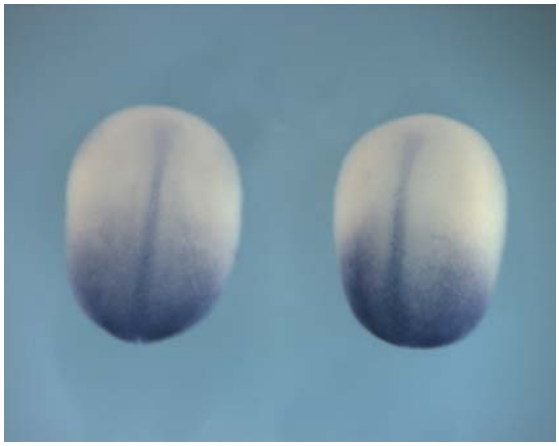
RIKEN Center for Developmental Biology (CDB)
2-2-3 Minatojima minamimachi, Chuo-ku, Kobe 650-0047, Japan

NRH1 and Wnt Signaling Come Together in Convergent Extension

July 20, 2004 – The African clawed frog, *Xenopus laevis*, begins development as a compact ball of cells that undergoes a dramatic transformation through cell migrations and positional rearrangements that result in the separation of the embryo into three distinct germ layers, which go on to give rise to all of the tissues and structures in the adult animal's body. During this transformation, known as gastrulation, the embryo changes from a roughly spherical shape to an elongated, streamlined form through a process called convergent extension (CE), in which polarized cells migrate to and merge at the embryo's midline, driving it to lengthen along its anterior-posterior axis.

A number of genes involved in the regulation of convergent extension have been identified in amphibians and other vertebrates, such as zebrafish, but the picture of the underlying molecular mechanisms remains incomplete. In a report published in *Nature Cell Biology*, Noriaki Sasai and colleagues in the RIKEN CDB Laboratory for Organogenesis and Neurogenesis (Kobe, Japan) show that the product of the gene *NRH1* is essential to the regulation of CE movements in the frog.



Expression of NRH1 at the neurula stage (dorsal view; anterior at top)

While performing a screen of genes expressed in the posterior neuroectoderm, Sasai et al. identified a gene encoding a protein that showed similarities to $p75^{NTR}$, a neurotrophin receptor. (Neurotrophins are molecules that function in the survival, growth and migration of neurons.) However, on testing its affinity for neurotrophin ligands, the group found that, unlike $p75^{NTR}$, NRH1 did not bind with neurotrophins, which led them to seek other biological roles for the protein.

Contact: Douglas Sipp sipp@cdb.riken.jp TEL: +81-78-306-3043
RIKEN CDB, Office for Science Communications and International Affairs

Experiments in which NRH1 was overexpressed by injecting its messenger RNA directly into very early (4-cell stage) embryos resulted in shortening of the body axis and the failure of mesodermal and neural plate marker gene expression to converge on the midline or extend axially. Interestingly, interfering with *NRH1* function by introducing morpholinos to block production of the NRH1 protein had similar effects – the inhibition of convergent extension. That both gain and loss of *NRH1* function resulted in the failure of CE activity suggested that the gene's function in this process is tightly regulated.

Embryo injected with NRH1 morpholino (top) shows shortened body axis due to failure of convergent extension. Wild type embryo is shown below.

These first findings led Sasai to investigate possible interactions between NRH1 and genes involved in the Wnt/PCP (planar cell polarity) signaling pathway, which is also known to play an important role in the regulation of CE movements in both fish and frog through the activity of downstream small GTPases. This protein family, which includes Rho, Rac and Cdc42, interacts with the cytoskeleton and plays important roles in the dynamics of cell morphology and motility. Overexpression and loss-of-function of NRH1 in the marginal zone (where convergent extension originates) respectively resulted in increased and decreased Rho, Rac and Cdc42 activity, confirming the link between NRH1 and Rho-family small GTPases. Loss of NRH1 function could be rescued by the co-injection of Frz7, a Wnt receptor functioning upstream of Rho, Rac and Cdc42 in the PCP pathway and, similarly, NRH1 complemented a dominant-negative Frz7 phenotype, indicating the two proteins play compensatory and mutually independent roles in the activation of small GTPases.

Further experiments showed that NRH1's effects on CE movements are also mediated by a second branch of the Wnt/PCP pathway, in which MKK7 and JNK work to phosphorylate c-Jun in the animal cap (a region of prospective ectoderm located on the roof of the blastocoel, a hollow in the spherical early embryo). As with the

small GTPases, the activation of the MKK7-JNK cascade could also be effected by either Frz7 or NRH1, but it was found that NRH1 functioned independently of *Xdsh*, another upstream regulator of Rho-family small GTPase activity in the Wnt/PCP pathway. The transduction mechanisms by which NRH1 interacts with Wnt/PCP signaling factors remain to be worked out, as do the specifics of the inter-related but apparently independent roles of NRH1 and PCP signaling in the control of cell movements within the developing embryo.

Contact: Douglas Sipp sipp@cdb.riken.jp TEL: +81-78-306-3043
RIKEN CDB, Office for Science Communications and International Affairs