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Perspective

A New Look at TCF and β-Catenin through the Lens of a Divergent *C. elegans* Wnt Pathway

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The canonical Wnt/β-catenin pathway is extensively characterized, broadly conserved, and clinically important. In this review, we describe the *C. elegans* Wnt/β-catenin asymmetry pathway and suggest that some of its unusual features may have important implications for the canonical Wnt/β-catenin pathway.

Introduction

Wnt signaling is crucial for many aspects of metazoan development, including controls of stem cells, proliferation, and differentiation (Clevers, 2006; Nusse, 2005). Indeed, defects in Wnt signaling are associated with a variety of diseases, including colon cancer and osteoporosis (MacDonald et al., 2009, this issue of *Developmental Cell*; Moon et al., 2004). The best known Wnt signaling pathways include the Wnt/β-catenin, Wnt/planar cell polarity (PCP), and Wnt/calcium pathways (James et al., 2008). The Wnt/β-catenin pathway, which we abbreviate Wβ for brevity, is often called the "canonical" Wnt pathway. This canonical Wβ pathway is found in all animals, including flies, vertebrates, and nematodes.

The canonical Wβ pathway is distinguished from other Wnt pathway variants by its control of transcription via two terminal effectors, a DNA binding protein of the TCF/LEF family and its β-catenin transcriptional coactivator. Active Wβ signaling stabilizes cytoplasmic β-catenin, permits formation of a nuclear β-catenin/TCF complex, and activates selected target genes (Figures 1A and 1B). In the absence of Wnt signaling, most β-catenin is located in adherens junctions where it functions in adhesion; nonjunctional β-catenin is degraded by a complex that includes glycogen synthase kinase (GSK) 3β, the tumor suppressor adenomatous polyposis coli (APC), and the Axin scaffolding protein (MacDonald et al., 2009). In the absence of nuclear β-catenin, TCF represses transcription of Wnt target genes (Cavallo et al., 1998). This barebones description is obviously an oversimplification but sufficient for our focus in this review on TCF and β-catenin. In *C. elegans,* the canonical Wβ pathway operates through the single TCF homolog, called POP-1 (Lin et al., 1995; Thorpe et al., 1997), and the canonical β-catenin homolog BAR-1, which is one of three *C. elegans* β-catenins identified by sequence homology (Table 1; Eisenmann et al., 1998; Korswagen et al., 2000).

In this review, we highlight the less familiar *C. elegans* Wnt/β-catenin asymmetry pathway (Mizumoto and Sawa, 2007), henceforth abbreviated WβA, and discuss possible implications for the canonical pathway. The WβA pathway resembles the Wβ pathway in that both activate transcription of target genes using TCF and a β-catenin-like transcriptional coactivator. Conversely, the "noncanonical" Wnt pathways, Wnt/PCP and Wnt/calcium, function independently of TCF and β-catenin, regulating instead the actin cytoskeleton and intracellular calcium levels, respectively (James et al., 2008). Although the *C. elegans* canonical Wβ pathway controls fates in a few cells during development (e.g., Q neuroblasts, vulval precursor cells; Korswagen, 2002), the WβA pathway regulates most asymmetric cell divisions and their linked cell fate specifications in the nematode lineage, from the four-celled early embryo through larval development, making it the primary *C. elegans* Wnt pathway (Bertrand and Hobert, 2009; Herman et al., 1995; Kaletta et al., 1997; Lin et al., 1995; Phillips et al., 2007; Siegfried and Kimble, 2002; Takeshita and Sawa, 2005). Figure 2 illustrates four particularly well-understood examples where the WβA pathway controls specific asymmetric cell divisions.

A major goal of this review is to clarify central features of the WβA pathway, which have been confusing because the pathway was missing a central component when first discovered. That central component is SYS-1, a β-catenin-like transcriptional coactivator. The discovery of SYS-1 transformed early models for WβA pathway function into the now broadly accepted interpretation of how this pathway operates. Another confusing aspect of the WβA pathway is its reliance on two β-catenin-like proteins. In addition to the SYS-1 transcriptional coactivator, WRM-1 regulates TCF localization (see below). Other *C. elegans* β-catenin-like proteins that are not in the WβA pathway include BAR-1/β-catenin, the effector of the canonical Wβ pathway (Eisenmann et al., 1998), and HMP-2/β-catenin, which is specialized for adhesion (Costa et al., 1998; Korswagen et al., 2000; Table 1). Here, we briefly describe the current view of the WβA pathway and then consider results from diverse organisms that suggest, or in some cases only hint, that the *C. elegans* WβA pathway may help us understand key aspects of canonical Wnt signaling more broadly. For the most part, we use vertebrate names for pathway components in an attempt to simplify nomenclature and avoid acronyms, with Table 2 providing names of the *C. elegans* homologs.

The WβA Pathway Is Forked and Controls the Ratio of β-Catenin and TCF

The major defining feature of the *C. elegans* WβA pathway is its two branches (Figures 1C and 1D). Upstream of both branches is a common trunk, including Wnt ligands, Frizzled receptors, and Dishevelled scaffolding proteins. Downstream of that trunk, one

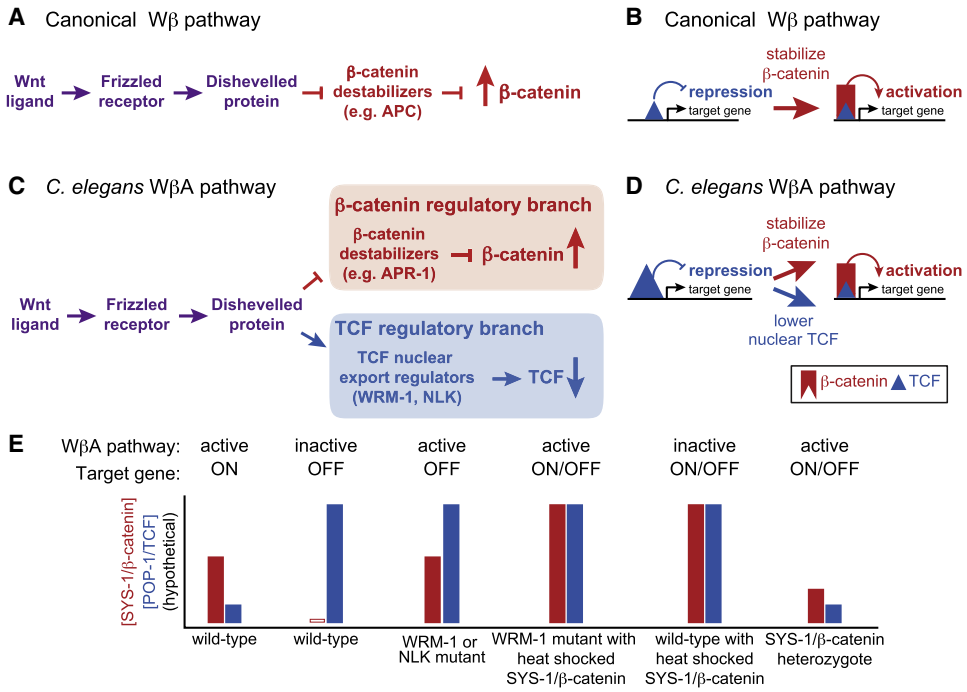

Figure 1. The WβA Pathway Controls Both SYS-1/β-Catenin and POP-1/TCF

(A) The canonical Wβ pathway, highly simplified view.

(B) The canonical Wβ pathway transforms TCF from a transcriptional repressor to a transcriptional activator of target genes.

(C) The *C. elegans* WβA pathway is forked, highly simplified view. The β-catenin and TCF regulatory branches are indicated. Worm-specific names are not used except (1) APR-1, a homolog of APC and (2) WRM-1, a divergent β-catenin. A Wnt ligand has not been identified for all WβA-dependent asymmetric divisions.

(D) The *C. elegans* WβA pathway transforms TCF from a transcriptional repressor to a transcriptional activator of target genes.

(E) The ratio of SYS-1/β-catenin and POP-1/TCF controls target gene expression. The relative protein concentrations on the graph are hypothetical, being inferred from genetic experiments and reporter gene analyses. SYS-1 is clearly higher in active than inactive cells, and POP-1 is clearly lower in active than inactive nuclei, but the relative concentration of SYS-1 to POP-1 in the same cell has not been measured. See text for explanation and references.

branch regulates TCF while the other branch controls β-catenin (Huang et al., 2007; Lin et al., 1995; Meneghini et al., 1999; Park and Priess, 2003; Phillips et al., 2007; Rocheleau et al., 1997; Thorpe et al., 1997). The branches work together to activate downstream target genes (Figure 1D; Bertrand and Hobert, 2009; Huang et al., 2007; Lam et al., 2006; Maduro et al., 2005; Shetty et al., 2005).

The TCF regulatory branch (Figure 1C, bottom branch) was discovered nearly a decade before the β-catenin regulatory branch (Figure 1C, top branch). This TCF regulatory branch was confusing in the absence of its β-catenin counterpart, but its main findings are easier to digest now that the pathway has acquired a shape more comparable to the canonical pathway. So what is important about the TCF regulatory branch? First and foremost, this branch lowers TCF in the nucleus of the actively signaled cell (Lin et al., 1998; Lin et al., 1995; Maduro et al., 2002; Meneghini et al., 1999; Park and Priess, 2003; Rocheleau et al., 1997; Siegfried and Kimble, 2002). Moreover, that reduction is required for expression of Wnt-responsive target genes (Arata et al., 2006; Bertrand and Hobert, 2009; Lam et al., 2006; Shetty et al., 2005). This finding was made possible by analyzing TCF in defined cells as they are being signaled in the anatomically simple *C. elegans*. TCF lowering was first seen at the EMS division during early embryogenesis (see Figure 2), but a similar lowering has now been seen broadly. At each asymmetric cell division controlled by the WβA pathway, nuclear TCF is lowered in the actively signaled daughter cell but remains high in the unsignaled daughter. An early interpretation of this TCF lowering invoked transcriptional derepression since abundant TCF represses target gene expression (Calvo et al., 2001; Cavallo et al., 1998). A more recent interpretation is that nuclear TCF must be low to activate transcription, perhaps because of a limiting coactivator (Kidd et al., 2005). This interpretation is consistent with dosage effects in the pathway (see below). Therefore, the current model for the WβA pathway is that TCF activates transcription when complexed with its transcriptional coactivator, but that it represses transcription when not bound to its transcriptional coactivator.

A second important feature of the TCF regulatory branch is that Nemo-like kinase (NLK) activates the pathway by lowering TCF. NLK is a serine/threonine kinase that, in *C. elegans*, acts genetically downstream of TGFβ activated kinase (TAK1) and

Table 1. *C. elegans* β-Catenin-like Proteins

β-Catenin	% Identity to Human β-Catenin	Function
HMP-2	29%	Adhesion
BAR-1	25%	Transcriptional activation (adhesion?)
WRM-1	19%	Regulation of TCF nuclear export
SYS-1	9%	Transcriptional activation (adhesion?)

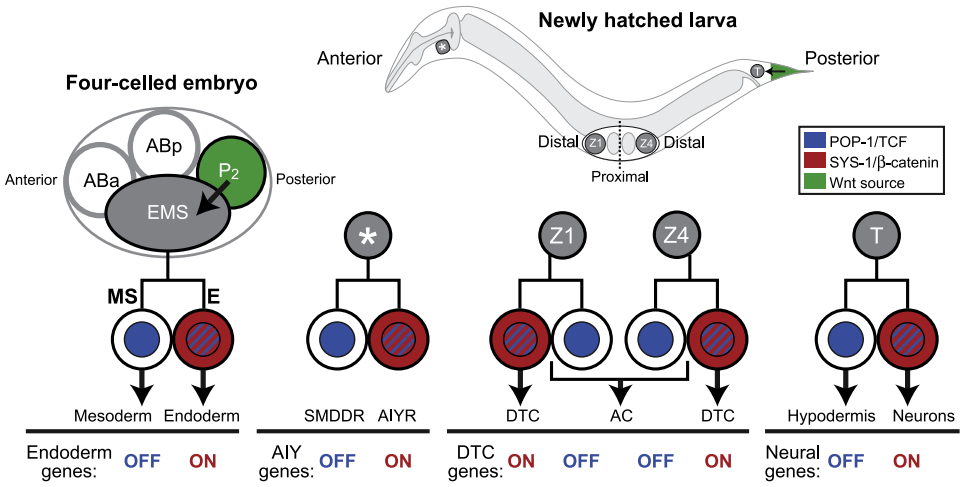

Figure 2. Key Asymmetric Cell Divisions Controlled by the *C. elegans* WβA Pathway

Left, EMS blastomere in the four-celled embryo. A Wnt signal from the P2 blastomere (green) polarizes EMS, which divides along the anterior-posterior (A-P) axis into MS (mesoderm) and E (endoderm) progenitor cells (Korswagen, 2002). Right, newly hatched larva showing positions of key progenitor cells. Middle left, ABprpapaaa neuronal progenitor cell (*). An unknown signal polarizes ABprpapaaa along the A-P axis to generate two distinct neurons, SMDDR and AIYR (Bertrand and Hobert, 2009). Middle right, two somatic gonadal precursors (SGPs), called Z1 and Z4 as individuals. An unknown signal polarizes each SGP along the gonadal proximal-distal (P-D) axis to generate daughters with distal tip cell (DTC) or anchor cell (AC) potential (Kimble and Crittenden, 2007). Far right, T progenitor cell. A posterior Wnt signal (green) polarizes the T cell along the A-P axis to produce daughters with hypodermal or neural potential (Herman and Wu, 2004; Mizumoto and Sawa, 2007). In each example, nuclear POP-1/TCF is low (blue cross hatching) and SYS-1/β-catenin is high (solid red) in the posterior/distal daughter cell that has an activated WβA pathway, whereas nuclear POP-1/TCF is high (solid blue) and SYS-1/β-catenin (no red) is absent or barely detectable in the sibling anterior/proximal daughter that has an inactive WβA pathway. In each case, the WβA pathway, including POP-1/TCF and SYS-1/β-catenin, activates identified direct target genes: the *end-1* endodermal gene in E (Shetty et al., 2005), the *ceh-10* gene, which specifies AIYR fate (Bertrand and Hobert, 2009), the *ceh-22* DTC-inducing gene in the distal daughters of Z1 and Z4 (Lam et al., 2006), and the *psa-3* gene in the posterior daughter of T (Arata et al., 2006). In addition to these well-studied examples, reciprocal asymmetry of POP-1/TCF and SYS-1/β-catenin occurs in most other asymmetric divisions throughout embryonic and larval development, suggesting a broad role for the WβA pathway.

upstream of TCF (Kaletta et al., 1997; Meneghini et al., 1999). An elegant series of experiments revealed that NLK works together with the divergent WRM-1 β-catenin to phosphorylate the single *C. elegans* TCF homolog, called POP-1, and promote its nuclear export (Lo et al., 2004; Rocheleau et al., 1999; Shin et al., 1999). Indeed, WRM-1 was the only β-catenin-like protein known for this pathway in its early days. An important and counterintuitive feature of this TCF nuclear export is its activation of the pathway. Although one might think a priori that nuclear export would inactivate TCF, the opposite appears to be true—the TCF nuclear export machinery is required to activate transcription of Wnt responsive genes. The current model is that export is incomplete and therefore decreases nuclear TCF rather than depleting it completely. By this model, nuclear TCF must be lowered to activate downstream genes, but TCF cannot be removed entirely.

Table 2. *C. elegans* WβA Proteins

C. elegans WβA Protein	Vertebrate Homolog	Function
MOM-2, LIN-44	Wnt ligand	Signaling ligand
MOM-5, LIN-17	Frizzled receptor	Receptor
DSH-2, MIG-5	Dishevelled	Adaptor
MOM-4	TGFβ activated kinase	Activates LIT-1/NLK
LIT-1	Nemo-like kinase	TCF nuclear export
WRM-1	β-catenin	TCF nuclear export
POP-1	TCF	DNA binding protein
APR-1	APC	β-catenin stability
SYS-1	β-catenin	Transcriptional coactivator

The β-catenin regulatory branch of the WβA pathway was only recently discovered (Figure 1C, top branch), in large part because it controls a β-catenin-like protein that could not be recognized by amino acid sequence, SYS-1 (for symmetrical sisters; see section below and Kidd et al., 2005). The control of SYS-1/β-catenin is not as well understood as that of β-catenin in the canonical pathway, but several lines of evidence demonstrate that the WβA pathway stabilizes SYS-1, much as the canonical Wβ pathway stabilizes canonical β-catenin (Huang et al., 2007; Phillips et al., 2007). As mentioned above, the WβA pathway is active in one daughter of each asymmetric cell division and inactive in the other daughter. During those asymmetric divisions, SYS-1 is present in the mother cell just before division and localized equally to centrosomes during division. After cytokinesis, SYS-1 appears to increase in the activated daughter cell, compared to what it receives on its centrosome, while SYS-1 is absent or barely detectable in the inactive daughter cell. That pattern, dubbed "SYS-1 asymmetry," depends on the SYS-1 protein rather than any sequences in its promoter or mRNA (Phillips et al., 2007). Therefore, SYS-1 is controlled posttranslationally. Moreover, SYS-1 disappearance requires proteasome activity and APR-1, the *C. elegans* homolog of APC (Huang et al., 2007). Therefore, the β-catenin branch of the WβA pathway controls SYS-1/β-catenin stability and may use the same regulators that control canonical β-catenin stability.

The control of both POP-1/TCF and SYS-1/β-catenin by the two branches of the WβA pathway focuses attention on the ratio

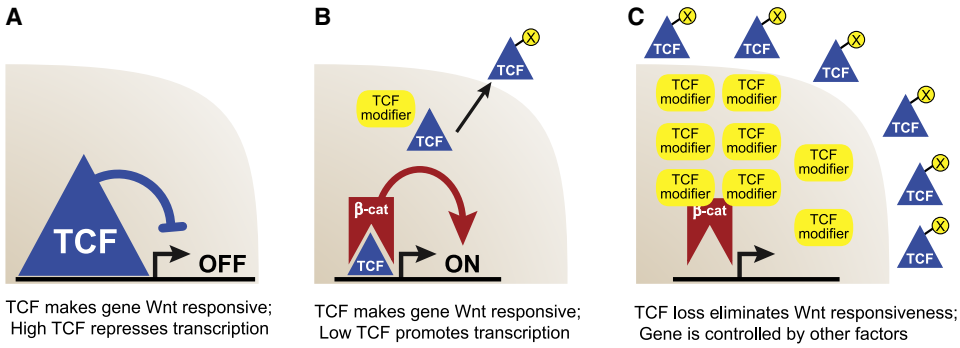

Figure 3. Model for TCF Modification and Transcriptional Control

This model is based on the NLK phosphorylation of TCF in the *C. elegans* WβA pathway, but it is shown in a more generic form to suggest implications for other modifiers. The idea put forward in (C) has not been tested and is only one possible explanation for loss of gene activity upon TCF modification. The brown shaded area depicts the promoter region of a Wnt-dependent target gene. Interpretations are written below each figure and discussed in the text.

of these two regulators rather than their absolute protein levels (Figure 1E). The importance of that ratio has been investigated by manipulating POP-1/TCF and SYS-1/β-catenin levels in nematodes or in tissue culture cells and subsequently analyzing either cell fate or WβA-responsive transcriptional reporters (Bertrand and Hobert, 2009; Huang et al., 2007; Kidd et al., 2005; Lam et al., 2006). In wild-type cells that are not activated by the WβA pathway, the SYS-1:POP-1 ratio is low, but when the cell is activated, that ratio is increased in a concerted fashion, by decreasing POP-1/TCF and increasing SYS-1/β-catenin (Figure 1E, left). In *wrm-1* or *lit-1/nlk* mutant cells, which are defective for TCF nuclear export, the ratio does not increase sufficiently and the pathway is inactive (Figure 1E, middle left). However, SYS-1 overexpression can overcome high nuclear TCF and drive the transcription of WβA-responsive target genes, either in an actively signaled daughter defective for the TCF regulatory branch or in a wild-type daughter cell that has not been activated by the WβA pathway (Figure 1E, middle right). Finally, the pathway is compromised in *sys-1* heterozygotes, which likely have less SYS-1 protein than normal (Figure 1E, far right; Kidd et al., 2005). Consistent with these genetic experiments, TOPFLASH assays in tissue culture cells respond to the ratio of SYS-1 and POP-1 (Kidd et al., 2005). Taking these lines of evidence together, the model emerges that WβA readout reflects the ratio rather than the absolute amounts of SYS-1/β-catenin and POP-1/TCF.

The similarities of the Wβ and WβA pathways are striking; the fundamental difference is presence of the TCF regulatory branch in the WβA pathway (Figure 1). *C. elegans* possesses both Wβ and WβA pathways, but, as mentioned above, its canonical Wβ pathway affects only a handful of cell fate decisions while the WβA pathway regulates asymmetric cell divisions and cell fates throughout development. What advantages might a forked pathway offer? The answer is likely to be a combination of robustness and speed. If either branch were wholly compromised, it is true that the pathway would fail. However, if either branch were partially compromised for a short time due to some stochastic fluctuation, the other branch could compensate and ensure the proper response. The forked pathway also allows a rapid change to the SYS-1:POP-1 ratio because numerator and denominator are reciprocally altered. We suggest that the forked WβA pathway may be specialized in *C. elegans* for asymmetric cell divisions but that its key features may inform our thinking about Wβ pathways more generally. Interestingly, Wβ signaling also regulates asymmetric divisions in the annelid *Platynereis dumerilii,* suggesting that Wnt signaling may be an ancient means of controlling binary fate specification (Schneider and Bowerman, 2007). However, as we discuss in the next section, the existence of a TCF regulatory branch in organisms outside nematodes remains an open question.

Hints that a TCF Regulatory Branch May Exist Outside Nematodes

The discovery of the WβA TCF regulatory branch was a surprise and opened the possibility that TCF/LEF proteins might be similarly controlled in other organisms. Perhaps TCF transcriptional activity (repression versus activation) can be regulated by altering TCF at the promoters of WβA-responsive target genes. This idea has been explored in other organisms with controversial results. Figure 3 presents a model for thinking about those results. This figure represents a generic form of ideas gleaned from the *C. elegans* WβA pathway. In this next section, we review three different TCF modifications and their effects on either TCF transcriptional activity or Wnt-dependent biological events. We first consider TCF phosphorylation by Nemo-like kinase, which is the mechanism used to lower nuclear TCF in the nematode WβA pathway, and then briefly summarize effects of TCF sumoylation and acetylation.

TCF Phosphorylation by NLK

Vertebrate NLK can phosphorylate vertebrate TCF family proteins TCF4 and LEF1 in vitro using recombinant proteins, and the resultant phosphorylated TCF is degraded in mammalian cells (Ishitani et al., 1999; Yamada et al., 2006). However, the role for NLK in Wnt signaling remains controversial. A study in zebrafish identifies the only case in vertebrates where NLK has been found to promote Wnt signaling. In this case, NLK depletion by morpholinos enhanced effects of Wnt8 depletion, suggesting that the two regulators act synergistically (Thorpe and Moon, 2004). Further, this NLK loss-of-function phenotype requires the presence of TCF, suggesting that NLK acts through TCF. One explanation might be that zebrafish NLK derepresses Wnt-dependent target genes, an idea similar to that proposed

in early models for the WβA TCF control. However, an idea more in line with our current understanding of the WβA pathway is that NLK phosphorylation of TCF promotes Wnt signaling by inducing TCF's ability to activate target genes.

Other studies that employed NLK overexpression yielded opposite results. In mammalian cells and *Xenopus*, NLK overexpression decreased Wnt signaling—either turning off target gene expression or suppressing a β-catenin-induced secondary axis (Ishitani et al., 2003; Ishitani et al., 1999). NLK overexpression in a TOPFLASH assay reduces LEF1 transcriptional activity, but nonphosphorylatable LEF1 is insensitive to NLK overexpression. One simple interpretation is that NLK phosphorylation of TCF normally antagonizes the Wnt pathway. However, an alternative explanation, which unifies these results with those using zebrafish morpholinos, is that overexpressed NLK drives TCF to an abnormally low level and thereby depletes TCF from the promoter (Figure 3C).

In flies, NLK loss promotes Wnt signaling but NLK overexpression inhibits it (Zeng and Verheyen, 2004). Effects on Pangolin/TCF were not explored, but NLK affected β-catenin stability. From these results and others, the authors suggest that fly NLK is a negative feedback inhibitor of Wnt signaling. NLKs may therefore regulate Wnt pathways in diverse ways.

TCF Sumoylation

Vertebrate TCF family members can be modified by the small ubiquitin-like modifier (SUMO). That sumoylation relies on either of two SUMO E3 ligases, the protein inhibitor of activated STAT (PIASy) or RanBP2 (Sachdev et al., 2001; Shitashige et al., 2008; Yamamoto et al., 2003). The effect of TCF sumoylation on its transcriptional activity can be either positive or negative. Sachdev et al. (2001) found that sumoylated LEF-1 was sequestered in nuclear bodies and that Wnt-dependent target gene expression was decreased as a result. Others found that sumoylated TCF-4 enhanced β-catenin binding and activated target gene expression (Shitashige et al., 2008; Yamamoto et al., 2003). These differing results can be explained in a variety of ways, including the analysis of distinct TCF homologs, diverse cell types, or perhaps the effects of low versus no TCF at the promoter (Figures 3B and 3C).

TCF Acetylation by CBP/p300

Some TCF homologs can be acetylated by the CREB binding protein (CBP)/p300. In *C. elegans*, acetylation of POP-1/TCF increases its nuclear retention and a nonacetylatable POP-1 cannot rescue a *pop-1* mutant (Gay et al., 2003). Therefore, POP-1/TCF acetylation appears to counteract the effect of POP-1 phosphorylation, which drives nuclear export. In *Drosophila*, acetylation of pangolin/TCF decreases its binding to β-catenin and is predicted to antagonize Wnt signaling (Waltzer and Bienz, 1998). Therefore, TCF acetylation may antagonize Wnt signaling, and do so by either of two mechanisms—increasing nuclear TCF or decreasing TCF affinity for β-catenin. In either case, TCF acetylation would nudge the transcriptional balance toward repression of TCF target genes.

In sum, TCF regulation is widespread, but our understanding of that regulation remains in its infancy. One unresolved issue highlighted in this review is whether individual TCF modifications promote or antagonize TCF-dependent transcription. Evidence exists for both, often with the same modification. However, in most cases, nuclear TCF (or perhaps more importantly, promoter-associated TCF) was not quantified, which we suggest is critical to interpret the results. A second unresolved issue is how these various modifications are themselves controlled. In *C. elegans*, the WβA pathway controls NLK phosphorylation, but what about in other organisms? In sea urchins, Notch signaling controls NLK phosphorylation of TCF (Röttinger et al., 2006), a finding that links the Wnt and Notch pathways at the promoter of Wnt-responsive genes. It is easy to imagine that dual controls of TCF and β-catenin have evolved to meet needs specific to developmental contexts. For example, control by a single pathway might be optimal at asymmetric divisions, while two or more pathways might be optimal to coordinate events in a more complex setting such as organogenesis.

Recognizing β-Catenin Coactivators by Criteria Other Than Amino Acid Sequence

β-catenins have been classically recognized by sequence similarity to canonical β-catenins in flies or humans, but that standard method failed for *C. elegans* SYS-1/β-catenin. In this section, we briefly review how SYS-1 was identified as a bona fide β-catenin transcriptional coactivator and then consider the possible existence of other β-catenins that cannot be recognized by their amino acid sequence. The amino acid sequences of SYS-1 and human β-catenin are highly dissimilar (Table 1; Liu et al., 2008). Nonetheless, SYS-1 and canonical β-catenin are regulated similarly, as mentioned above, and SYS-1 possesses the functional and structural hallmarks of canonical β-catenins.

Functionally, the SYS-1 protein is a key terminal effector of the WβA pathway (Bertrand and Hobert, 2009; Huang et al., 2007; Miskowski et al., 2001; Phillips et al., 2007; Siegfried and Kimble, 2002; Siegfried et al., 2004). In addition, SYS-1 rescues a *bar-1* mutant when placed under control of the *bar-1* promoter; it binds to POP-1/TCF; and it works together with POP-1/TCF as a transcriptional coactivator (Kidd et al., 2005). Its coactivator function has been assayed using both TOPFLASH assays in tissue culture cells and reporter assays in transgenic nematodes (Huang et al., 2007; Kidd et al., 2005; Lam et al., 2006). Therefore, SYS-1 clearly possesses a β-catenin-like coactivator function. However, SYS-1 has not yet been found to play a β-catenin-like role in adhesion. (Canonical β-catenins have a dual role as both transcriptional coactivators and adhesion proteins [reviewed by Nelson and Nusse, 2004; Table 1].) In contrast to SYS-1, the *C. elegans* HMP-2 β-catenin homolog is specialized for adhesion, but it is an extremely poor transcriptional coactivator (Costa et al., 1998; Korswagen et al., 2000; Natarajan et al., 2001). Therefore, SYS-1 may be redundant with HMP-2 for its adhesive function, or it may simply lack that adhesive function.

Structurally, the SYS-1 protein possesses twelve armadillo repeats that stack upon one another to form a superhelix, much like canonical β-catenin (Figure 4A; Huber et al., 1997; Liu et al., 2008). In addition, the SYS-1/POP-1 and human β-catenin/TCF complexes are nearly identical in other ways. Perhaps most importantly, the interaction between the two proteins is anchored in both complexes by a conserved aspartate to lysine salt bridge, termed the charged button (Graham et al., 2000; Liu et al., 2008; Poy et al., 2001). Indeed, an EMS-induced *pop-1* missense mutation changes its charged button amino acid from an aspartate to a glutamate, and abrogates POP-1 function in vivo as well as in binding studies in vitro (Liu et al., 2008; Siegfried and Kimble, 2002).

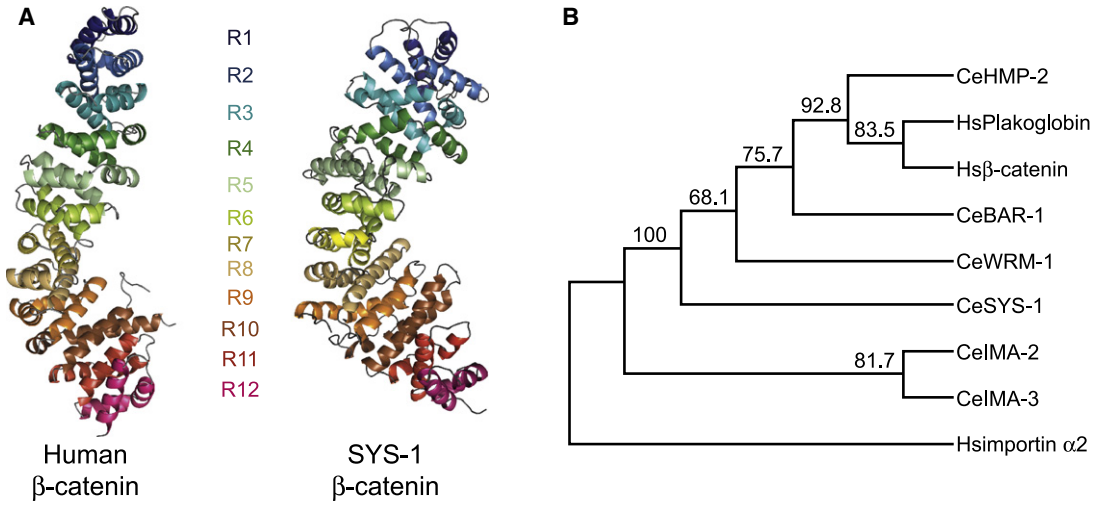

Figure 4. SYS-1 Is a Bona Fide β-Catenin

(A) The crystal structures of both human β-catenin and *C. elegans* SYS-1 reveal 12 armadillo repeats despite their negligible sequence identity (modified from Liu et al., 2008). R1, first armadillo repeat.

(B) Phylogenetic analysis place SYS-1 in the β-catenin clade, using other armadillo proteins for comparison (modified from Liu et al., 2008). Ce, *C. elegans;* Hs, *Homo sapiens*; IMA, importin-α.

The identification of SYS-1 as a β-catenin-like protein raises two issues. First, is SYS-1 a true β-catenin homolog—did SYS-1 and β-catenin derive from a common ancestor or is SYS-1 a product of convergent evolution? By phylogenetic analyses, SYS-1 groups robustly within the β-catenin clade, using the sequence of its structurally most similar armadillo repeats (Liu et al., 2008; Figure 4B). However, the identification of divergent β-catenin intermediates will be required to address this question definitively. One such intermediate may be WRM-1, which is recognizable as a highly divergent β-catenin from its amino acid sequence (Rocheleau et al., 1997). However, intermediates in other species would lend more weight to the idea that SYS-1 is a divergent β-catenin.

The second question is whether SYS-1 is a deviant β-catenin that is specific to a small group of nematodes or an emissary β-catenin that heralds discovery of similarly divergent β-catenins throughout the animal kingdom. At the current time we cannot answer this question—no SYS-1 homologs are recognized by sequence searches, except in the genomes of closely related nematodes. Instead, functional criteria must be used to explore the idea that more divergent β-catenins exist, a process that is both time consuming and risky. Nonetheless, the possibility that such β-catenins may exist, even in vertebrates, is tantalizing given the importance of Wnt signaling in both development and disease.

Quick Recap and Ideas for Future Experiments

This review focuses on the *C. elegans* WβA pathway and its possible implications for the canonical Wnt pathway. We discuss several key features of the WβA pathway. The first is a simple one—readout of the WβA pathway relies on the ratio of its two terminal effectors, β-catenin and TCF. The Wβ pathway is likely to rely on the β-catenin to TCF ratio as well, but most research has focused on controls of the β-catenin numerator. By contrast, controls of the TCF denominator remain poorly characterized, and experiments designed to study them have yielded contradictory and often confusing results. A possible explanation of those confusing results comes from a second feature of the WβA pathway—that high TCF represses, while low TCF activates, transcription of Wnt target genes. This idea is counterintuitive and its generality for TCF proteins remains unknown. However, the basic idea that lowering the abundance of a transcriptional activator increases its target gene expression has been demonstrated in systems as diverse as yeast and mammals (Kim et al., 2003; Lipford et al., 2005; Muratani et al., 2005; von der Lehr et al., 2003). The emerging theme is that turnover of a transcriptional activator may be a general requirement for target gene expression. Therefore, it would be informative to know if abundant TCF in a vertebrate nucleus reflects an activated or repressed state. Or more treacherously, does high TCF mean that Wnt signaling was active in that nucleus at some point in its developmental history, but now has induced high TCF as part of a negative feedback loop? The ability to investigate the WβA pathway in individual cells as they receive their signal was crucial for learning that low TCF is the active form in *C. elegans*, and similar analyses will be critical in other systems. If low TCF were the active form in vertebrates as it is in *C. elegans*, both experimental interpretation and drug design would be impacted dramatically. A third feature of the WβA pathway is that its primary β-catenin transcriptional coactivator had to be recognized by functional rather than sequence criteria. We suggest that SYS-1 is not alone on the planet and that other divergent β-catenins await discovery. In fact, the amino acid sequence typical of canonical β-catenins is strongly conserved in HMP-2 and plakoglobin, two β-catenin-like proteins specialized for adhesion. Perhaps those sequence constraints relate to the role of β-catenin in adhesion rather than transcriptional coactivation. We suggest that the remarkable progress already made understanding the canonical Wβ pathway may be expanded further with a fresh look at the terminal regulators using a WβA lens.

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