A RING finger to wed TCF and β -catenin

Claudio Cantù, Tomas Valenta & Konrad Basler

In complex organisms, determination of cell fate and behaviour is governed by a few fundamental molecular signalling pathways. Among them, the Wnt pathway has a crucial role in almost all aspects of animal development, including the regulation of homeostasis in adult tissues and, if deregulated, in cancer formation and progression [1]. β -catenin, the central player of the Wnt pathway, is degraded in the cytoplasm if it is not associated with adherens junctions. Binding of Wnt ligands leads to stabilization and translocation of β -catenin to the nucleus where it interacts with members of the TCF/LEF family of transcription factors, which bind to the Wnt responsive elements (WRE) within promoters or enhancers of Wnt target genes. In a 'Wnt-OFF' state, TCF/LEF factors occupy the WREs together with transcriptional co-repressors—for example, TLE/Groucho. Nuclear β -catenin displaces the co-repressors from TCF/LEF factors, and serves as a scaffold to attract transcriptional coactivators, such as CBP/p300, mixed lineage leukaemia, Bcl9/Bcl9L and Pygopus [1].

In this issue of *EMBO reports*, Wu and colleagues [2] add a new piece to the puzzle of Wnt signalling. They identify the RING zinc-finger-containing protein RNF14 as a new enhancer of the Wnt-dependent transcriptional outputs acting at the level of the TCF/LEF- β -catenin complex. The authors show that RNF14 binds to all four mammalian TCF/LEF members. RNF14 is an E3 ubiquitin ligase that acts on unknown substrates and has autoubiquitination activity [3]. It has at least two functional domains: the RING domain that mediates the interaction between E2 ubiquitin-conjugating enzymes, and the androgen receptor-binding domain through which it activates androgen receptor-mediated signalling [4].

Wu and co-workers show that RNF14 can specifically enhance Wnt-dependent

transcription in colon cancer cells by affecting the transcription of reporter constructs containing TCF-binding motifs, as well as the transcription of endogenous Wnt target genes, which are connected to decreased survival of colon cancer cell lines. Of note, the androgen receptor-binding domain is irrelevant for inducing TCF-dependent transcription, suggesting a new nuclear receptor-independent role of RNF14. More interestingly, a domain in the carboxyl terminus (amino acids 380–474) previously reported to be important for RNF14 homodimerization, although dispensable for TCF binding, is crucial for enhancing the transcription of Wnt target genes. Thus, it is probable that monomeric RNF14 can still bind to TCF, but only as a homodimer can it support TCF/ β -catenin transcriptional activity (Fig 1).

The authors investigate the relevance of RNF14 *in vivo* by using a GFP-based Wnt reporter in zebrafish and find that RNF14 requires an already functional Wnt environment—even when overexpressed RNF14 cannot force ectopic activation of the Wnt reporter. In other words, RNF14 overexpression—or downregulation—modulates the reporter activity only in a pre-existing 'Wnt-ON' context. The behaviour of the reporter mirrors the changes in the transcription of endogenous Wnt target genes. For example, knockdown of RNF14 mildly, but significantly, downregulates Wnt target gene transcription, which supports the idea that RNF14 acts as an accessory protein to operate as a TCF-dependent transcriptional modulator.

RNF14 is the latest addition to a growing list of factors that influence Wnt-dependent transcription by binding to TCF/LEF and β -catenin directly. Other examples are the poly(ADP-ribose) polymerase 1, which interacts physically with TCF4 and augments the transcriptional activity of the β -catenin–TCF4 complex in colon cancer

cells [5], and the transducin β -like protein 1 (TBL1) and its related protein TBLR1, which are required for Wnt/ β -catenin-mediated transcription through mutually dependent recruitment of TBL1–TBLR1 and β -catenin to Wnt target gene promoters [6]. One possibility is that some of these coactivators are relevant in certain tissues or cell types where they modulate the intensity of the transduced Wnt signal and thereby influence, for example, the proliferation potential. However, what is the molecular mechanism by which RNF14 enhances Wnt pathway-dependent transcription?

When overexpressed, RNF14 even boosts the transcription induced by a constitutively active form of β -catenin (S33Y β -cat). This indicates that its role lies downstream from β -catenin stabilization. The special feature of RNF14 is its biochemical behaviour: although RNF14 does not bind to β -catenin directly, it enhances the interaction between β -catenin and TCF proteins like a wedding ring. Supporting this idea, the effect of RNF14 depletion on cancer cell viability is rescued by the expression of a TCF4- β -catenin fusion protein, implying that the main RNF14 function is to stabilize the TCF- β -catenin interaction. In addition, chromatin immunoprecipitation experiments indicate that RNF14 depletion reduces the presence of β -catenin, but not TCF, at the WREs. Indeed, the authors show that even in the absence of any Wnt signal RNF14 occupies the WREs to the same extent as TCF/LEF.

This is reminiscent of what has been described in *Drosophila* for Pygopus [7]. Pygopus was shown to occupy Wnt target gene loci together with Pangolin, the *Drosophila* TCF. On the basis of this it was suggested that, at the onset of a Wg/Wnt signal, Pygo captures Armadillo (the β -catenin homologue in flies), thus ensuring that the signal is transduced efficiently. Subsequently, it was reported that the

Fig 1 | Possible mechanism by which RNF14 modulates Wnt/ β -catenin signalling. (**A,B**) The enhancers and promoters of Wnt/ β -catenin target genes contain many Wnt-responsive elements (WREs) that are recognized by TCF/LEF transcription factors, which bind to them even in the Wnt-OFF state. In the absence of a Wnt stimulus, TCF/LEF associates with Groucho/TLE co-repressors, which repress transcription through recruitment of histone deacetylases (HDACs). If RNF14 is expressed it binds to TCF/LEF as a dimer or multimer, without affecting the association of TCF/LEF with WREs (**B**). (**C,D**) In the Wnt-ON state, β -catenin enters the nucleus and binds to TCF/LEF, thus displacing co-repressors from target promoters. β -catenin then recruits additional transcriptional coactivators, thus changing TCF/LEF from a transcriptional repressor to an activator. RNF14 can facilitate (strengthen) the interaction between β -catenin and TCF/LEF allowing high transcriptional activity even at low levels of nuclear β -catenin (**D**). When RNF14 is not expressed, the TCF- β -catenin interactions are weaker and β -catenin might partly dissociate from target promoters (**C**). CTTA, carboxy-terminal transcriptional coactivator; NTTA, amino-terminal transcriptional coactivator; RNF14, ring finger protein 14; TCF, T-cell factor; TLE, transducin-like enhancer of split.

genetic loss of Groucho renders *Pygopus* dispensable for Wg/Wnt transcriptional outputs [8], suggesting that *Pygopus* works as an antirepressor. This raises the possibility that RNF14 has a similar function, antagonizing the binding between TCF and its co-repressors, whilst enhancing the one with β -catenin. However, the Waterman group described previously that the co-repressor TLE is barely detectable in the HCT116 cancer cell line [9], yet in the same cell type RNF14 seems to have a strong effect on the Wnt/ β -catenin dependent transcription. If RNF14 can occupy the WRE together with TCF before any Wnt stimulus, a tripartite molecular complex—that is RNF14, TCF and TLE—with no binding competition among the three factors, could be envisioned, and this possibility still needs to be tested.

The mechanism of action of RNF14 might be simpler—RNF14 could induce TCF to maintain a conformation that increases its affinity for β -catenin (Fig 1). This hypothesis is all the more compelling if one considers the ubiquitin ligase enzymatic activity of RNF14. TCF factors have been shown to be affected by post-translational modifications. Notably, TCF4 could be sumoylated at the Lys297, enhancing its affinity for β -catenin and

hence increasing TCF/ β -catenin dependent transcription in mammalian cells [10]. Similarly, β -catenin stability, as well as its partner recognition, are known to be regulated by post-translational modifications such as phosphorylation and ubiquitination [1]. A plausible and exciting working hypothesis based on these facts is that RNF14 regulates the transcriptional output of Wnt signalling by modulating, through ubiquitination, the affinity between TCF and β -catenin.

Much more has to be learned about transcriptional coactivators such as RNF14—is it a general, evolutionarily conserved, constituent of the pathway? Is it ubiquitously expressed, or does it support Wnt-dependent transcription in a tissue- or stage-specific manner? Does it contribute, when misregulated, to cancer initiation and aggressiveness?

Signalling pathways that govern cell proliferation and fate are much more than a linear series of components; rather they are constituted by a complex network of modulators capable of specifically influencing the transcriptional output in a tissue- or cell-specific manner. When such networks are misregulated, even a single cell might become cancerogenic. A new potential therapeutic approach could

aim at modulating the modulators: breaking the RING finger-containing RNF14 could promote a divorce between TCF and β -catenin, thereby slowing down or stopping the ‘brakeless’ sprint that drives cancer formation and progression.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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EMBO reports (2013) **14**, 295–296; published online 12 March 2013; doi:10.1038/embor.2013.21