Cell
Leading Edge
Previews

Tiki Casts a Spell on Wnt

Jean-Paul Vincent[1,*] and Tony Magee[2]
[1]MRC's National Institute for Medical Research, London NW7 1AA, UK
[2]National Heart and Lung Institute, Imperial College London, London SW7 2AZ, UK
*Correspondence: jvincen@nimr.mrc.ac.uk
DOI 10.1016/j.cell.2012.06.007

Negative feedback is a widespread feature of signaling pathways. In an unexpected twist described in this issue, He and colleagues identify a membrane-tethered metalloprotease named Tiki that inhibits Wnt signaling by removing an essential eight-residue fragment from Wnt itself.

Developmental biology has contributed substantial knowledge about the signaling pathways that allow cells to communicate. A prime example is the pioneering screen of Smith and Harland (1991) aimed at identifying specific mRNAs that affect axial patterning in frog (*Xenopus)* embryos. The first clone they reported encoded X-Wnt8, a member of the Wnt family of secreted lipoproteins. Subsequently, additional Wnt-signaling components, as well as other types of developmental regulators, were similarly identified. Remarkably, 20 years on, the same basic approach has yielded a new and unusual modulator of Wnt signaling (Zhang et al., 2012).

Zhang et al. converted an arrayed set of *Xenopus tropicalis* cDNAs to mRNAs and injected pools of mRNAs into embryos to identify those that cause anterior defects upon overexpression. One such clone led to head enlargement, and the corresponding gene was named *Tiki1*, after the first man in Polynesian mythology who is often represented as a large head. A close relative of this gene, *Tiki2*, was identified bioinformatically. The enlargement of anterior-neural structures is characteristic of reduced Wnt signaling (Houart et al., 2002; Kiecker and Niehrs, 2003), suggesting that Tiki1 could act as a Wnt inhibitor. Indeed, Tiki1 mRNA suppresses the ability of ectopic Wnt to cause embryonic axis duplication. It does not, however, prevent axis duplication by overexpressed β-catenin, a key effector of Wnt signaling, or activated LRP6, an essential receptor (for a review on Wnt signaling, see van Amerongen and Nusse, 2009). These findings suggest that Tiki functions upstream of the receptors, either in the extracellular space or within Wnt-expressing cells, to dampen Wnt signaling. This activity is essential for normal head development as Tiki knockdown leads to a strong reduction in the size of head structures. The pattern of Tiki expression is consistent with regulation by Wnt signaling, and ectopic Wnt signaling activates Tiki expression. Accordingly, Tiki appears to be yet another Wnt feedback inhibitor. Interestingly, Tiki does not interfere with maternal Wnt signaling. Therefore, the regulatory interactions mediated by Tiki allow maternal Wnts to dampen subsequent pathway activation by zygotic Wnts.

Numerous feedback inhibitors of Wnt signaling, acting outside and inside responding cells, have been identified (van Amerongen and Nusse, 2009). Extracellular inhibitors include Wif-1, which binds Wnts, preventing access to their receptors; sFRP, which binds and inactivates Frizzled; and Dkk, which with Kremen triggers endocytosis and downregulation of LRP6. To elucidate the mode of action of Tiki, the authors turned to cultured mammalian cells. Human embryonic kidney 293 (HEK293) cells naturally express Tiki2, and small interfering RNA against Tiki2 boosts the effect of exogenously added Wnt3a, an indication that the inhibitory role of Tiki is conserved, at least between frogs and humans. The key mechanistic insight came from analyzing Wnt3a produced by mouse L cells (fibroblasts). These cells, which normally do not express Tiki, secrete active Wnt3a upon transfection. When Tiki is cotransfected, Wnt3a is still secreted but is no longer active and, crucially, runs slightly faster than active Wnt3a in a polyacrylamide gel. This is a first clue that Wnt3a could undergo Tiki-dependent proteolytic cleavage.

Tiki-modified Wnt3a is water soluble, whereas active Wnt3a appears hydrophobic in a phase separation assay. Acylation of Wnt was first discovered by Willert et al. (2003), who specifically suggested that C77 of Wnt3a is palmitoylated. This was followed by another report demonstrating the presence of palmitoleic acid at S209 (Takada et al., 2006). It has therefore been thought for some time that Wnt3a, as well as many other Wnts, carries two acyl groups. However, although palmitoleic acid modification of S209 has been demonstrated convincingly by mass spectrometry, the evidence for palmitoylation at C77 remains largely indirect and has in fact been called into question (Komekado et al., 2007; Tang et al., 2012). Despite these uncertainties, it is clear that Wnts are hydrophobic and carry at least one acyl group. Given that Tiki-modified Wnt3a is soluble, it is conceivable that Tiki inactivates Wnt by preventing acylation or by removing the acylated residue(s). Edman sequencing and quantitative mass spectrometry show that Wnt3a is indeed cleaved in the presence of Tiki; however, only eight N-terminal residues of the mature protein are removed, leaving both C77 and S209 in place. Moreover, the authors show by metabolic labeling that Tiki has no measurable impact on the acylation state of Wnt3a. How Tiki affects Wnt3a solubility remains unknown.

The authors show that, following cleavage, Wnt3a becomes oxidized and forms large covalent disulfide-bonded oligomers. Wnts contain a remarkably high number of cysteines (24 in mature Wnt3a), many of which are likely to be disulfide bonded. Therefore, Tiki cleavage may somehow upset disulfide pairing, hence causing the oligomerization, possibly in denaturated form. There is no evidence as yet that Wnt oligomers form in vivo and/or are physiologically relevant.

Nevertheless it is clear that, directly or indirectly, Tiki acts through an unusual mechanism, removing a stretch of eight residues that are essential for Wnt3a's signaling activity.

An unusual behavior of Tiki is that it causes an increase in the size of Wnt3a C77A despite catalyzing the removal of eight residues. Somehow, this mutant form of Wnt3a becomes hyperglycosylated in response to Tiki-mediated cleavage. One possible target of this extra glycosylation is the NCT sequon located nearby at positions 87–89. It is conceivable that this sequon's highly conserved asparagine 87 is not normally glycosylated because the adjacent highly conserved cysteine 88 pairs with C77. In the C77A mutant, normal pairing would not occur, freeing up C88. Somehow, cleavage by Tiki could provide the trigger for C88 to become glycosylated. This scenario therefore suggests an alternative view of the role of C77. Loss of C77 would not prevent acylation but rather cause disruption of disulfide pairing and loss of activity. Overall, there is a need to further investigate the complex interplay between Tiki-mediated cleavage, acylation, glycosylation, and disulfide bond formation in Wnts.

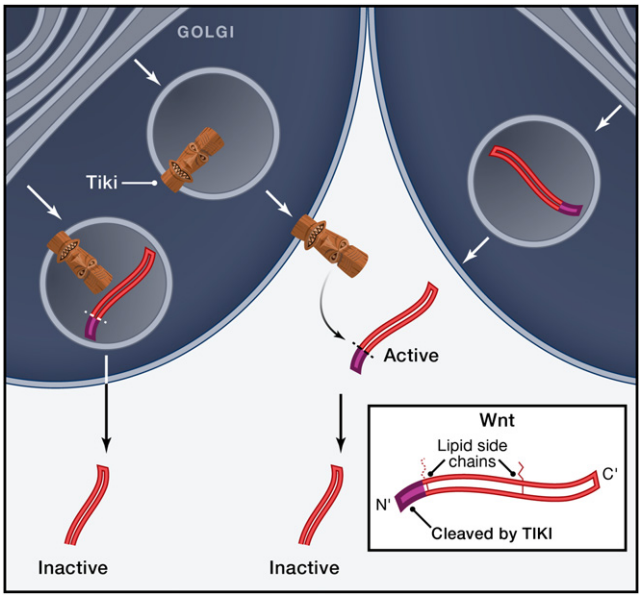

Figure 1. Tiki Is a Transmembrane Protein with an Extracytoplasmic Protease Domain

A fraction of Wnt coexpressed with Tiki (represented by a stylized Polynesian head) is secreted in a cleaved (inactive) form. In this configuration, proteolytic cleavage of Wnt presumably occurs both in the secretory pathway (shown in the lower left) as well as at the cell surface (not shown). The ratio of cleaved to uncleaved Wnt likely depends on the relative levels of expression of Tiki and Wnts and also, possibly, on posttranslational regulation of Tiki. Mosaic analysis shows that Tiki presented at the cell surface can also cleave Wnt produced from a neighboring cell (right).

Tiki is a type I transmembrane glycoprotein with most of the protein residing on the extracytoplasmic side, either on the extracellular surface or within the secretory pathway. The authors present evidence that Tiki can act at both locations (Figure 1). For example, a mutated form that is retained in the Golgi inhibits the activity of cotransfected Wnt. Conversely, mosaic analysis shows that Tiki can act non-cell-autonomously, consistent with an extracellular role. Indeed, the purified extracellular domain of Tiki can cleave purified Wnt3a in vitro, an indication that it has proteolytic activity despite a lack of homology to known proteases. All the evidence so far suggests that Tiki is a new metalloprotease. This is consistent with the presence of two highly conserved histidines and three cysteines, which could act as metal-coordinating ligands. Tiki appears to have broad specificity for various Wnts. One notable exception is Wnt11, and this may explain why Tiki has no effect on maternal Wnt signaling, which relies on Wnt11 (Tao et al., 2005).

Sequence comparison between all the cleavable Wnts does not reveal a consensus cleavage site, and the N-terminal sequence of Wnt11 is not dramatically different from that of other Wnts, although unlike cleavable Wnts, it contains a positively charged lysine. This could be relevant because a mutation that introduces four negatively charged residues abrogates cleavage. However, more refined experiments are needed to identify features of the cleavage site that impart specificity. Intriguingly, the residues of Wnt3a excised by Tiki are relatively conserved in *Drosophila* Wingless even though no Tiki ortholog can be recognized in this species. This is particularly surprising in light of the fact that Tiki is found all the way down to sponges.

Overall, the work of Zhang et al. is likely to trigger interest among a broad spectrum of biomedical researchers. Biochemists will want to figure out the mode of action of this new metalloprotease, the basis for specificity, and the nature of the cleavage site. Other outstanding issues concern the role of subcellular localization and the developmental significance of specificity, as it is likely to impact on how early embryos manage Wnt signaling during the transition between maternal and zygotic gene expression.

REFERENCES

Houart, C., Caneparo, L., Heisenberg, C., Barth, K., Take-Uchi, M., and Wilson, S. (2002). Neuron *35*, 255–265.

Komekado, H., Yamamoto, H., Chiba, T., and Kikuchi, A. (2007). Genes Cells *12*, 521–534.

Kiecker, C., and Niehrs, C. (2003). The role of Wnt signaling in vertebrate head induction and the organizer-gradient model dualism. In Wnt Signalling in Development, M. Khul, ed. (Austin, TX: Biosciences).

Smith, W.C., and Harland, R.M. (1991). Cell *67*, 753–765.

Takada, R., Satomi, Y., Kurata, T., Ueno, N., Norioka, S., Kondoh, H., Takao, T., and Takada, S. (2006). Dev. Cell *11*, 791–801.

Tang, X., Wu, Y., Belenkaya, T.Y., Huang, Q., Ray, L., Qu, J., and Lin, X. (2012). Dev. Biol. *364*, 32–41.

Tao, Q., Yokota, C., Puck, H., Kofron, M., Birsoy, B., Yan, D., Asashima, M., Wylie, C.C., Lin, X., and Heasman, J. (2005). Cell *120*, 857–871.

van Amerongen, R., and Nusse, R. (2009). Development *136*, 3205–3214.

Willert, K., Brown, J.D., Danenberg, E., Duncan, A.W., Weissman, I.L., Reya, T., Yates, J.R., 3rd, and Nusse, R. (2003). Nature *423*, 448–452.

Zhang, X., Abreu, J.G., Yokota, C., MacDonald, B.T., Singh, S., Coburn, K.L.A., Cheong, S.-M., Zhang, M.M., Ye, Q.-Z., Hang, H.C., et al. (2012). Cell *149*, this issue, 1580–1592.

Note Added in Proof

Since submission of this article, an online contribution showed that C55 in Xwnt8 (=Wnt3a C77) is not palmitoylated but instead forms a disulfide bridge with C66 in Xwnt8 (=Wnt3a C88), as predicted here. See Janda et al., Science, published online May 31, 2012. 10.1126/science.1222879.