Development 122, 2987-2996 (1996)
Printed in Great Britain © The Company of Biologists Limited 1996
DEV9490

Maternal β-catenin establishes a 'dorsal signal' in early *Xenopus* embryos

C. Wylie[1,2], M. Kofron[1], C. Payne[1], R. Anderson[1], M. Hosobuchi[4], E. Joseph[4] and J. Heasman[1,3]

[1]Institute of Human Genetics, [2]Department of Pediatrics, [3]Department of Cell Biology and Neuroanatomy, University of Minnesota School of Medicine, Box 206 UMHC, 420 Delaware Street SE, Minneapolis, MN 55455, USA
[4]Department of Molecular and Cellular Biology, Harvard University, 7 Divinity Avenue, Cambridge, MA 02138, USA

SUMMARY

In previous work, we demonstrated that maternally encoded β-catenin, the vertebrate homolog of armadillo, is required for formation of dorsal axial structures in early *Xenopus* embryos (Heasman, J., Crawford, A., Goldstone, K., Garner-Hamrick, P., Gumbiner, B., Kintner, C., Yoshida-Noro, C. and Wylie, C. (1994). *Cell* 79, 791-803). Here we investigated, firstly, the role(s) of β-catenin in spatial terms, in different regions of the embryo, by injecting β-catenin mRNA into individual blastomeres of β-catenin-depleted embryos at the 32 cell stage. The results indicate that β-catenin can rescue the dorsal axial structures in a non-cell-autonomous way and without changing the fates of the injected cells. This suggests that cells overexpressing β-catenin send a 'dorsal signal' to other cells. This was confirmed by showing that β-catenin overexpressing animal caps did not cause wild-type caps to form mesoderm, but did cause isolated β-catenin-deficient marginal zones to form dorsal mesoderm. Furthermore β-catenin-deficient vegetal masses treated with overexpressing caps regained their ability to act as Nieuwkoop Centers.

Secondly, we studied the temporal activity of β-catenin. We showed that zygotic transcription of β-catenin starts after the midblastula transition (MBT), but does not rescue dorsal axial structures. We further demonstrated that the vegetal mass does not release a dorsal signal until after the onset of transcription, at the midblastula stage, suggesting that maternal β-catenin protein is required at or before this time. Thirdly we investigated where, in relationship to other gene products known to be active in axis formation, β-catenin is placed. We find that BVg1, bFGF, tBR (the truncated form of BMP2/4R), siamois and noggin activities are all downstream of β-catenin, as shown by the fact that injection of their mRNAs rescues the effect of depleting maternally encoded β-catenin. Interference with the action of glycogen synthase kinase (GSK), a vertebrate homolog of the *Drosophila* gene product, zeste white 3 kinase, does not rescue the effect, suggesting that it is upstream.

Key words: *Xenopus*, β-catenin, midblastula transition, armadillo, dorsal signal

INTRODUCTION

There is growing evidence that a wnt-initiated signalling pathway is involved in the formation of dorsal structures in the early *Xenopus* embryo. Several wnt family members can cause an ectopic dorsal axis when their mRNAs are injected into cleavage stage embryos (McMahon and Moon, 1989; Christian et al., 1991; Smith and Harland, 1991; Sokol et al., 1991; Ku and Melton, 1993). In addition, a number of homologs of genes active in the wingless-initiated signalling pathway leading to segmental patterning in *Drosophila* embryos are present in early stages of *Xenopus*. These include glycogen synthase kinase (GSK, a homolog of zeste white3, He et al., 1995; Pierce and Kimelman, 1995), Xdsh (a homolog of dishevelled, Sokol et al., 1995) and β-catenin (a homolog of armadillo, Demarais and Moon 1992; Funayama et al., 1995). The effects of overexpession of wild-type or dominant negative forms of these mRNAs in *Xenopus*, support the idea that they are involved in a similar pathway required for dorsal axis formation. Throughout this paper the word 'dorsal' is used to describe a type of mesoderm or endoderm. **Dorsal mesoderm** is derived from the dorsal equatorial zone of the blastula, differentiates into notochord and somite and has the ability to induce neural tissue. **Dorsal endoderm** is derived from the dorsal vegetal cells and differentiates into pharyngeal endoderm. These dorsal vegetal cells have the ability at the blastula stage to induce dorsal mesodermal cell types in the adjacent equatorial cells. A **dorsal axis** consists of a notochord, somites, neural tissue and head structures.

In a previous paper, we showed that β-catenin encoded by maternal mRNA is absolutely required in *Xenopus* for the formation of dorsal mesoderm and, in the absence of this, the dorsal axis does not form (Heasman et al., 1994). We also found that one of the *Xenopus* wingless homologs, Xwnt8, cannot rescue β-catenin-depleted embryos that lack a dorsal axis, even though Xwnt8 causes an ectopic axis in control embryos. This indicates that β-catenin is indeed downstream of an endogenous wnt-like signal. Overexpression of β-catenin has been shown to cause similar dorsalization effects as those caused by Xwnt8 mRNA (Guger and Gumbiner, 1995).

What is the role of β-catenin in dorsal axis formation? Classical studies using Nieuwkoop recombinants of midblastula-stage animal caps and vegetal masses suggest that dorsal mesoderm is signalled to form in the marginal (equatorial) zone of the blastula by the dorsal side of the vegetal mass, known as the Nieuwkoop Center, which itself is destined to become endoderm (Boterenbrood and Nieuwkoop, 1973). The responding cells, known as the Spemann Organizer, induce

more dorsal mesoderm in the marginal zone (Smith and Slack, 1983) and induce neural tissue in the adjacent ectoderm. Thus, the vegetal mass changes the fate of cells of the animal cap from ectoderm to mesoderm, including dorsal mesoderm. In a previous paper, we used Nieuwkoop recombinants to demonstrate that, when **either** the animal **or** the vegetal components (or both) of such recombinants lack β-catenin, the amount of dorsal mesoderm formed is dramatically reduced (Heasman et al., 1994). This suggests that β-catenin is required for more than the establishment of the vegetal Nieuwkoop Center and may play a role throughout the embryo. The experiments presented here confirm this and serve to determine where, when and how β-catenin acts.

To identify where in the blastula β-catenin is required, and whether it has different functions in different regions, we show the results of rescue experiments in which β-catenin mRNA was injected into different individual blastomeres of 32 cell stage, β-catenin-depleted embryos. We find that β-catenin-overexpressing cells rescue dorsal mesoderm in a non-cell-autonomous manner, without altering their own germ layer specification. We also show that β-catenin-overexpressing animal caps can convert β-catenin-deficient mesoderm to dorsal mesoderm, and β-catenin-deficient endoderm into a Nieuwkoop Center. These experiments indicate that the primary role of cells with an active β-catenin-containing pathway is to signal other cells to become dorsal ie. to differentiate into dorsal-type derivatives of their respective germ layers. Thus, β-catenin causes cells to release a 'dorsal' signal, rather than a 'dorsal mesoderm' signal. Using timed recombinations of animal caps and vegetal masses, we further show that the endogenous dorsal signal, which requires β-catenin, does not pass from vegetal masses to animal cells until after the midblastula stage. Since transcription starts at the mid-blastula stage in *Xenopus*, this fits well with a model where β-catenin lies upstream of a maternal transcription factor, responsible for the release of a zygotic dorsal signal.

To investigate where β-catenin is placed in relationship to other known molecular activities involved in dorsal axis formation, we attempted to rescue β-catenin-depleted embryos by injection of the following mRNAs: **dn-gsk**, a dominant negative form of the serine/threonine kinase GSK3β, whose *Drosophila* homolog, zeste white 3, lies upstream of armadillo and is inhibitory to a wingless signal (Peifer et al. 1994). Expression of dn-gsk on the ventral side of the *Xenopus* embryo causes ectopic axis formation (He et al. 1995; Pierce and Kimelman 1995). **BVg1**, a modified form of the endogenous Vg1 (Thomsen and Melton, 1993). Vg1 is localized in the vegetal pole of the full-grown oocyte (Rebagliati et al., 1985). Its modified form, BVg1, or a non-modified zebrafish Vg1 mRNA have dorsal axis forming activity (Dohrmann et al. 1996), although *Xenopus* Vg1 itself does not. **bFGF**, a growth factor present in early *Xenopus* embryos (Kimelman and Kirschner, 1987; Slack et al., 1987). bFGF has been shown to induce animal caps to form dorsal mesoderm and overexpression of a dominant negative form of the FGF receptor causes embryos to develop with reduced trunk and tail structures (Amaya et al. 1991). **Dominant negative BMP2/4 receptor**, **tBR** is a truncated form of the maternally expressed BMP receptor. Expression of tBR converts ventral mesoderm to dorsal mesoderm in marginal zone explants (Graff et al., 1994; Hawley et al., 1995). **siamois** is a homeobox-containing transcription factor expressed in dorsal vegetal cells of embryos after MBT. It has potent dorsal axis forming activity (Lemaire et al., 1995). **noggin** is a secreted protein whose zygotic transcript is initially restricted to the presumptive dorsal mesoderm and is found in highest concentration in the dorsal blastopore lip (Smith and Harland, 1992). We conclude from these, and our previously published experiments, that β-catenin is downstream of wnts and GSK, but upstream of the other molecules tested.

MATERIALS AND METHODS

Oocytes and embryos

Oocytes were prepared, injected and fertilised as in previous experiments (Holwill et al., 1987; Heasman et al., 1991, 1994). Oocytes were cultured throughout in oocyte culture medium (OCM) consisting of the following: per 400 ml: Liebovitz medium 240 ml, sterile distilled water 160 ml, bovine serum albumen 0.16 g (Sigma A 9647), glutamine 2 ml (200 mM), gentamicin 200 μl (10 mg/ml GibcoBRL) pH 7.6-8.

Oligos and RNAs

The oligo used was identical in structure to that published previously; 5′-T*G*C*C*TTTCGGTCTG*G*C*T*C-3′, in which * indicates phosphorothioate linkages. RNAs injected were synthesized by linearizing the plasmid vectors containing subcloned cDNAs, and transcribing the linear template with SP6 RNA polymerase in the presence of m7G(5′)ppp(5′)G CAP analog (Ambion, Inc). Injections were carried out exactly as in Heasman et al. (1994). The sources and amounts of mRNA injected were as follows:

RNA	Linearized	Dose	Origin	Reference
β-catenin	*Eco*RI	150 pg	B. Gumbiner	(Funayama et al., 1995)
tBR	RNA Gift	900 pg	D. Melton	(Graff et al., 1994)
dn-gsk3	RNA Gift	650 pg	D. Kimelman	(Pierce et al., 1995)
siamois	RNA Gift	40 pg	P. Lemaire	(Lemaire et al., 1995)
BVg1	*Eco*RI	10 pg	D. Melton	(Thomsen et al., 1993)
β-gal	*Xho*I	300 pg	D. Melton	(Thomsen et al., 1993)
noggin	*Sac*I	1 pg	R. Harland	(Smith et al., 1992)

Northern blots

Probes were synthesized by random priming of excised inserts of MyoD (*Eco*RI and *Bam*HI), Xwnt8 (*Eco*RI), NRP1 (*Eco*RI). RNA was prepared and analysed as in Hopwood et al. (1989). MyoD was a gift from John Gurdon, Xwnt8 from Randall Moon and NRP1 from Richard Harland.

Recombinations

Embryos were dissected as shown diagrammatically in Fig. 3 in 1× MMR on 2% agarose plates, washed in OCM and recombined for the time stated in the text in OCM on agarose plates.

Histology

Embryos were fixed in MEMFA for 2 hours, rinsed in PBS and stained using X-gal (Hemmati-Brivanlou and Harland, 1989). For sectioning, embryos were dehydrated and embedded in polyethylene distearate wax (Polysciences).

RESULTS

Where in the embryo is β-catenin required for dorsal mesoderm formation?

In previous experiments, we showed that embryos depleted of

β-catenin lacked dorsal axial structures and could be rescued by the injection of β-catenin mRNA into depleted oocytes (Heasman et al., 1994). Here we tested where in the embryo β-catenin is required in the following way. We generated β-catenin-depleted embryos by injecting 750 pg antisense oligo 303 into oocytes and fertilizing them. The embryos were then injected at the 32 cell stage with β-catenin mRNA by injecting into single blastomeres on the dorsal side, as indicated in Fig. 1. When these embryos reached the tailbud stage, we found that dorsal axial structures were rescued compared to controls (Fig. 2A), but to different extents. Injection into equatorial cells gave the most complete rescue (Fig. 2D,E), followed by vegetal (Fig. 2C,G) then animal cells (Fig. 2B,F; Table 1). However, histological examination showed that, even in animal-injected embryos, the neural tube, notochord and somites had formed in the trunk (not shown), but most of the embryos lacked heads, and had not elongated as much as controls. In one experiment, β-galactosidase mRNA was co-injected with the rescuing β-catenin mRNA, to study whether the progeny of the β-catenin-injected blastomere contributed to the rescued dorsal axis ie. to ask whether the rescue was a cell autonomous event or not. Five specimens from each group were stained using X-gal, dehydrated, cleared and examined. When individual animal cells were injected, their descendants were found in ectoderm when the axis was rescued(Fig. 2F; 3/5 cases). When there was no rescue (2/5 cases), the blue cells formed a large clone both in the epidermis and surrounding the non-obliterated blastocoel (data not shown). When equatorial cells were injected, their descendants were found in dorsal mesoderm and endoderm (Fig. 2E; 5/5 cases). When vegetal cells were injected, their descendants were found in endoderm (Fig. 2G; 5/5 cases). This shows that β-catenin expression generated new dorsal axial structures, in a non-cell autonomous manner, without altering the fates of the expressing cells with respect to their germ layer specification.

The role of β-catenin-expressing cells is to produce a 'dorsal signal'

The previous experiment showed that expression of β-catenin in a cell does not, by itself, tell the cell or its progeny to form dorsal mesoderm. The simplest interpretation is that the β-catenin-overexpressing cells produce a 'dorsal signal' ie. signal to other cells not to alter the germ layer specification of the responding cells, but to cause them to become dorsal derivatives of their normal germ layer. To test this, we carried out three recombination experiments, in each case using MyoD expression as an index of the formation of dorsal mesoderm. Midblastulae were dissected into three components (Fig. 3A). Firstly, we cultured β-catenin-depleted marginal zones (which are unable to form dorsal mesoderm) together with uninjected or β-catenin-overexpressing animal caps until the late neurula stage. If the overexpression of β-catenin in animal caps causes them to send a dorsal signal, then depleted marginal zones should now make dorsal mesoderm. Without the animal caps they should not make the dorsal mesodermal marker, and with wild-type caps there should be a smaller endogenous signal. Fig. 3A lanes 3-8 shows that this is the case. Lanes 3 and 4 indicate the expression of MyoD in wild-type and β-catenin-overexpressing equatorial zones, compared to β-catenin-depleted marginal zones (lane 5 and6). Lane 7 shows a low level of MyoD expression in wild-type cap/β-catenin-deficient

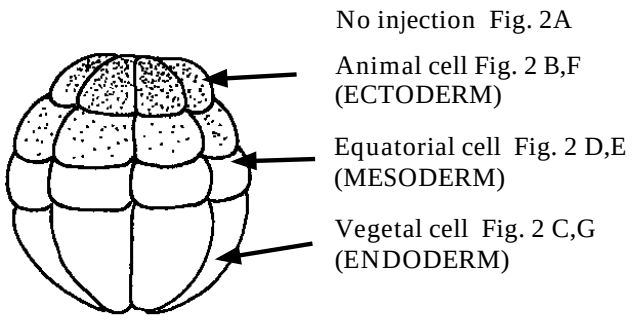


Fig. 1. The sites of injection of 150 pg of β-catenin mRNA into 32 cell-stage β-catenin-deficient embryos. Injections were on the dorsal side, as judged by pigment differences in 1 cell, into one cell in the positions indicated. Representative embryos cultured after injection into these sites are shown in Fig. 2.

Table 1. Aggregated numbers of embryos examined in the experiments reported in Figs 2 and 6

Experiment	Number of embryos with dorsal structures	Number of embryos with no dorsal structures	Number of embryos with a second ectopic axis
uninjected	26	0	0
β-catenin oligo	0	51	0
β-catenin animal injection	9	4	0
β-catenin vegetal injection	8	0	0
β-catenin equatorial injection	8	0	0
uninjected + BVg1 RNA	23	0	10
β-catenin oligo + BVg1 RNA	38	7	0
uninjected + gskdn RNA	10	0	15
β-catenin oligo + gskdn RNA	0	12	0
uninjected + BMPdn RNA	10	0	0
β-catenin oligo + BMPdn RNA	8	3	0
uninjected + siamois RNA	18	0	10
β-catenin oligo + siamois RNA	24	12	0
uninjected + β-catenin RNA	8	0	15
β-catenin oligo + β-catenin RNA	30	2	0
uninjected + noggin mRNA	17	0	16
β-catenin oligo + noggin mRNA	12	0	0

recombinants and lanes 8 and 9 show the strong expression of MyoD in β-catenin-depleted recombinants treated with over-expressing caps. Secondly, we cultured untreated animal caps together with β-catenin-overexpressing caps from the mid-blastula stage until the late neurula stage. If β-catenin-overexpressing cells signal other cells to form dorsal mesoderm, the overexpressing caps will be able to signal the untreated animal caps to form dorsal mesoderm. Fig. 3A lane 10 demonstrates that this is not the case. No MyoD formed in the animal:animal combinations. Thirdly, we tested the ability of uninjected or overexpressing animal caps to dorsalize β-catenin-depleted vegetal masses. To do this we cultured ten recombinants together for 1 hour, removed the animal caps (which is very easy after only 1 hour, see Fig. 5), and combined the 'conditioned' vegetal masses with new untreated animal caps. Fig. 3A lane 16 shows that, after treatment with the overexpressing animal caps, the vegetal masses now had the ability to induce dorsal mesoderm to form in untreated animal caps. β-catenin-depleted vegetal masses treated with control animal caps induced much reduced amounts of MyoD in untreated animal caps (lane 15). The animal caps used to condition the vegetal

masses for 1 hour did not themselves express MyoD (lane 17 and 18). In a separate experiment, we showed that β-catenin-deficient animal caps do not produce a dorsal signal and have the ability to cause MyoD expression when combined with either equatorial regions or vegetal masses of β-catenin-deficient embryos (data not shown).

These recombination experiments indicate that β-catenin-expressing animal cells have the capacity to tell other cells to be dorsal (the marginal and vegetal combinations), but not to be mesoderm (the animal:animal combinations).

To confirm that β-catenin-overexpressing caps were themselves dorsalized, we made Nieuwkoop recombinants consisting of wild-type bases (brown dye) combined with either wild-type caps (brown) or overexpressing caps (blue) and cultured them until siblings reached the neurula stage (stage 16). Fig. 3bi and ii show that the overexpression of β catenin leads to a dramatic elongation of the caps compared to controls, even though they received the same endogenous signal from the vegetal mass. Elongation of animal caps is a reliable indicator of dorsal mesodermal gastrulation-type movements, and has been shown to correlate with dorsal mesoderm induction (Symes and Smith, 1987; Sokol, 1993).

In a previous study, which has since been repeated many times, we showed that a β-catenin-depleted vegetal mass combined with a wild-type cap induced little or no dorsal mesoderm. Also, a β-catenin-depleted animal cap formed much reduced amounts of dorsal mesoderm in response to a wild-type vegetal mass (Heasman et al., 1994). We report here that injection of β-catenin mRNA into a single blastomere will generate dorsal mesoderm in an otherwise β-catenin-depleted embryo. The apparent contradiction here may be explained by the differences in the amounts of β-catenin present in each experiment. In the recombinant experiment reported previously, the vegetal mass is expressing only endogenous levels of β-catenin, whereas in the 32-cell-injection experiment, β-catenin is overexpressed far above endogenous levels in the progeny of 1 blastomere of the 32 cell stage embryo. Here we tested whether this explanation was correct by examining whether overexpression (instead of wild-type levels) of β-catenin in the vegetal component of a recombinant would cause dorsal mesoderm to form in a β-catenin-depleted animal cap. Fig. 3A lane 12 shows that β-catenin-overexpressing vegetal masses do induce dorsal mesoderm in depleted animal caps compared to wild-type combinations (lane 11) and that the converse is also the case: overexpressing caps enhance the expression of MyoD when combined with β-catenin-deficient vegetal masses (lane 13 and 14).

In summary, these experiments all indicate that the expression of β-catenin in cells causes those cells to produce ‘dorsal signal/s’.

The timing of the ‘dorsal signal’

(i) After mRNA depletion, β-catenin mRNA is resynthesized by the gastrula stage

Fig. 4 shows the time period of development in which β-catenin mRNA is depleted, and thus synthesis of the protein is reduced, after oligo-mediated depletion of mRNA in oocytes. We have shown previously that this treatment substantially reduces the amount of β-catenin protein (Heasman et al., 1994) and here we demonstrated this by immunostaining blastulae from this experiment (data not shown). After the blastula stage, precise staging of β-catenin-depleted embryos is difficult due to the delay in formation of the blastopore. No dorsal blastopore lip forms and instead the onset of gastrulation is delayed until a blastopore lip appears simultaneously all round the blastopore. After gastrulation, no neural folds form in the β-catenin-depleted embryos. Because of this, we left β-catenin-depleted embryos until uninjected sibling embryos reached the stages shown. Thus these are shown as ‘sibling stages’ in Fig. 4. We found that zygotic β-catenin mRNA reappeared during the late blastula and gastrula stages, and wild-type levels are restored by sibling

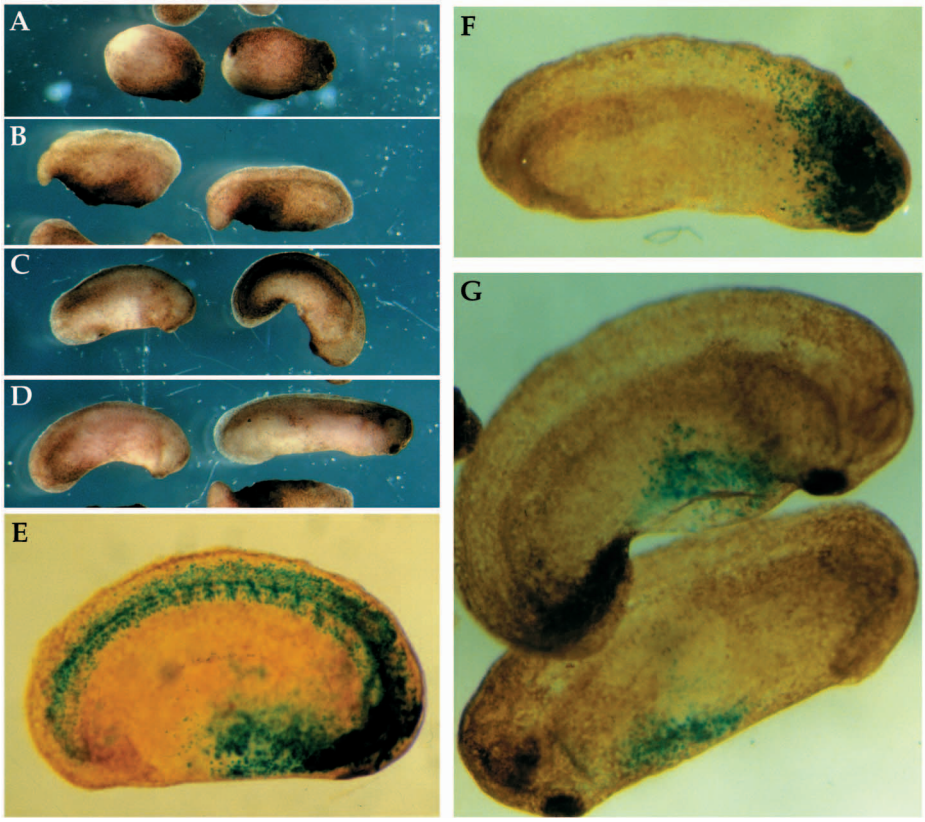
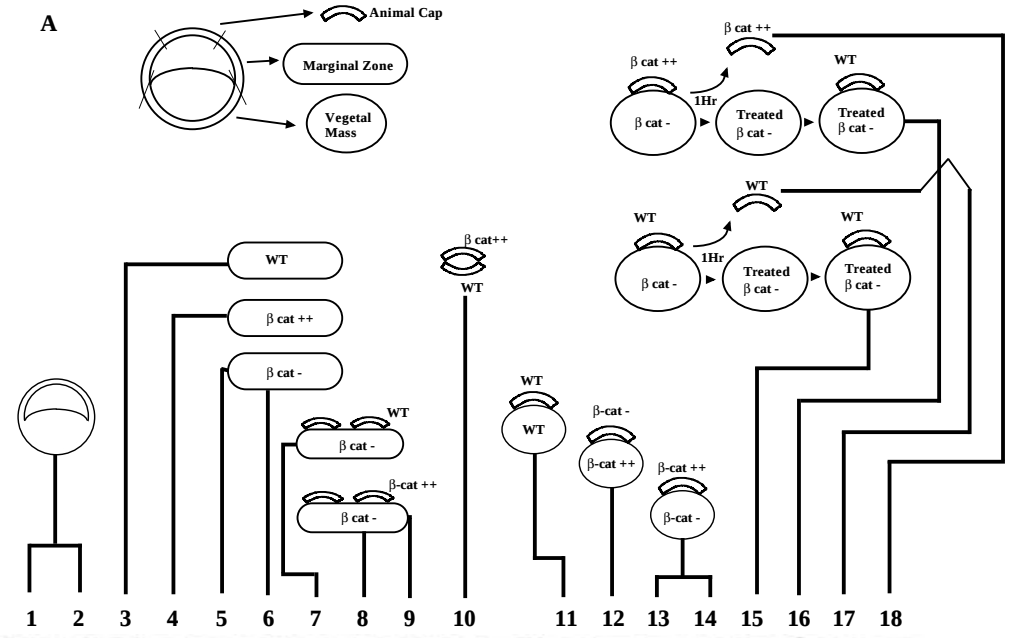


Fig. 2. β-catenin mRNA rescues the dorsal axis when injected into single animal, equatorial or vegetal cells of β-catenin-depleted 32-cell stage embryos.The degree of rescue is shown in A-D. (A) No injection, (B) animal cell injection, (C) vegetal cell injection, and (D) marginal cell injection of 150 pg of β-catenin mRNA. The descendant cells of the single injected cells are shown by X-gal staining. (E) The progeny of an equatorial cell overexpressing β-catenin are in axial mesoderm (notochord and somite) and anterior endoderm. (F) Progeny of an animal-injected cell overexpressing β-catenin are in the skin over the head, and (G) the progeny of a vegetal-injected blastomere are in endoderm.



1 = Sibling whole embryos of caps used to treat vegetal masses
2 = Sibling whole embryos of rest of experiment
3 = Control marginal zones
4 = Overexpressing marginal zones
5 = Depleted marginal zones (600pg oligo)
6 = Depleted marginal zones (800pg oligo)
7 = Depleted marginal zones + control animal caps
8 = Depleted marginal zones + overexpressing animal caps (2ng mRNA)
9 = Depleted marginal zones + overexpressing animal caps (1ng mRNA)
10 = Control animal caps + overexpressing animal caps
11 = Control animal caps + control vegetal masses
12 = Depleted animal caps + overexpressing vegetal masses
13 = Overexpressing animal caps + depleted vegetal masses
14 = Overexpressing animal caps + depleted vegetal masses
15 = Control caps + control cap-treated depleted vegetal masses
16 = Control caps + overexpressing cap-treated vegetal masses
17 = Control caps used to treat vegetal masses
18 = Overexpressing caps used to treat vegetal masses

stage 12 (the end of gastrulation). Thus maternal β-catenin may function at any point before zygotic β-catenin replaces it. This zygotic protein is not able to rescue the maternal deficiency, suggesting that the maternal protein acts at least before the gastrula stage.

(ii) The 'dorsal signal' starts to pass between vegetal masses and animal caps after stage 7 (early blastula stage)

If the primary role of β-catenin is to cause cells to emit a 'dorsal signal', when does this happen? To test this, we compared the abilities of stage 7 (early blastula) and stage 9 (late blastula) vegetal masses to stimulate stage 9 animal caps to form dorsal mesoderm (indicated by expression of MyoD), and ventral and lateral mesoderm (by the expression of Xwnt8). This was done by co-culturing the tissues for only 1 hour, and then separating them, so that the stage 7 vegetal masses had not reached stage 9 before the animal caps were taken away (Fig. 5A shows sibling embryos to the stage 7 caps at the beginning of the culture period, and Fig. 5C the same embryos at the end of this

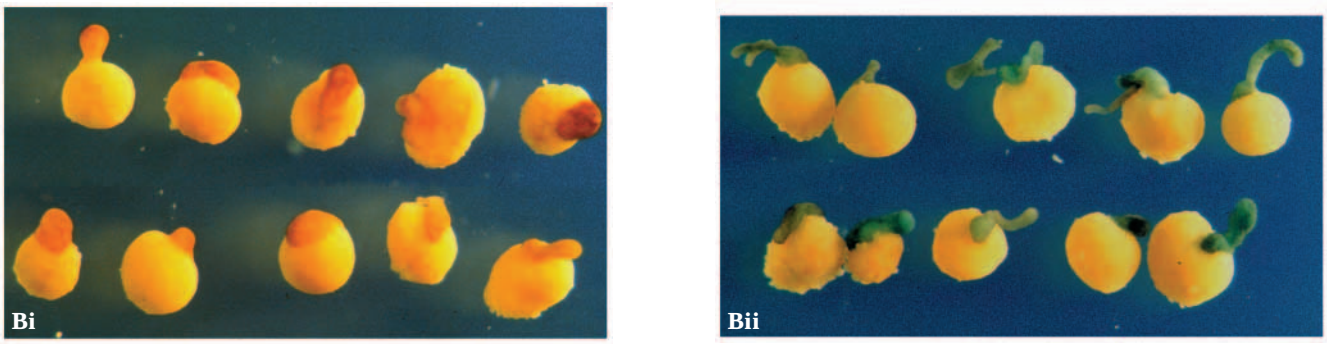


Fig. 3. (A) Animal caps from β-catenin overexpressing embryos produce a 'dorsal signal' but will not induce animal caps to form mesoderm. The design of the experiments are described in the text and illustrated here diagrammatically. WT, wild type; β cat++, overexpressing β-catenin mRNA; β cat–, β-catenin-depleted by the injection of antisense oligo into oocytes. RNA was prepared from groups of 5 marginal zones, 10 animal caps or 10 animal/vegetal recombinants at the late neurula stage, and northern blots were probed for the dorsal mesodermal marker MyoD. Ef1α was used as a loading control. (B**)** Overexpression of β-catenin in animal caps causes them to be dorsalized. Nieuwkoop recombinants consisiting of either wild-type caps and wild-type vegetal masses (Bi), or β-catenin-overexpressing caps and wild-type vegetal masses (Bii) were made at the midblastula stage and cultured until siblings reached the neurula stage (stage 16). Overexpressing caps elongated dramatically compared to controls.

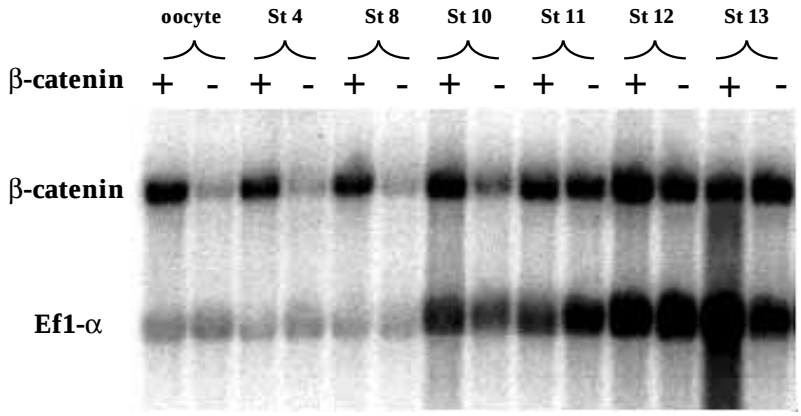


Fig. 4. Zygotic β-catenin mRNA is synthesized from the early gastrula stage onwards. Maternal β-catenin was depleted from oocytes by injection of antisense oligo, and then oocytes and embryos derived from the same batch of oocytes were frozen at the stages shown. The developmental northern blot was probed with a β-catenin probe and with Ef1α as a loading control. +, uninjected; –, depleted of maternal β-catenin mRNA. Zygotic β-catenin mRNA has appeared by stage 10 (early gastrula), and reaches wild-type levels during gastrulation.

period).The caps were then cultured until sibling stage 20 so that they had time to express mesodermal markers. Stage 9 animal caps elongated, and expressed MyoD, in response to to stage 9 vegetal masses (Fig. 5G,L), but not in response to stage 7 vegetal masses (Fig. 5F,L). They did express very low levels of the ventral mesoderm marker Xwnt8 in response to stage 7 vegetal masses, but at significantly lower levels than in response to stage 9 vegetal masses. This unexpectedly low expression of Xwnt8 in recombinants of stage 9 caps and stage 7 bases was found in three repeats of this experiment.

In order to eliminate the possibility that there is a dorsal signal from the stage 7 vegetal masses, but below the sensitivity of the assay, we included, in the same experiment, vegetal masses that were overexpressing β-catenin, and therefore should have an enhanced 'dorsal signal'. Stage 9 overexpressing vegetal masses caused an exaggerated dorsal mesoderm response in animal caps, measured by elongation (Fig. 5I) and MyoD expression (Fig. 5L). However, they did not cause a response when used at stage 7 (Fig. 5H) and (Fig. 5L). This experiment shows that before the midblastula stage, vegetal masses do not signal animal caps to form dorsal mesoderm.

To find when embryonic transcription started in this experiment, we took siblings of the embryos from which the stage 7 vegetal masses were dissected, immediately after the co-culture period (shown in Fig. 5C), and tested them for the expression of GS17, whose expression starts at the MBT. Fig. 5M left lane shows a very low level of GS17 in the sibling whole embryos at the end of the co-culture period used for the stage 7 vegetal masses (Fig. 5C), indicating the very recent onset of zygotic transcription. In comparison, there is a much higher level of expression in the stage 9 embryos used for stage 9 animal caps and vegetal masses (Fig. 5M right lane : embryos frozen at the time of development shown in Fig. 5B).

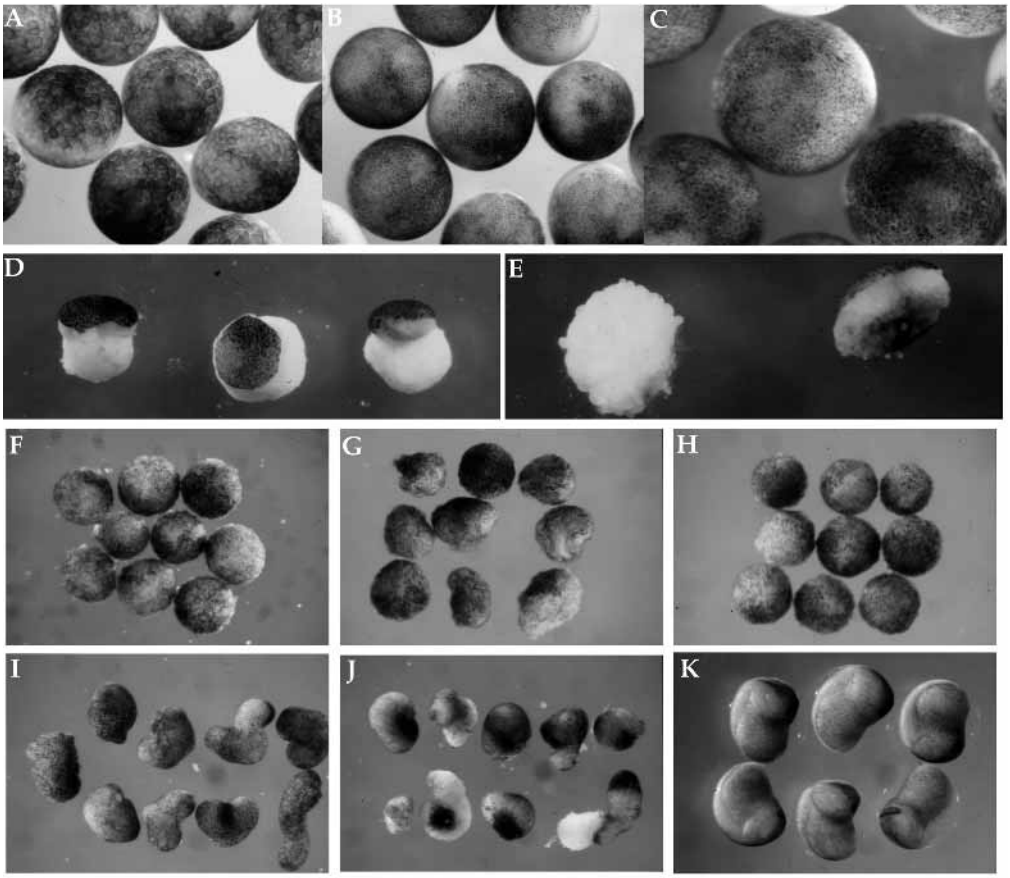
Taken together, the experiments suggest that the 'dorsal signal' is not passed from vegetal to animal cells before MBT. The results suggest a model where β-catenin expression in dorsal cells activates a maternal transcription factor/s at MBT, causing the release of a dorsal signal at this time. Zygotic β-catenin appears too late to activate the transcription factor/s.

The relationship of β-catenin to other molecules known to have activity in dorsal axis formation

To determine when β-catenin acts with respect to other molecules known to be capable of dorsal axis formation (see Introduction), we tested the ability of these molecules to rescue the dorsal axis lost by β-catenin depletion. We generated a series of embryos depleted of β-catenin by fertilizing antisense oligo-injected oocytes, and injected mRNAs for β-catenin, BVg1, dn-gsk, tBR, siamois and noggin at the 8-16 cell stages, into the dorsal equatorial region. In each case, we showed that the mRNAs were translated by their ability to cause ectopic dorsal axes when injected into the ventral sides of sibling wild-type embyos. We judged whether β-catenin-deficient embryos were rescued by their morphology, and by the expression of the dorsal mesoderm marker MyoD, the neural marker NRP1 and the ventral mesoderm marker Xwnt8 at the late neurula stage (stage 23). Table 1 contains the aggregated numbers of embryos scored morphologically. The fates of the descendants of the injected cells were followed in each case by coinjection of β-galactosidase mRNA.

Fig. 6A demonstrates that expression of a dominant negative GSK3β (dn-gsk) mRNA caused an extra dorsal axis in control embryos, (upper two embryos), but did not rescue the effect of β-catenin depletion (lower two embryos). Fig. 6B and C show that the dn-gsk-injected cells, coinjected with β-galactosidase mRNA, contributed to both mesoderm and endoderm of the ectopic axis in the controls (6B), and remained as a large clone in the β-catenin-depleted embryos at the same stage (6C). In contrast, Fig. 6D-F illustrate the effect of a molecule that does rescue dorsal axial structures in β-catenin-depleted embryos. Expression of BVg1 caused an ectopic, second dorsal axis in control embryos (Fig. 6D) and rescued a single dorsal axis in β-catenin-depleted embryos (compare Fig. 6E and F). Similar results were found with β-catenin, tBR, siamois and noggin (not shown). Not all the mRNAs rescued to the same extent. Siamois and tBR injection caused less complete axes than the others, with less head structures.

The expression of molecular markers in these experiments is shown in Fig. 7. The northern blot demonstrates that MyoD and NRP1 were not expressed in β-catenin-depleted embryos (lane 2 and 3), while Xwnt8 expression was increased, compared to controls. The identical expression pattern was seen in β-catenin-depleted embyos injected with dn-gsk (lane 8 and 9). In contrast, injection of β-catenin (lane 4 and 5), BVg1 (lane 6 and 7), siamois (lane 12-15) and noggin (lanes 16-20) mRNAs, rescued the expression of both MyoD and NRP1. tBR injection into β-catenin-depleted embryos caused them to express wild-type levels of MyoD and to overexpress Xwnt8, but did not rescue significantly the neural marker, NRP1(lane 11). Lastly, we showed that bFGF induced MyoD



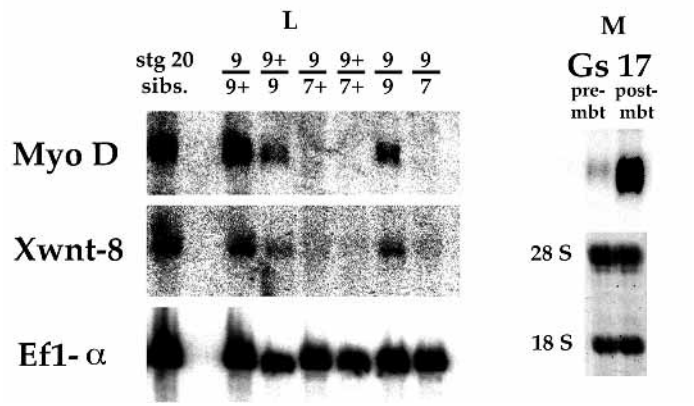


Fig. 5. Animal caps from stage 9 embryos elongate and make MyoD in response to stage 9, but not stage 7 vegetal masses. The stage 7 embryos at the time the 9/7 animal and vegetal masses were put together are shown in A, and when they were taken apart again in C. The stage 9 embryos at the time the 9/7 and 9/9 animal and vegetal masses were put together are shown in B. Stage 9 caps on stage 7 vegetal masses are shown in D and separated after 1 hour in E, to show that the two components are easy to distinguish and can be completely separated. Stage 9 animal caps elongate and express MyoD to a dramatically greater extent in response to wild-type stage 9 vegetal masses (G, and 9/9 in L), but do not respond to stage 7 vegetal masses, either untreated (F, and 9/7 in L), or overexpressing β-catenin (H, and 9/7+ in L). Combinations in which either vegetal masses (I and 9/9+ in northern) or animal caps (J and 9+/9 in L) were overexpressing β-catenin show an increased response in the animal caps. Sibling embryos at the time the animal caps were frozen are shown in K, and their level of expression of MyoD (one embryo) in the right hand lane of the northern blot. M illustrates the degree to which zygotic transcription is underway at the time when recombinants were seperated, as measured by the expression of Gs17 in northern blots. RNA was prepared from the embryos shown in B and C. Those in B expressed high levels of Gs17, showing that embryonic transcription had started. Those in C expressed only low levels, indicating that the embryos were at the midblastula transition.

expression in animal caps taken from β-catenin-depleted embryos (Fig. 7 lane 22).

DISCUSSION

The results presented here tell us about the role of β-catenin in the early *Xenopus* embryo, and when and where it is required for the formation of dorsal mesoderm. The first experiment was designed to test whether β-catenin acts in a cell-autonomous manner or not, and whether it causes the cells in which it is expressed to become dorsal mesoderm or not. The conclusion is that β-catenin acts in a non-cell autonomous manner, when overexpressed in animal and vegetal cells, and in both autonomous and non-autonomous manner when over-expressed in equatorial cells. It does not cause animal or vegetal cells to become dorsal mesoderm, when it is expressed in those cells. However, some progeny of β-catenin-overexpressing cells in the marginal zone become dorsal rather than ventral mesodermal cells.

The second experiment tested the nature of the signal produced by β-catenin-overexpressing animal caps. When β-catenin-depleted fragments of blastulae were co-cultured with overexpressing animal caps, they recovered their dorsal properties, but in different ways characterised by their germ layer specification. Marginal zones made dorsal mesoderm; vegetal masses regained the ability to signal animal caps to make dorsal mesoderm (ie to become Nieuwkoop Centers). We also found that wild-type caps cultured with β-catenin-deficient equatorial parts or vegetal masses produced a much reduced but reproducible MyoD signal compared to wild-type combinations. In comparison, equatorial parts from β-catenin-deficient embryos expressed no MyoD, and animal/vegetal combinations where both parts are deficient in β-catenin, also showed no detectable transcription of MyoD (Heasman et al., 1994 and data not shown). The likely interpretation is that the endogenous ‘dorsal signal’ emanating from the wild-type cap

is sufficient to rescue, to a detectable extent, dorsal mesoderm in the marginal zone and a Nieuwkoop Center in the vegetal mass. This could also explain the fact that dorsal marginal zones can induce ventral marginal zones to become more dorsal (the foundation of the third signal in the three-signal model for mesoderm induction and patterning, Smith and Slack, 1983).

These experiments support the hypothesis that β-catenin acts to produce a 'dorsal signal'over the entire dorsal side of the embryo rather than the other two possible interpretations of β-catenin activity: that β-catenin could act only in the Nieuwkoop Center, or that β-catenin could act as a 'competence modifier'.

β-catenin is unlikely to act only in the Nieuwkoop Center because we have shown previously and subsequently repeated, that β-catenin-depleted animal caps have a much reduced response in terms of MyoD expression to wild-type vegetal masses. Also, Sokol and Melton (1991) have shown that dorsal and ventral halves of animal caps make different responses to activin, only dorsal halves making dorsal mesoderm. This is consistent with the possibility that there is a dorsal/ventral difference in the animal cap of the blastula stage embryo in the ability to send a dorsal signal. We interpret this experiment in the following way. Activin induces the animal cap to form mesoderm. Only cells on the dorsal side produce a 'dorsal signal' that causes mesoderm to develop with dorsal characteristics ie. to form notochord and somites.

The term 'competence modifier' is used to describe a second signal received by equatorial cells that modifies their response to a primary mesoderm-inducing signal from the vegetal mass, causing them to form dorsal rather than ventral mesoderm. An Xwnt pathway has been suggested to act as a competence modifier, to explain the fact that Xwnt-overexpressing animal caps make a more dorsal response to mesoderm inducers than wild-type caps (Christian et al., 1992; Sokol, 1992; Moon et al., 1993). However, the experiments described here do not support this explanation for the activity of β-catenin. We find that β-catenin-depleted embryos can be rescued, in a non-cell autonomous fashion, by the injection of β-catenin mRNA into single animal, marginal or vegetal cells. Here, the rest of the embryo lacks maternal β-catenin, and yet makes dorsal mesoderm. So maternal β-catenin cannot be required in the responding, dorsal mesoderm-forming cells. This does not exclude the possibility that there is a later pathway, mediated by zygotic β-catenin, that does act in responding cells, although such a pathway cannot rescue the ventralized phenotype caused by depleting maternal β-catenin.

The third and fourth experiments described here address the question, when is β-catenin required for dorsal mesoderm to form in the embryo? Previous experiments have suggested that mesoderm induction in *Xenopus* starts before MBT (Gurdon et al., 1985; Jones and Woodland, 1987), although the assays in these cases did not involve separating recombinants before zygotic transcription started. Various factors including bFGF, activin and BVg1 have been considered as candidate inducers. The concept of early blastula inducers has had a great influence over thinking about the signalling pathways that lead to dorsal mesoderm formation. However, four observations made us revisit this issue. Firstly, the results presented here and elsewhere show that β-catenin expressing cells produce a 'dorsal signal', without telling other cells to become mesoderm. So it does not necessarily have to be on the same time scale as general mesoderm induction. Secondly, in *Drosophila* embryonic epidermis, the wingless signal has been to shown to increase cytosolic levels of armadillo (Peifer et al., 1994) and thus to maintain the transcription of the engrailed transcription factor (Martinez-Arias et al., 1988), although it is not known how increased cytosolic levels of armadillo lead to maintenance of gene expression. Thirdly, it has been reported that overexpressed β-catenin accumulates in nuclei of blastula stage *Xenopus* embryos (Funayama et al. 1995). Fourthly, several gene products, including the transcription factors goosecoid (Cho et al., 1991), siamois (Lemaire et al., 1994) and Xlim1(Taira et al., 1992), are zygotically expressed after MBT, in the presence of protein synthesis inhibitors, in specific and localized fashions. This suggests that maternal transcription factors must be in place to initiate the synthesis of such localized zygotic genes directly. The role of β-catenin may be to localize such a maternal transcription factor/s in dorsal nuclei. The experiments presented here show that neither wild-type nor β-catenin overexpressing vegetal masses from early blastulae are able to send a 'dorsal signal' to late blastulae animal caps, suggesting that there is no 'dorsal signal' released before MBT. These results are in agreement with the experiments of Sokol (1993), who showed that animal caps do not elongate in response to Xwnt8 mRNA

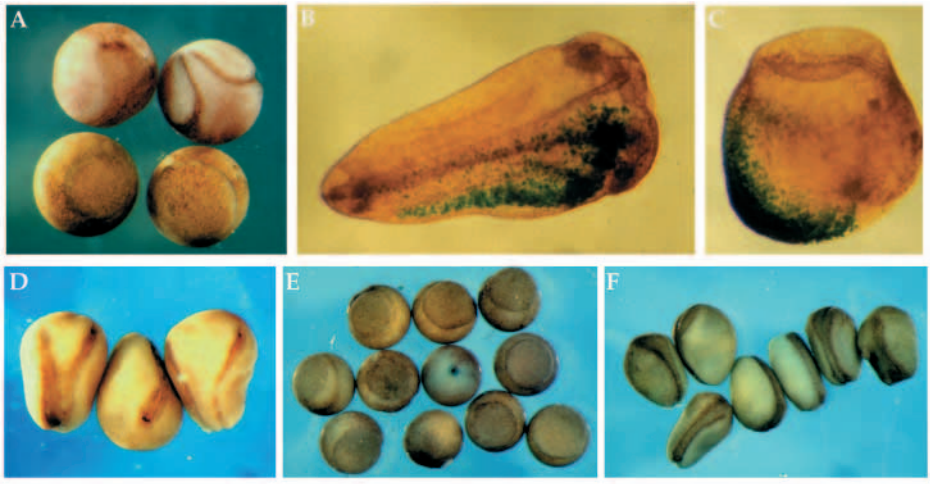


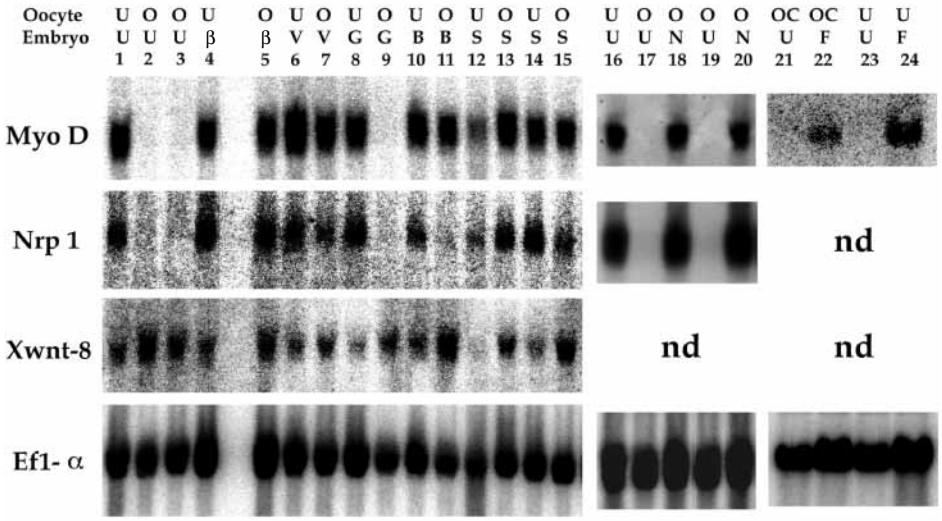
Fig. 6. Only GSK, of the molecules tested, lies upstream of β-catenin. (A) Two untreated embryos (upper two embryos) and two β-catenin-depleted embryos (lower two embryos), injected at the 32-cell stage with dn-gsk, and photographed at the neurula stage. Overexpression of dn-gsk causes an ectopic axis in the untreated, but does not rescue the β-catenin-depleted embryo. B and C show the distribution of descendants of the injected cells later in development, in the untreated and β-catenin-depleted embryos, respectively. D-F show that overexpression of BVg1 causes a second dorsal axis in control embryos (D), and generates a single axis in β-catenin-depleted embryos (F). Embryos from the same batch of β-catenin-depleted embryos, but which were not injected with BVg1, are shown in E.

if they are isolated before the late blastula stage (Sokol, 1993). This suggests that earlier caps have not received an endogenous inducing signal. While the nature of the putative maternal transcription factor with which β-catenin may associate is unknown, there are a number of candidates for the zygotic gene products downstream, (siamois, gsc, Xlim1) and for the secreted 'dorsal signal/s', including noggin, Xnr 1or 2 (Jones et al., 1995), Xnr3 (Smith et al., 1995), BVg1, bFGF and activin.

The heterochronous recombination experiments also showed that before the midblastula stage there is only a small degree of ventral mesodermal signalling, as indicated by Xwnt8 expression (and also Xbra; data not shown) in 9/7 recombinants compared to 9/9. This suggests that both ventral and dorsal mesoderm induction signals coming from the vegetal mass may occur after MBT.

While many of the experiments described here and elsewhere concerning dorsal mesoderm formation, use animal and vegetal recombinants or animal cap assays, it is clear from the fate map that dorsal mesoderm actually forms from cells in the equatorial zones of blastulae. Activin and bFGF can cause MyoD to be expressed in β-catenin-deficient animal caps and yet whole embryos lacking only maternal β-catenin are not able to use endogenous activin or bFGF to form a dorsal axis. This suggests there is no redundancy of maternal dorsal signalling pathways in the equatorial zones, and that activin and bFGF responsive events are either downstream of the β-catenin-initiated 'dorsal signal' or not involved. The same is true for Vg 1, noggin, siamois and tBR activity. Whether the timing of the release of the endogenous 'dorsal signal' in the equatorial zone is after MBT, as it is for the vegetal signal, has not been assessed here because of the difficulty of knowing whether an equatorial dissection at the early blastula stage is completely free from vegetal cells. As described above, cells in this region overexpressing β-catenin respond in both an autonomous and non-autonomous fashion, reminiscent of cells responding to wingless signals in the *Drosophila* embryonic epidermis (Hooper 1994, Yoffe et al., 1995). The fact demonstrated here that Xgsk-3β lies upstream of β-catenin is further support of the conservation of a wingless-type pathway in *Xenopus*.

In conclusion, the simplest model suggested by the present available data is that a maternal Xwnt-initiated pathway stabilizes β-catenin (by analogy with the effects of wingless on armadillo protein, Van Leeuwen et al., 1994) on the dorsal side of the embryo and leads to the release of 'a dorsal signal/s' that is released by both animal and vegetal cells and may be required in all presumptive germ layers. In the case of the 'dorsal signal' from the vegetal mass to the



Sample	Oocyte treatment	Embryo treatment
1	uninj	uninj
2	600pg. β-catenin oligo	uninj
3	800pg. β-catenin oligo	uninj
4	uninj	β-catenin mRNA 150 pg
5	800pg. β-catenin oligo	β-catenin mRNA 150 pg
6	uninj	BVg1 mRNA 10 pg
7	800pg. β-catenin oligo	BVg1 mRNA 10 pg
8	uninj	dn-gsk mRNA 650 pg
9	800pg. β-catenin oligo	dn-gsk mRNA 650 pg
10	uninj	tbR mRNA 900 pg
11	800pg. β-catenin oligo	tbR mRNA 900 pg
12	uninj	Siamois mRNA 40 pg
13	800pg. β-catenin oligo	Siamois mRNA 40 pg
14	uninj	Siamois mRNA 80 pg
15	800pg. β-catenin oligo	Siamois mRNA 80 pg
16	uninj	uninj
17	500pg. β-catenin oligo	uninj
18	500pg. β-catenin oligo	noggin mRNA 1 pg
19	700pg. β-catenin oligo	uninj
20	700pg. β-catenin oligo	noggin mRNA 1 pg
21	800pg. β-catenin oligo	caps -bFGF
22	800pg. β-catenin oligo	caps +bFGF 100 ng/ml
23	control	caps -bFGF
24	control	caps +bFGF 100 ng/ml

Fig. 7. Only GSK is unable to cause the expression of dorsal mesodermal and neural markers in β-catenin-deficient embryos. This is a northern blot of RNA from neurulae derived from embryos untreated and treated with ologo. The blot was probed for the dorsal markers MyoD (skeletal muscle) and NRP1(neural tissue), the ventral marker Xwnt8 and the loading control Ef1α. Each lane is described both in terms of the treatment each sample had as oocytes (i.e. injected with oligo or not) and in terms of the treatment after fertilization (ie. the injection of a specific mRNA at the 8-16 cell stage or not). This information is also summarized above each figure as oocyte treatment: U, uninjected or O, antisense oligo-injected; and embryo treatment: β, β-catenin mRNA-injected; V, BVg1; G, dn-gsk; B, tBR; S, siamois; N, noggin. Lanes 21-24 are of animal cap-derived tissue rather than whole embryos. F, bFGF-treated caps.

animal cap in recombinants, the signal is not released until after the midblastula transition. The data are consistent with the suggestion that β-catenin is required for the activation or maintenance of the expression of a maternal transcription factor(s) that, in turn, leads to both cell autonomous and non-autonomous effects when zygotic trancription starts.The immediate questions are to characterize both the nature of the initiating wingless-type signal (the presumed primary dorsal signal) and the nature of the maternal transcription factor/s.

We are grateful to the NIH (grant number: 1 RO1 HD33002-01), the Institute of Human Genetics and the Harrison Fund, for financing this work. Siamois was a gift from Patrick Lemaire, dn-gsk3 from David Kimelman, noggin from Richard Harland, tBR from Ken Cho. Many thanks also to Dr D. A. Melton for collaboration and advice.

REFERENCES

Amaya, E., Musci, T. and Kirschner, M. (1991). Expression of a dominant negative mutant of the FGF receptor disrupts mesoderm formation in Xenopus embryos. *Cell* **66**, 257-270.

Boterenbrood, E. C. and Nieuwkoop, P. D. (1973). The formation of the mesoderm in urodelean amphibians. *Willhelm Roux' Archiv. Dev. Biol.* **173**, 319-332.

Christian, J., McMahon, J., McMahon, A. and Moon, R. (1991). *Xwnt-8*, a *Xenopus Wnt1/int*-related gene responsive to mesoderm inducing factors may play a role in ventral mesoderm patterning during embryogenesis. *Development* **111**, 1045-1056.

Christian, J., Olson, D. and Moon, R. (1992). Xwnt-8 modifies the character of mesoderm induced by bFGF in isolated Xenopus ectoderm. *EMBO J.* **11**, 33-41.

Cho, K. W. Y., Blumberg, D., Steinbeisser, H. and DeRobertis, E. M. (1991). Molecular nature of Spemann's Organizer: the role of the Xenopus homeobox gene goosecoid. *Cell* **67**, 1111-1120.

DeMarais, A. and Moon, R. (1992). The armadillo homologs β-ctenin and plakoglobin are differentially expressed during early development of Xenopus lavis. *Dev. Biol.* **153**, 337-346.

Dohrmann, C. E., Kessler, D. S. and Melton, D. A. (1996). Induction of axial mesoderm by zDVR-1, the zebrafish orthologue of Xenopus Vg1. *Dev. Biol.* **175**, 108-117.

Funayama, N., Fagotto, F., McCrea, P. and Gumbiner, B. (1995). Embryonic axis formation by the armadillo repeat domain of β catenin, evidence for intercellular signalling. *J. Cell Biol.* **128**, 959-968.

Graff, J. M., Thies, R. S., Song, J. J., Celeste A. J. and Melton D. A. (1994). Studies with a Xenopus BMP receptor suggest that ventral mesoderm-inducing signals override dorsal signals in vivo. *Cell* **79**, 169-179.

Guger, K. A. and Gumbiner, B. M. (1995). beta-Catenin has Wnt-like activity and mimics the Nieuwkoop signaling center in Xenopus dorsal-ventral patterning. *Dev. Biol.* **172**, 115-125.

Gurdon, J. B., Fairman, S., Mohun, T. J. and Brennan, S. (1985). Activation of muscle-specific actin genes in Xenopus development by an induction between animal and vegetal cells of a blastula. *Cell* **41**, 913-22.

Hawley, S., Wunnenberg-Stapleton, K., Hashimoto, C., Laurent, M., Watabe, T., Blumberg, B. and Cho, K. (1995). Disruption of BMP signals in embryonic Xenopus ectoderm leads to direct neural induction. *Genes Dev.* **9**, 2923-2935.

He, X., Saint-Jeannet, J.-P., Woodgett, J., Varmus, H. and Dawid, I. (1995). Glycogen synthase kinase-3 and dorsoventral patterning in Xenopus embryos. *Nature* **374**, 617-622.

Heasman, J., Crawford, A., Goldstone, K., Garner-Hamrick, P., Gumbiner, B., Kintner, C., Yoshida-Noro, C. and Wylie, C. (1994). Overexpression of cadherins, and underexpression of β catenin inhibit dorsal mesoderm induction in early Xenopus embryos. *Cell* **79**, 791-803.

Heasman, J., Holwill, S. and Wylie, C. C. (1991). Fertilization of cultured Xenopus oocytes and use in studies of maternally inherited molecules. *Methods Cell Biol.* **36**, 213-230.

Hemmati-Brivanlou, A. and Harland, R. (1989). Expression of an engrailed-related protein is induced in the anterior neural ectoderm of early Xenopus embryos. *Development* **108**, 611-617.

Holwill, S., Heasman, J., Crawley, C. and Wylie, C. C. (1987). Axis and germ line deficiencies caused by u.v irradiation of Xenopus oocytes cultured in vitro. *Development* **100**, 735-743.

Hooper, J. E. (1994). Distinct pathways for autocrine and paracrine Wingless signalling in Drosophila embryos. *Nature* **372**, 461-464.

Jones, C. M., Kuehn, M. R. Hogan, B. M. Smith, J. C. and Wright C. V. E. (1995). Nodal-related signals induce axial mesoderm and dorsalize mesoderm during gastrulation. *Development* **121**, 3651-3662

Jones, E. A. and Woodland, H. R. (1987). The development of animal cap cells in *Xenopus*: a measure of the start of animal cap competence to form mesoderm. *Development* **101**, 557-563.

Kimelman, D. and Kirschner, M. (1987). Synergistic induction of mesoderm by FGF and TGF-beta and the identification of an RNA coding for FGF in the early Xenopus embryo. *Cell* **51**, 869-877.

Ku, M. and Melton, D. A. (1993). Xwnt-11: a maternally expressed *Xenopus* wnt gene. *Development* **119**, 1161-1173.

Lemaire, P., Garrett, N. and Gurdon, J. (1995). Expression cloning of Siamois, a Xenopus homeobox gene expressed in dorsal-vegetal cells of blastulae and able to induce a complete secondary axis. *Cell* **81**, 85-94.

Martinez Arias, A., Baker, N. and Ingham, P. W. (1988). Role of segment polarity genes in the definition and maintenance of cell fates in the *Drosophila* embryo. *Development* **103**, 157-170.

McMahon, A. and Moon, R. (1989). Ectopic expression of the proto-oncogene int-1 in Xenopus embryos leads to duplication of the embryonic axis. *Cell* **58**, 1075-1084.

Moon, R., Christian, J., Campbell, R., McGrew, L., DeMarais, A., Torres, M., Lai, C.-J., Olson, D. and Kelly, G. (1993). Dissecting Wnt signalling pathways and Wnt-senstive developmental processes through transient misexpression analyses in embryos of *Xenopus laevis. Development* **Supplement**, 85-94.

Peifer, M., Sweeton, D., Casey, M. and Wieschaus, E. (1994). Wingless signal and Zeste-white 3 kinase trigger opposing changes in the intracellular distribution of Armadillo. *Development* **120**, 369-380.

Pierce, S. and Kimelman, D. (1995). Regulation of Spemann organiser formation by the intracellular kinase Xgsk-3. *Development* **121**, 755-765.

Rebagliati, M. R., Weeks, D. L., Harvey, R. P. and Melton D. A. (1985). Identification and cloning of localized maternal mRNAs from Xenopus eggs. *Cell* **42**, 769-777.

Slack, J., Darlington, B., Heath, J. and Godsave, S. (1987). Mesoderm induction in early Xenopus embryos by heparin-binding growth factors. *Nature* **326**, 197-200.

Smith, J. C. and Slack, J. M. W. (1983). Dorsalization and neural induction: Properties of the organizer in *Xenopus laevis*. *J. Embryol. exp. Morph.* **78**, 299-317.

Smith, W. C. and Harland, R. M. (1991). Injected Xwnt-8 RNA acts early in Xenopus embryos to promote formation of a vegetal dorsalizing centre. *Cell* **67**, 753-765.

Smith, W. C. and Harland, R. M. (1992). Expression cloning of noggin, a new dorsalizing factor localised to the Spemann organiser in *Xenopus* embryos. *Cell* **70**, 828-840.

Smith, W. C., McKendry, R., Ribisi S. and Harland, R. M. (1995). A nodal-related gene defines a physical and functional domain within the Spemann organizer. *Cell* **82,** 37-46.

Sokol, S. (1992). Interaction of Wnt and activin in dorsal mesoderm induction in Xenopus. *Dev. Biol.* **154**, 348-355.

Sokol, S., Christian, J. L., Moon R. T. and Melton, D. A. (1991). Injected Wnt RNA induces a complete body axis in Xenopus embryos. *Cell* **67**, 741-752.

Sokol, S., Klingensmith, J., Perrimon, N. and Itoh, K. (1995). Dorsalising and neuralising properties of Xdsh, a maternally expressed *Xenopus* homolog of dishevelled. *Development* **121**, 1637-1647.

Sokol, S. and Melton, D. (1991). Pre-existent pattern in *Xenopus* animal pole cells revealed by induction with activin. *Nature* **351**, 409-411.

Sokol, S. Y. (1993). Mesoderm formation in *Xenopus* ectodermal explants overexpressing Xwnt8: evidence for a cooperating signal reaching the animal pole by gastrulation. *Development* **118**, 1335-42.

Symes, K. and Smith, J. C. (1987). Gastrulation movements provide an early marker of mesoderm induction in *Xenopus laevis. Development* **101**, 339-349.

Taira, M., Jamrich, M., Good, P. J. and Dawid, I. B. (1992). The LIM domain-containing homeo box gene Xlim-1 is expressed specifically in the organizer region of Xenopus gastrula embryos.*Genes Dev.* **6**, 356-366.

Thomsen, G. and Melton, D. (1993). Processed Vg1 protein is an axial mesoderm inducer in Xenopus. *Cell* **74**, 433-441.

Yoffe, K. B., Manoukian, A. S., Wilder, E. L., Brand, A. H. and Perrimon N. (1995). Evidence for engrailed-independent wingless autoregulation in Drosophila. *Dev. Biol.* **170**, 636-650.

Van Leeuwen, F., Samos, C. H. and Nusse, R. (1994). Biological activity of soluble wingless protein in cultured Drosophila imaginal disc cells. *Nature* **368**, 342-344.

(Accepted 17 July 1995)