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Switch-like behavior enables Wnt11 concentration specific response during dorso-ventral axis formation in *Xenopus laevis*

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ABSTRACT

Wnt signaling plays a role in diverse processes such as cell proliferation, differentiation, migration or cell polarity. Dysfunction of Wnt signaling is associated with human diseases and aging. Wnts can activate several interacting intracellular signaling pathways, in particular the so called canonical and non-canonical pathways. The canonical Wnt response leads to a stabilization of cytoplasmic β–catenin whereas non-canonical Wnt signaling can result in the activation of calcium–calmodulin dependent kinase II (CamKII). Earlier data revealed that those signaling pathways can inhibit each other in a concentration dependent manner. During *Xenopus laevis* dorsal axis formation, Wnt11 has been shown to activate both, β–catenin signaling as well as CamKII activity. In line, Wnt11 is required for dorsal as well as ventral cell fates. Here, we show that the concentration dependent mutual inhibition of CamKII and β–catenin signaling is sufficient to obtain a switch-like behavior as opposed to a graded response. We present a model that faithfully recapitulates the activity of Wnt11 during dorso-ventral axis formation in *Xenopus laevis* on the basis of the Wnt switch behavior.

1. Introduction

Wnt proteins are secreted glyco–proteins that activate different intracellular signaling branches including Wnt/β–catenin and Wnt/calcium signaling (Sheldahl et al., 1999; Slusarski et al., 1997a; 1997b). During Wnt/β–catenin signaling, cytoplasmic β–catenin is stabilized and activates target gene transcription together with TCF/LEF transcription factors. In contrast, Wnt mediated intracellular calcium release triggers activation of calcium sensitive enzymes including calcium calmodulin dependent kinase II, CamKII (Kühl et al., 2000). Recent work indicated that a given particular Wnt ligand can activate different Wnt signaling branches in a concentration dependent manner (Grumolato et al., 2010; Koval and Katanaev, 2011; Kühl et al., 2000; Nalesso et al., 2011). Whereas high concentrations of Wnt favor activation of β–catenin signaling, lower concentrations of Wnt rather trigger calcium release and activation of calcium dependent enzymes (Nalesso et al., 2011). As both pathways can inhibit each other (see Fig. 1), a switch like behavior has been hypothesized (Kestler and Kühl, 2011). Dorso–ventral axis formation during embryogenesis might represent an *in vivo* example for such a Wnt switch.

Patterning along the dorso–ventral body axis is a key event during early embryogenesis. Major contributions to our understanding of how patterning along this axis occurs have been gained in amphibians including the South African clawed frog *Xenopus laevis* (Dale and Slack, 1987; Kimelman et al., 1992; Moon et al., 1997; Slack, 1993; Smith and Slack, 1983). On the dorsal side of the embryo, Wnt/β–catenin signaling is activated to ensure proper gene expression in the Spemann organizer such as goosecoid (Gsc) (Blumberg et al., 1991) or the BMP antagonist Noggin (Zimmerman et al., 1996). Wnt11 has been suggested to be the relevant Wnt ligand *in vivo* (Ku and Melton, 1993) and Wnt11 has been shown to be enriched on the dorsal side of the embryo (Schroeder et al., 1999). Wnt mediated activation of CamKII is in contrast required for proper ventral development (Kühl et al., 2000). Major determinant of ventral patterning is a member of the TGF–β super family, BMP4 (Fainsod et al., 1994; Köster et al., 1991; Schmidt et al., 1995). BMP4 forms a gradient with highest concentrations on the ventral side (Dale and Wardle, 1999). BMP4 itself is counteracted by the secreted protein Noggin (Smith and Harland, 1992; Zimmerman et al., 1996). Relevant target genes of BMP4 during dorso–ventral axis formation are Vent1 and Vent2 (Gawantka et al., 1995; Onichtchouk et al., 1996).

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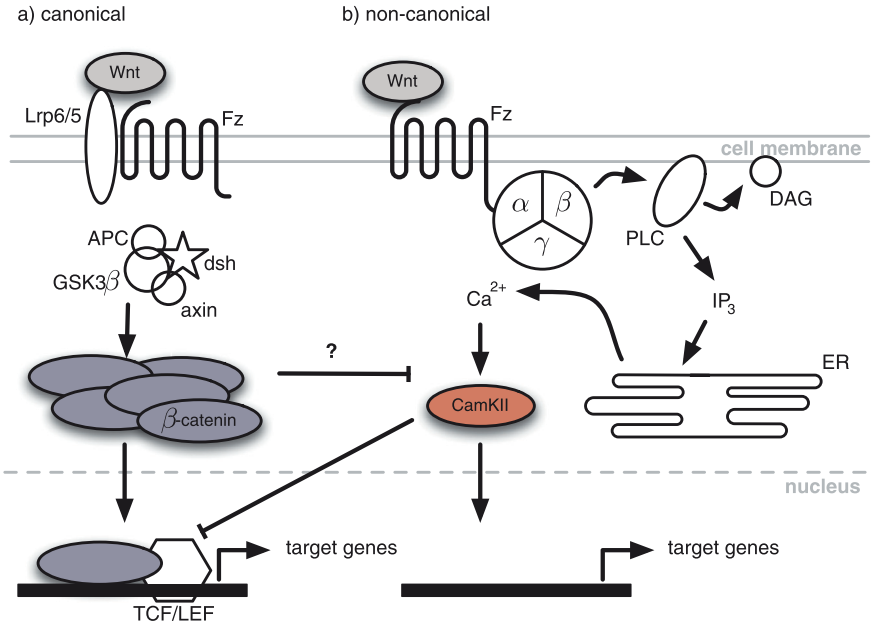

Fig. 1. Schematic description of a) the canonical Wnt signaling pathway mediated by β-catenin and b) the non–canonical calcium dependent Wnt pathway and their interactions. The inhibitory mechanisms of canonical Wnt signaling on the non-canonical pathway are not yet understood on a molecular level.

Targeted micro-injection of RNA into the early *Xenopus* embryo can be used to interfere with Wnt11 function or CamKII activity (Kühl et al., 2000). For this purpose, two CamKII constructs generated by point mutations were previously used (Kühl et al., 2000). Exchange of lysine to methionine on amino acid position 42 (K42M) results in a kinase dead variant of CamKII. CamKII functions as a multimer (e.g. dodecamers) (Kühl, 2004) and the kinase dead variant is incorporated into these multimers. This impairs target protein phosphorylation and results in an overall reduced CamKII activity. From now on we call this variant of CamKII "k.d. CamKII" throughout the manuscript. In contrast, exchange of threonine 286 to aspartate mimics phosphorylation and therefore activation of CamKII. Overexpression of this construct results in elevated CamKII activity. We call this constitutively active variant of CamKII from now on "c.a. CamKII".

These manipulations are normally done at the 4-cell stage of early *Xenopus* development when the dorsal and ventral sides are already fully separated. Manipulation of either the two dorsal or the two ventral blastomeres thus affect the two targeted cells but also all descendants. Injected RNAs coding for the constructs described will be translated into the functional proteins and will be active until stage 7 of development when enzymatic activity assays were performed. At this stage the embryo consists of roughly 10^3 cells. The asymmetry in Wnt signaling during early embryonic development is subsequently transferred into spatially differential gene expression several hours later at stage 10 (Fig. 2, WT for wild type condition). Changes in marker gene expression at stage 10 can therefore be considered as a readout for pathway activities.

As a result of manipulating CamKII activity for example, marker gene expression along the dorso-ventral axis changes in a characteristic manner (Kühl et al., 2000) (Fig. 2).

Together with the *in vitro* measurements of CamKII activity, we here mathematically model the interplay of Wnt and BMP4 signaling during early dorso–ventral axis formation and are now able to investigate this switch like behavior.

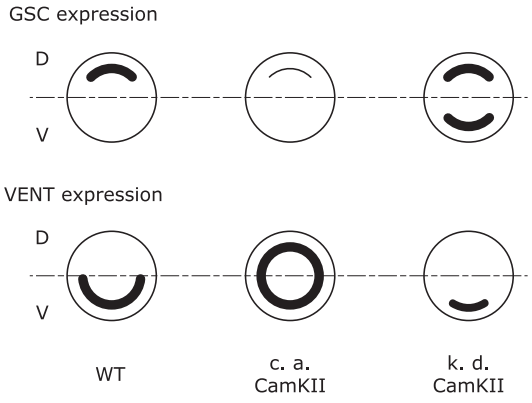

Fig. 2. Schematic representation of spatial expression of Vent1/2 and Gsc in *Xenopus laevis* stage 10. Vegetal views of embryos are shown. The dorsal (D) and ventral (V) sides of embryos are given. WT=wild type. Dorsal injection of RNA coding for a constitutively active version of CamKII (c.a. CamKII) or ventral injection of a kinase dead variant of CamKII (k.d. CamKII) resulted in changes of gene expression as shown earlier (Kühl et al., 2000). Note that the reduced expression of Gsc or Vent upon dorsal injection of RNA coding for c.a. CamKII or ventral injection of RNA coding for k.d. CamKII, respectively, can even be absent in affected embryos.

2. Model

In the following section we formulate a mathematical model of the "Wnt switch" building on an earlier hypothesis (Kestler and Kühl, 2011). This includes a mutual inhibition between the CamKII and β-catenin dependent branches of the Wnt signalling network. This model is then used to describe dorso-ventral patterning during embryogenesis in the South African clawed frog, *Xenopus laevis. Patterning along the dorso-ventral axis includes additional molecules and pathways such as* BMP4 and Noggin (Fig. 3). This extension of the model ist required to better reflect *in vivo* situation and to match in silico generated predictions with *in vivo* experiments.

In a first step we describe as outlined before the Wnt11 gradient, the respective Wnt11 dependent responses of CamKII and β–