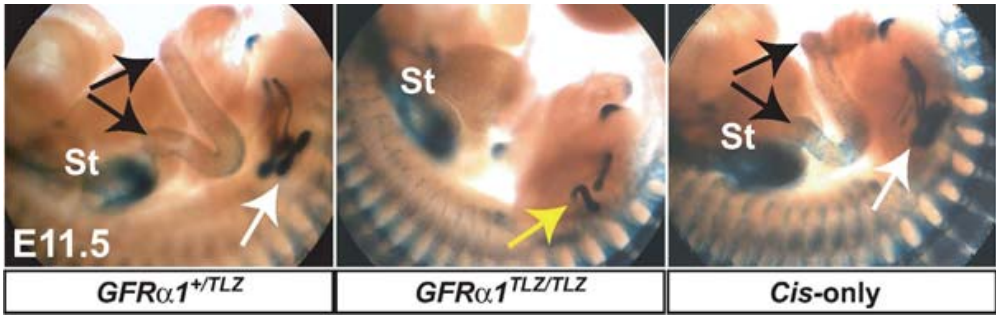
RIKEN Center for Developmental Biology (CDB)
2-2-3 Minatojima minamimachi, Chuo-ku, Kobe 650-0047, Japan

No getting around RET: Researchers find no role for RET-independent GFRα in development or regeneration

November 18, 2004 – Neurons depend on external molecular signals for their very survival. These molecules, collectively referred to as neurotrophic factors, include a family of four GDNF Family Ligands (GFLs) that bind to specific receptor sites on the surfaces of neural cells. These sites allow GFLs to signal through a receptor complex composed of the RET tyrosine kinase and a GFRα-family receptor. Tyrosine kinases, such as RET, are well-known for their function in phosphorylation cascades that span the cell membrane. The role of the GFRα co-receptors in these complexes was long thought to be limited to as a co-receptor for RET, but GFRs have recently been suggested to play other roles as well.

The individual functions of the RET and GFRα subunits in these receptor complexes, which are important in developmental milieux from peripheral neurogenesis to the developing kidney, remains a thorny question complicated by the fact that GFRα is expressed in many cells lacking RET in vivo (RET-independent GFRα) and that, in vitro, cells expressing GFRα1 without RET have been shown to respond to GDNF signals. A report by Hideki Enomoto (Team Leader, Laboratory for Neuronal Differentiation and Regeneration) and colleagues at the RIKEN Center for Developmental Biology and the Washington University School of Medicine published in the November 18 issue of *Neuron* now challenges the view that RET-independent GFRα1 plays a significant physiological role in either development or regeneration.



GFRα1-expressing cells (blue) in heterozygote (left), homozygote (middle) and Cis-only (right) mutants. Enteric neurons and kidneys fail to develop in the null-mutant, but are normal in heterozygote and Cis-only mice.

Enomoto first devised an elegant experimental system to make it possible to generate mice specifically lacking RET-independent GFRα1. The study of GFRα deficiencies in vivo is dogged by the lethality of the phenotype, in which the absence of enteric neurons and functioning kidneys results in death soon after birth. In vitro studies and the proximity of RET-independent GFRα and RET-expressing cells in some developmental regions, however, have prompted strong speculation that GFRα might be able to operate even in the absence of RET indigenous to the cell. It has been suggested that this might take the form of either *trans* signaling, in which the GFRα receptor captures diffusible GFLs and presents them to a neighboring RET-expressing cell, or through a separate signaling mechanism mediated by GFL-activated neural cell adhesion molecules (NCAMs).

Given this body of work showing the likelihood of a physiological role for RET-independent GFRα1 activity, Enomoto et al. decided to test whether the in vitro evidence would be borne out in living mice. The team showed that mice homozygous

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

for a transgene deleting an important segment of the GFRα1 gene died, while heterozygotes (which carried only a single copy of the transgene) were healthy and fertile. On comparing specific embryonic regions in hetero- and homozygous mice, they found associations between RET-expressing and RET-independent GFRα1 cells in kidney, enteric and motor neurons, as well as the expected disturbances in development. However, when they next generated mice that were only capable of expressing GFRα1 only in the RET-expressing cells (by cloning GFRα1 cDNA into a region under the control of the *Ret* promoter and crossbreeding the resulting animals with GFRα1 heterozygotes), they were surprised to discover the mice were born healthy and free of any evident developmental defects in the kidney or nervous system. They found no trace of GFRα1 mRNA in non-*Ret*-expressing cells in these mice (which they named Cis-only mice, for their lack of *trans* signaling), while GFRα1 transcripts were detected as expected in RET-positive cells, proving that the conditional expression scheme had worked.

Analysis of individual regions known to be susceptible developmental failure on loss of GFRα1 function, such as the kidneys, motor and enteric neurons and certain parts of the central nervous system during development and following injury, showed that Cis-only mice develop and regenerate structures that are both morphologically normal and fully functional.

Investigating the second question of a possible alternate RET-independent GDNF receptor complex thought to involve neural cell adhesion molecules, they next examined Cis-only mouse olfactory bulbs. These bulbs are reduced in size in NCAM-deficient mice as the result of impaired migration of neural precursors through a zone called the rostral migratory stream and swell with cells that have failed to reach their normal destination; this phenotype is seen only weakly in mice lacking GFRα1 (which is thought by some to regulate NCAM-mediated cell adhesion), but not in mice lacking RET. Again, the Cis-only mice showed no discernible differences from wild type.

This comprehensive series of experiments makes a convincing case against any essential physiological role for RET-independent GFRα1, but leaves the question of why GFRα1 would be more widely expressed if it indeed plays no role without RET. It may be the case that GFRα receptors associate with other partners that have yet to be identified. Whatever the answer, by laying to rest a theory that had been strongly supported by in vitro evidence, the Enomoto report serves to underscore the importance of differences between the behavior of cells in the body and cells in a dish.

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