# Dickkopf1 and the Spemann-Mangold head organizer

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**ABSTRACT** Work in amphibians indicates that inhibition of Wnt and BMP signals is essential for head development and that head induction by the Spemann-Mangold organizer may be mediated by secreted Wnt antagonists. Wnts are potent posteriorizing factors and antagonize the Spemann-Mangold organizer. *Dickkopf1* (*dkk1*) encodes a secreted effector expressed in head organizing centers of *Xenopus,* mouse and zebrafish. It acts as a Wnt inhibitor and is able together with BMP inhibitors to induce the formation of ectopic embryonic heads in *Xenopus*. It anteriorizes both mesendoderm and neuroectoderm, promoting prechordal plate and forebrain fates. Injection of inhibitory antibodies leads to microcephaly and cyclopia. Dkk1 thus is an essential mediator of the vertebrate head organizer.

KEY WORDS: *Spemann-Mangold organizer; dkk1; head induction; Wnt; BMP; cyclopia; prechordal plate.*

## Introduction

Spemann established that the organizer can be subdivided into a head and a trunk organizer, corresponding to the early and late dorsal upper blastopore lip (Spemann, 1931). Similarly, Mangold investigating regional specificity of induction by organizer derivatives at the end of gastrulation found that head and trunk inducing activity reside in the prechordal plate and chordamesoderm, respectively (Mangold, 1933). These results have been confirmed in *Xenopus* and chicken (Pera and Kessel, 1997; Zoltewicz and Gerhart, 1997). In addition, in mouse distinct head and trunk organizing centers can be distinguished (Thomas and Beddington, 1996; Beddington and Robertson, 1999). Classical experiments by Nieuwkoop (Nieuwkoop, 1952) predicted the presence of an anterior neural inducer ("activator") and a posteriorizing agent ("transformer") with different antero-posterior (a-p) distributions. The similarity of their inductions suggests that Nieuwkoop's activator and transformer are related or identical to Spemann's head and trunk inducers.

## Simultaneous inhibition of Wnts and BMPs required for head induction

Work on the secreted BMP antagonists Noggin, Chordin and Follistatin established, that a central molecular mechanism of induction by the amphibian trunk organizer resides in the inhibition of BMP signalling (Harland and Gerhart, 1997). An important step in understanding the molecular nature of the head organizer was the identification of Cerberus, a multifunctional secreted protein which can induce entire ectopic heads (Bouwmeester *et al.*, 1996). *Cerberus* is expressed in the anterior endoderm in *Xenopus* and in the mouse AVE, a head organizing center. Cerberus acts by inhibiting signalling of TGF-β (BMPs, nodal) and Wnt growth factors (Piccolo *et al.*, 1999). Similar to *cerberus*, the Wnt antagonist *frzb* is expressed in the *Xenopus* head organizer and is capable of inducing heads in conjunction with BMP inhibitors (Leyns *et al.*, 1997; Wang *et al.*, 1997; Glinka *et al.*, 1998). Wnt glycoproteins are implicated in diverse processes during embryonic patterning in all metazoa tested (Cadigan and Nusse, 1997; Moon *et al.*, 1997) and are candidates for Nieuwkoop's posteriorizing factor. They inhibit dorsal mesoderm formation and can directly posteriorize anterior neuroectoderm (Christian and Moon, 1993; McGrew *et al.*, 1995).

The activities and the expression of the head inducers *cerberus* and *frzb* along with the observation that simultaneous inhibition of BMP and Wnt signals is sufficient to induce ectopic heads suggested a molecular interpretation of the Nieuwkoop two-signal concept, as a two-inhibitor model (Fig. 1). It proposes that the distinguishing feature of the head vs. trunk organizer is the presence of Wnt inhibitors, in addition to BMP inhibitors which are required both for head as well as trunk induction (Glinka *et al.*, 1997; Glinka *et al.*, 1998; Niehrs, 1999). It should be noted, though, that in the direct developing frog *eleutherodactylus coqui* ectopic expression of the BMP antagonist *noggin* alone is sufficient to induce complete secondary heads, suggesting that Wnt inhibitors are induced secondarily (Fang *et al.*, 1999).

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| | Organizer | Induction |
|---|---|---|
| **Endomesoderm** | dkk1<br>cerberus<br>frzb<br>chordin<br>noggin<br>follistatin | → Head<br>→ |
| **Chordamesoderm** | chordin<br>noggin<br>follistatin | → Trunk<br>→ |

**Fig. 1. Two-inhibitor model for Organizer regionalization.** *Trunk induction requires inhibition of BMP-signalling while head induction requires dual inhibition of Wnt and BMP-signalling. The organizer produces factors that inhibit both types of signals (anti-wnt: Dkk1, Cerberus, Frzb; anti-BMP: Cerberus, Noggin, Chordin, Follistatin). Regional specificity of induction results from differential expression of Wnt- and BMP-inhibitors in endomesoderm and chordamesoderm. Note that Cerberus inhibits also Nodal and Activin signalling, which may be important to maintain anterior endomesodermal fate.*

## Role of *dickkopf1* in the head organizer

Based on these findings we devised an expression screen to isolate potential head inducing Wnt-inhibitors and have identified a novel protein *dickkopf1*(*dkk1*), member of a new family of secreted proteins. *Dkk1* is expressed in the *Xenopus* head organizer, the anterior endoderm and prospective prechordal plate (Fig. 2B) (Glinka *et al.*, 1998). *Dkk1* mRNA coexpression with BMP inhibitors leads to induction of complete head structures in *Xenopus* embryos (Fig. 3A). In contrast, injection of inhibitory anti Dkk1 antibodies leads to microcephaly (Fig. 3B). *Dkk1* is thus the first growth factor antagonist of the Spemann-Mangold organizer required for head formation. It encodes a potent Wnt antagonist, inhibiting the ability of injected *Xwnt8* mRNA to induce secondary embryonic axes as well as the ability of injected *Xwnt8* plasmid DNA to posteriorize the embryonic axis in *Xenopus*. *Dkk1* functions upstream of the first intracellular component of the Wnt pathway,
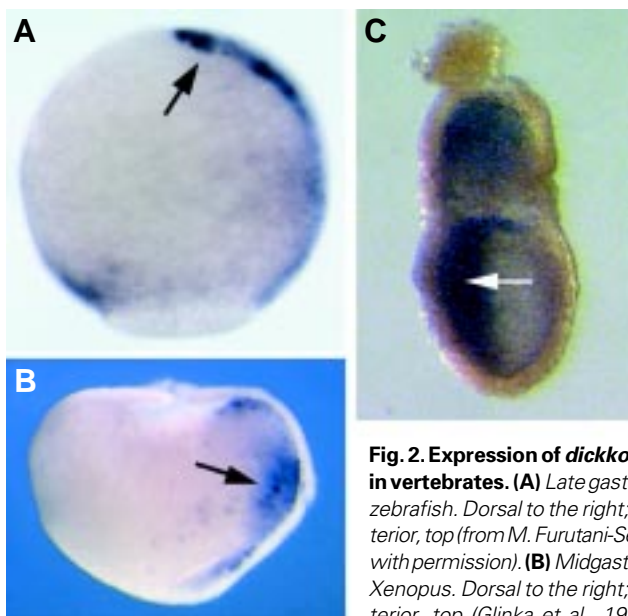
**Fig. 2. Expression of *dickkopf1* in vertebrates. (A)** *Late gastrula zebrafish. Dorsal to the right; anterior, top (from M. Furutani-Seiki, with permission).* **(B)** *Midgastrula Xenopus. Dorsal to the right; anterior, top (Glinka et al., 1998).* **(C)** *Early gastrula mouse. Anterior, left (Glinka et al., 1998). Arrows point to dkk1 expression in presumptive prechordal endomesoderm.*

*dishevelled*, suggesting that it interacts with Wnt ligands or their seven-transmembrane *frizzled* receptors (Bhanot *et al.*, 1996).

Like *Xenopus dkk1*, mouse (Glinka *et al.*, 1998) and zebrafish *dkk1* (Hashimoto *et al.*, 2000) are expressed in the prechordal plate (Fig. 2 A,C) as well as the equivalents of the amphibian anterior endoderm, the mouse AVE (Pearce *et al.*, 1999) and zebrafish hypoblast (Hashimoto *et al.*, 2000). *Dkk1* expression is regulated by early Nodal and pre-MBT Wnt signalling, which set up dorso-ventral patterning (Hashimoto *et al.*, 2000). Similar to *Xenopus dkk1*, mouse (Glinka *et al.*, 1998), zebrafish (Hashimoto *et al.*, 2000) and human *dkk1* (Fedi *et al.*, 1999; Krupnik *et al.*, 1999) inhibit Wnt/β-catenin signalling.

*Dkk1* is member of a multigene family of secreted glycoproteins with at least four different members in humans which contain two
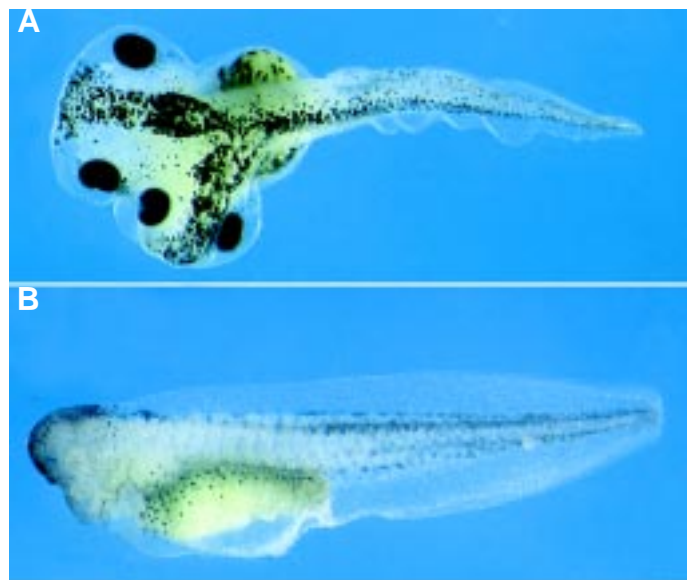
**Fig. 3. Head induction by *dickkopf1*. (A)** *A tadpole-stage embryo that has been injected with mRNA encoding a dominant-negative BMP receptor (tBR) and dkk1 forms a complete secondary head.* **(B)** *A tadpole embryo microinjected with inhibitory anti-Dkk1 antibodies. The experimental embryo lacks anterior head structures (Glinka et al., 1998 and unpublished).*

conserved cysteine-rich domains (Glinka *et al.*, 1998; Krupnik *et al.*, 1999). The C-terminal cysteine rich domain cys2 bears homology to proteins containing a colipase fold, a domain observed in secreted colipases and snake venoms (Aravind and Koonin, 1998). The significance of this homology is at present unclear. Furthermore, the glycoprotein Soggy of unknown function has homology to *dkk3* but lacks the cysteine rich domains and is unable to inhibit various Wnt signals (Krupnik *et al.*, 1999). The 8 kDa polypeptide Bv8 present in frog skin and snake venom has homology to the cys2 domain of *dkk*s (Mollay *et al.*, 1999). During vertebrate embryogenesis *dkk*s are differentially expressed in various neural and mesenchymal tissues (Grotewold *et al.*, 1999; Monaghan *et al.*, 1999; Hashimoto *et al.*, 2000) suggesting that the proteins are involved in inductive processes.

## Embryonic patterning effects of *dkk1*

*Dkk1*, unlike *frzb* and *cerberus*, induces heads with two eyes (Fig. 3A) and this is likely due to its ability to induce complete

prechordal plates (Kazanskaya *et al.*, 2000). The prechordal plate is required for splitting of the eye field and prechordal plate defects are known to induce cyclopia. Injection of inhibitory anti Dkk1 antibodies reveals that *dkk1* is not only sufficient but also required for prechordal plate- but not for notochord formation. Specifically, prechordal plate markers such as *XBlimp1*, *gsc*, and *Hex* are reduced following Ab injection (Kazanskaya *et al.*, 2000). These genes are super-induced and expanded following *dkk1* overexpression but they are not induced ectopically, e.g. in ventral mesoderm or posterior chordamesoderm. This indicates that other factors in addition to Wnt inhibitors are required for prechordal plate formation, most likely BMP inhibitors, since coexpression of *dkk1* with BMP inhibitors can induce prechordal plate markers ectopically in ventral mesoderm (Glinka *et al.*, 1998). *Dkk1* expression can rescue forebrain, eye and notochord formation in zebrafish mutant for *bozozok/dharma*, encoding a homeobox protein involved in early axis formation (Hashimoto *et al.*, 2000). This suggests that *dkk1* may mediate the Nieuwkoop center signal of *bozozok/dharma.*

In the neural plate *dkk1* is required for antero-posterior and dorso-ventral patterning between mes- and telencephalon, where it promotes anterior and ventral fates (Kazanskaya *et al.*, 2000). A role in d-v neural patterning is consistent with the observation that Wnts not only posteriorize but also dorsalize neuroectoderm, promoting neural crest formation (Saint-Jeannet *et al.*, 1997; Chang and Hemmati-Brivanlou, 1998; Dorsky *et al.*, 1998; LaBonne and Bronner-Fraser, 1998). *Dkk1* affects neuroectoderm directly: In zebrafish embryos where anterior endomesoderm formation is prevented by *antivin* mRNA injection *dkk1* can still anteriorize neural plates (Hashimoto *et al.*, 2000). Furthermore, *Xenopus* animal caps which are of purely ectodermal origin express anterior neural markers in response to *dkk1* mRNA injection (Glinka *et al.*, 1998).

*Xenopus* embryos posteriorized with bFGF, BMP4 and Smad1 are rescued by *dkk1*, indicating that these genes function by activating Wnt signalling. However, embryos treated with retinoic acid (RA) are not rescued and RA affects the central nervous system (CNS) more posterior than *dkk1*, suggesting that Wnts and retinoids may act to pattern anterior and posterior CNS, respectively, during gastrulation (Kazanskaya *et al.,* 2000).

## Conclusion

There is accumulating evidence for a physiological role of Wnt signalling in neural and mesendodermal regionalization, although the identity of the Wnts involved remains elusive. These Wnts are antagonized by secreted Wnt inhibitors emanating from head organizing centers, the prechordal plate and anterior endoderm. The Wnt inhibitor Dkk1 plays an important role in the head organizer, specifying anterior fates both in endomesoderm and neuroectoderm.

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