lack of an effect due to Shh was unexpected but it may be explained by the specific temporal window selected. Early inductive events caused by Shh probably occur before a significant amount of Frzb1 protein has reached the embryo. This is in agreement with our observation for a requirement of Shh together with Wnt for somitogenesis are congruent with the proposed role of Wnts in somitogenesis. The effects of Frzb1 described here on mouse embryos have already occurred whereas caudal structures are still undergoing critical events such as somitogenesis. The effects of Frzb1 described here on mouse somitogenesis are congruent with the proposed role of Wnts in *Xenopus* muscle development. Microinjection of Wnt8 cDNA expressed at gastrula and later stages promotes ectopic muscle differentiation (Christian and Moon, 1993). Conversely, inhibition of Wnt signaling by microinjection of a dominant-negative Wnt8 construct (Hoppler et al., 1996), dominant-negative dishevelled mRNA (Sokol, 1996) and of Frzb1 mRNA (Leyns et al., 1997) lead to inhibition of muscle development in the trunk region.

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gene activation, cell proliferation and survival. In the case of the Wnts, their activity on gene activation and on proliferation is well documented: it is therefore not surprising that increased concentration of Frzb1 may cause a marked effect in those tissues that already express high levels of the molecule. It is possible that a fine balance between Frzb1 and Wnts may be required to regulate myogenic determination as well as more generalized effects on growth. This is suggested by the morphology of the somites in Frzb1-treated embryos, where the dermomyotomal cell population appears to be still present and to express Pax3.

In conclusion, the novel method described here has allowed us to study the effect of a Wnt antagonist on mouse development in vivo: Frzb1 caused a marked effect on posterior mesoderm, consistent with the effects observed in amphibia with different methodologies. The inhibition of myogenesis by Frzb1, in vitro and in vivo, suggests that Wnts may regulate this process by a direct effect on gene activation. While we will apply this method to the study of other molecules active in the signaling of Wnt genes, inactivation studies for Wnt antagonists should confirm and extend the current scenario of Wnt action on early steps of vertebrate myogenesis.

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