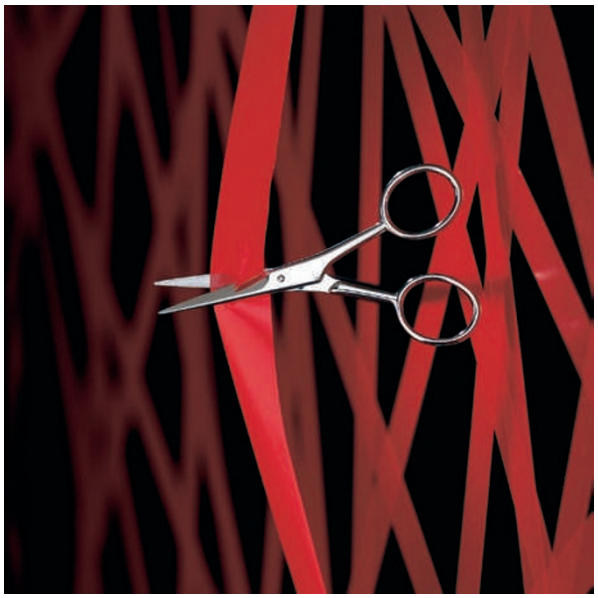
DEVELOPMENT

RYK's talents

Wnt genes are essential for the proliferation and differentiation of neuronal precursor cells (NPCs). RYK, a receptor tyrosine kinase, has been identified as a Wnt receptor. However, as RYK lacks intracellular kinase activity, it was unknown how signalling is transduced through this receptor. Reporting in *Developmental Cell*, Lu and co-workers have revealed that Wnt signalling through RYK uses an unconventional signalling pathway: on Wnt binding, a carboxy-terminal cleavage product of RYK translocates to the nucleus, where it initiates neuronal differentiation.

To test RYK's function in the developing cortex, the authors compared brain sections from $RYK^{+/+}$ and $RYK^{-/-}$ mice at embryonic day 14.5. They found that the number of proliferative NPCs and apoptotic cells did not differ between the two groups, but that the number of newly generated neurons (TUJ1-positive cells) was reduced in $RYK^{-/-}$ mice, suggesting that the differentiation of NPCs was impaired.



Immunostaining with an antibody that recognizes the C terminus of RYK revealed that the receptor localizes to the membrane in NPCs, whereas in TUJ1-positive cells it was found both at the membrane and in the nucleus, suggesting a link between nuclear localization of RYK and neuronal differentiation. Moreover, western blots from cortical lysates obtained at different developmental stages revealed that the abundance of a C-terminal fragment of RYK increases with development, whereas that of full-length RYK decreases. When RYK was expressed in HEK293T cells, western blots of subcellular fractions showed that full-length RYK was found in the membrane fraction, whereas a smaller C-terminal fragment was found exclusively in the cytosolic fraction. Mutational studies mapped the cleavage site that is targeted to produce this fragment to the transmembrane region of RYK. The protease inhibitor DAPT prevented RYK cleavage, identifying γ-secretase as the protease responsible for the cleavage.

Does Wnt stimulate the production and/or the nuclear translocation of the C-terminal fragment of RYK? The authors expressed RYK–green fluorescent protein in Cos7 cells together with WNT3 and observed that Wnt is required not for the cleavage of RYK, but for its nuclear translocation.

Interestingly, expression of *RYK* mRNA remains constant during development, but expression of *WNT3* mRNA correlates with the cleavage of RYK in the developing cortex and in cultured, differentiating NPCs. The authors tested whether WNT3 is a key factor in RYK signalling during neuronal differentiation of NPCs: they infected NPCs with a lentivirus expressing WNT3 and then cultured the cells under differentiation conditions. WNT3 increased neuronal differentiation twofold in these cultures, whereas cells from mice expressing a RYK mutant that lacks the C-terminal domain showed reduced differentiation.

These results demonstrate that Wnt signals through an unusual signalling mechanism, in which the C-terminal Ryk cleavage product is translocated to the nucleus. The identity of the target genes will undoubtedly be revealed in future studies.

Claudia Wiedemann

ORIGINAL RESEARCH PAPER Lyu, J., Yamamoto, V. & Lu, W. Cleavage of the Wnt Receptor Ryk regulates neuronal differentiation during cortical neurogenesis. *Developmental Cell* **15**, 773–780 (2008)