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xSyndecan-4 Regulates Gastrulation and Neural Tube Closure in *Xenopus* Embryos

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Gastrulation is the morphogenetic process in embryos of multicellular organisms by which the presumptive mesoderm and endoderm move inside the ectoderm to form a three-layered embryo[1]. Once gastrulation is completed, a process known as neurulation is responsible for correct formation of the neural tube, the embryonic precursor of the brain and spinal cord. The proper neural plate shape is acquired during gastrulation, in part, through a process named convergent extension (CE). CE involves active cell movements and rearrangements that are driven by intercalation of polarized cells and regulated, among others, by noncanonical Wnt signaling[2,3,4]. Failures during these movements result in severe congenital malformations, collectively known as neural tube defects (NTD). Spinal NTD include myelomeningocele (bifid spine), the severity of which varies, but can cause prenatal lethality and severe neurological deficit in surviving individuals. In humans, NTD are relatively common with a frequency of 0.2–3.5 per 1,000 pregnancies[5].

Genetic studies in *Drosophila*, mice, and zebrafish have begun to reveal specific functions for heparan sulfate proteoglycans (HSPGs) throughout embryonic development[6]. Although there is no clear evidence about a possible role of HSPGs during neural tube closure, reports on zebrafish and *Xenopus* embryos have suggested that HSPGs are involved in gastrulation movements[7,8]. In our laboratory, we have started to study the function of proteoglycans (PGs) in early patterning of the vertebrate embryo. We have demonstrated that biglycan, a chondroitin sulfate PG, regulates dorsal-ventral patterning through modulation of BMP4 signaling activity[9]. Our recent paper published in *Nature Cell Biology* (Muñoz, R., Moreno, M., Oliva, C., Orbenes, C., and Larraín, J. **8(5),** 492–500. 2006) shows that a cell-surface HSPG, Syndecan-4 (Syn4), is critical during *X. laevis* gastrulation and neural tube closure. At the gastrula stage, xSyn4 is expressed in the involuting dorsal mesoderm and the anterior neuroectoderm, both tissues that undergo CE. Both gain- and loss-of-function of xSyn4 impaired CE in *Xenopus* embryos, resulting in failure of neural tube closure (Fig. 1). Several studies have demonstrated that a functional noncanonical Wnt signaling is required for proper CE. This pathway regulates the polarized protrusive activity of dorsal mesodermal cells and either its activation or inactivation results in disruption of proper cell polarization and CE[10,11,12,13,14,15]. In accordance with a possible role of xSyn4 in noncanonical Wnt signaling, this paper demonstrates that xSyn4 is able to interact biochemically and functionally with the Wnt receptor

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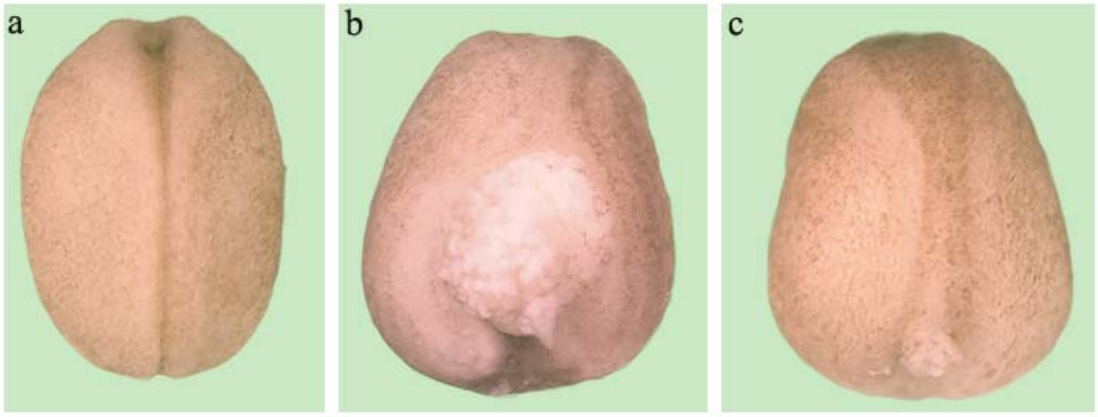

FIGURE 1. xSyn4 is essential for CE. Microinjection of morpholinos for xSyn4 (xSyn4MO) caused severe defects in gastrulation and neural tube closure in contrast with control embryos (a). The phenotypes induced by xSyn4MO were classified into those with severe gastrulation defects (b) and those with only neural tube closure defects (c).

Frizzled7 (xFz7) and its downstream effector Dishevelled (xDsh). Furthermore, xSyn4 is necessary and sufficient for translocation of xDsh to the plasma membrane, a hallmark of Wnt signaling activation. However, it has been suggested that the ability of xSyn4 to translocate xDsh is regulated by fibronectin. Syn4 binds to the heparin-binding domain of fibronectin through its glycosaminoglycan chains (GAG)[16] and is required, together with integrins, for proper stabilization of focal adhesion sites[17,18,19]. Recent reports have demonstrated that chronic depletion of fibronectin and acute disruptions of α5β1 integrin-fibronectin binding increases the frequency and randomizes the orientation of polarized cellular protrusions. Consequently, it has been suggested that integrin-fibronectin interactions normally repress frequent random protrusions in favor of fewer mediolaterally oriented ones. In the absence of α5β1 integrin binding to fibronectin, CE still occurs, but results in convergent thickening instead of extension[20].

In conclusion, we have demonstrated by gain- and loss-of-function assays that xSyn4 regulates and is essential for proper CE during early development of *Xenopus* embryos. We also showed that xSyn4 is a component of the noncanonical Wnt pathway, being necessary for translocation of xDsh to the plasma membrane, a process that requires its cytoplasmic domain. However, this function may be done by at least two mechanisms: xSyn4 interacts directly with xDsh or through complex with xFz7 (Fig. 2). Importantly, the ability of xSyn4 to interact with xDsh is regulated by fibronectin. Together, these results suggest a model where specific activation of the noncanonical Wnt pathway results from the activation of xSyn4 by fibronectin and xFz7 by Wnt ligands.

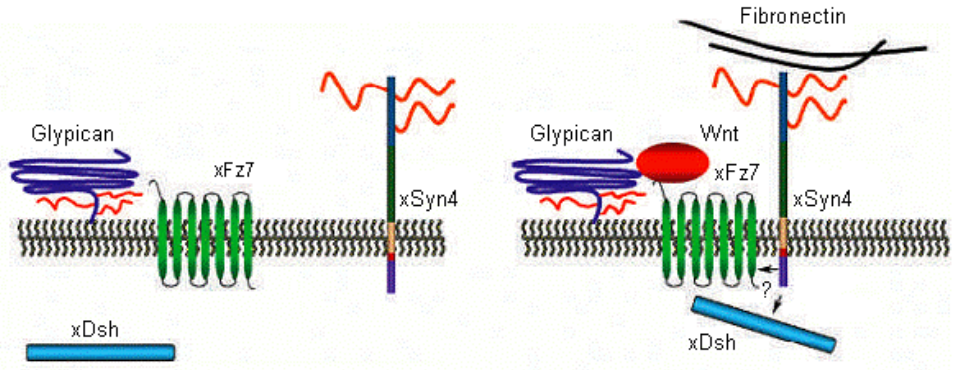

FIGURE 2. Schematic representation of the proposed role of xSyn4 in noncanonical Wnt signaling.

Two models have been proposed to explain how HSPGs modulate Wingless/Wnt signaling. In the first model, HSPGs are necessary for maintaining the local concentration of Wingless/Wnt ligand that is available for its receptor[21,22]. In the second model, HSPGs are proposed to act as coreceptors that directly facilitate the formation of Wingless/Wnt -Fz signaling complexes[23,24]. Recently, a coreceptor function for glypican4 (xgly4), a cell-surface HSPG, in CE movement and noncanonical Wnt signaling, has also been described in *Xenopus* embryos[7]. Xgly4 interacts physically with Wnt11, and this interaction results critical for activation of Wnt/PCP pathway[8]. Although it is not yet clear how GAGs and core protein of HSPG affects signaling, our experiments suggest that GAGs are important for interaction with fibronectin and the protein core for complexing with xFz7 and translocation of xDsh to plasma membrane (data not shown). It is also interesting to mention that a role for HSPGs in the modulation of canonical Wnt signaling during axis determination of early *Xenopus* embryos has been reported[25]. These, together with our findings, suggest a role for HSPG in the specific activations of the different branches of Wnt signaling.

Recently, the requirement for CE during the initiation of neural tube closure has become clear. Mouse mutants named *loop-tail*, *crash* and *circletail*, and *dishevelled-1*, *dishevelled-2* double knockdown, showed improper neural tube closure, which leads to craniorachischisis (type of NTDs). Each of the mutant genes in which craniorachischisis is observed encodes a protein that functions in a branch of noncanonical Wnt signaling: the so-called planar cell polarity pathway. The cause of craniorachischisis in these mutants has been inferred to be a failure of CE, because, in *Xenopus*, overexpression of wild-type or mutant forms of xDsh and Strabismus (which blocks convergent extension) usually results in similar neural tube closure defects[5,25]. It is important to note that overexpression and morpholino knockdown of xSyn4 produces open NTD in *Xenopus* embryos. We propose that Syn4 works as a coreceptor for specific activation of the noncanonical Wnt pathway.

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