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REVIEW ARTICLE

Molecular regulation and pharmacological targeting of the β-catenin destruction complex

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The β-catenin destruction complex is a dynamic cytosolic multiprotein assembly that provides a key node in Wnt signalling regulation. The core components of the destruction complex comprise the scaffold proteins axin and adenomatous polyposis coli and the Ser/Thr kinases casein kinase 1 and glycogen synthase kinase 3. In unstimulated cells, the destruction complex efficiently drives degradation of the transcriptional coactivator β-catenin, thereby preventing the activation of the Wnt/β-catenin pathway. Mutational inactivation of the destruction complex is a major pathway in the pathogenesis of cancer. Here, we review recent insights in the regulation of the β-catenin destruction complex, including newly identified interaction interfaces, regulatory elements and post-translationally controlled mechanisms. In addition, we discuss how mutations in core destruction complex components deregulate Wnt signalling *via* distinct mechanisms and how these findings open up potential therapeutic approaches to restore destruction complex activity in cancer cells.

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Abbreviations

APC, adenomatous polyposis coli; ARC, ankyrin repeat cluster; Arm, Armadillo; ASAD, APC-self-associating domain; CID, catenin inhibitory domain; CK1, casein kinase 1; CRC, colorectal carcinoma; DIX, dishevelled-axin domain; Dvl, dishevelled; FZD, frizzled; GSK3, glycogen synthase kinase 3; LRP5/6, lipoprotein-related protein 5/6; OD 1/2, oligomerization domain 1/2; PP1, protein phosphatase 1; PP2A, protein phosphatase 2A; RegB, region B; RGS, regulators of G-protein signalling; SAMP, Ser-Ala-Met-Pro; TNKS, tankyrase; β-TrCP, β-transductin repeat-containing protein

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The destruction complex is a key node for Wnt signalling regulation

Central to the signalling events within the Wnt/β-catenin pathway is the regulation of the dual function protein **β-catenin**. In epithelial cells, a continuous supply of β-catenin is required to secure its role as a stabilizer of adherens junction complexes, while at the same time, its task as a transcriptional coactivator of Wnt target gene expression remains under tight control (Clevers, 2006). Suppression of β-catenin-mediated transcription is accomplished by the destruction complex, a large cytosolic multiprotein assembly that mediates the rapid turnover of nonjunctional β-catenin (Stamos and Weis, 2013). The core components of the destruction complex include the scaffold proteins axin and adenomatous polyposis coli (APC), as well as the Ser/Thr kinases **casein kinase 1** (CK1) and **glycogen synthase kinase 3** (GSK3) (Figure 1).

The importance of a fully functional destruction complex to prevent Wnt pathway activation emerged in the mid 90's with the discovery that inherited and sporadic mutations in APC predispose to the development of colon cancer, due to uncontrolled β-catenin accumulation and Wnt target gene transcription (Munemitsu *et al.,* 1995; Korinek *et al.,* 1997; Morin *et al.,* 1997; Rubinfeld *et al.,* 1997a; Clevers, 2006). Later studies revealed that the destruction complex captures and phosphorylates β-catenin at its flexible N-terminus (Amit *et al.,* 2002; Liu *et al.,* 2002; Marin *et al.,* 2003), earmarking it for recognition by the F-box protein β-transductin repeat-containing protein (β-TrCP; Orford *et al.,* 1997; Jiang and Struhl, 1998; Wu *et al.,* 2003). Next, the β-TrCP-associated Skp1-Cullin F-box (SCF) ubiquitin ligase ubiquitinates β-catenin and delivers it to the proteasome to accomplish its destruction. Together, this proteolysis cascade serves to keep cytosolic β-catenin levels low and prevent its translocation to the nucleus (Figure 1).

Binding of **Wnt** to the cell surface **frizzled** (FZD) receptors and low density lipoprotein-related protein 5/6 (LRP5/6) interferes with β-catenin degradation *via* subcellular redistribution of destruction complex components (Clevers, 2006; MacDonald and He, 2012; Stamos and Weis, 2013). First, the Wnt-activated FZD receptor recruits the cytosolic effector protein Dishevelled (Dvl), providing an initial docking site for axin at the plasma membrane. In following steps, axin-bound kinases phosphorylate the cytosolic tail of LRP5/6, which creates additional interaction sites for axin and mediates the formation of stabilized, multimerized Wnt-receptor–Dvl–axin complexes (MacDonald *et al.,* 2009; MacDonald and He, 2012). As a result, the destruction complex is turned off and β-catenin accumulates in the cytosol (Figure 1). The molecular basis for Wnt-mediated destruction complex inactivation remains heavily debated, and for a

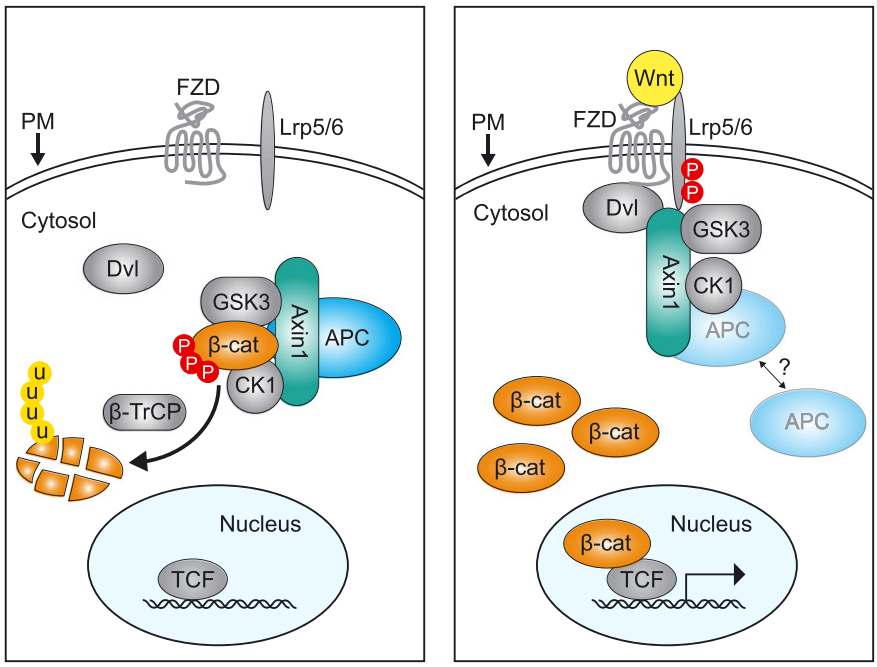

Figure 1

The β-catenin destruction complex is a central regulatory node in Wnt/β-catenin signalling. In the absence of Wnt, the β-catenin destruction complex facilitates continuous degradation of β-catenin. The destruction complex is comprised of the scaffold proteins APC and axin and the kinases CK1 and GSK3. These proteins act together to mediate phosphorylation (P) of β-catenin (β-cat). Phospho-β-catenin is recognized and ubiquitinated (U) by the β-TrCP Skp1-Cullin F-box (SCF) E3 ligase, after which the protein undergoes proteasomal degradation. In the presence of Wnt, the membrane-bound receptors FZD and Lrp5/6 are activated and phosphorylated leading to the recruitment of Dvl. Through subsequent recruitment of axin, the cytosolic β-catenin destruction complex undergoes inhibitory rearrangements, leading to the accumulation of β-catenin and its translocation to the nucleus where it acts as a co-transcription factor in complex with DNA-bound T-cell factor (TCF). The cell nucleus, cytosol and plasma membrane (PM) are indicated in the figure.

detailed discussion, we refer to a number of excellent reviews (Metcalfe and Bienz, 2011; MacDonald and He, 2012; Davidson and Niehrs, 2014). Briefly, proposed models include direct blockade of the catalytic site of axin-bound GSK3 by binding of phosphorylated LRP6 motifs (Cselenyi *et al.,* 2008; Piao *et al.,* 2008; Wu *et al.,* 2009), inhibition of GSK3 *via* Wnt-induced dissociation of APC (Valvezan *et al.,* 2012), or sequestration of GSK3 within multivesicular bodies *via* endocytosis of the receptor complex (Taelman *et al.,* 2010; Vinyoles *et al.,* 2014). In another model, the destruction complex remains intact and becomes saturated with phosphorylated β-catenin, while downstream ubiquitination is inhibited (Li *et al.,* 2012; Gerlach *et al.,* 2014). Notwithstanding the mechanism, the undisputed outcome of Wnt signalling is the stabilization of β-catenin and its translocation to the nucleus to associate with DNA-bound T-cell factor/Lef proteins and co-activate Wnt target gene transcription (Behrens *et al.,* 1996; Molenaar *et al.,* 1996).

Thus, the β-catenin destruction complex provides a critical regulatory node in the Wnt cascade. Not surprisingly, mutational inactivation of key destruction complex components is a frequent occurrence in cancer and, as a consequence, provides a highly attractive target for pharmacological intervention (Polakis, 2012; Zhan *et al.,* 2017). Below, we discuss recent insights in the molecular working mechanisms of the β-catenin destruction complex in healthy and cancer cells, focusing on the role of inter- and intramolecular interactions, post-translational modifications as well as newly emerging targeting strategies.

CK1 and GSK3 kinase activity initiate β-catenin destruction

The central activity of the destruction complex is executed by the axin-bound kinases CK1 and GSK3 (Ikeda *et al.,* 1998; Liu *et al.,* 2002; Xue *et al.,* 2013). CK1 first phosphorylates the flexible β-catenin N-terminus at Ser^{45}, which primes it for GSK3 phosphorylation at Thr^{41}, followed by Ser^{37} and Ser^{33} (Amit *et al.,* 2002; Liu *et al.,* 2002). The phosphorylation motif generated by Ser^{37} and Ser^{33} ultimately mediates the recognition, ubiquitination and proteasomal degradation by β-TrCP (Orford *et al.,* 1997; Wu *et al.,* 2003). In addition, both kinases phosphorylate other components of the destruction complex, including axin and APC. These modifications are key in the regulation of protein and complex function and will be discussed later in this review.

Mammalian cells express different CK1 isoforms, classified as **CK1α**, **CK1δ**, **CK1ε** and **CK1γ**. The α, δ and ε isoforms reside in the cytosol, while the γ isoform is membrane-tethered *via* C-terminal lipidation (Amit *et al.,* 2002; Davidson *et al.,* 2005). CK1α is the shortest variant, merely consisting of the catalytic kinase domain. Both the δ and ε isoforms carry an extended C-terminus that can be auto-phosphorylated, leading to auto-inhibition of catalytic activity (Cegielska *et al.,* 1998; Graves *et al.,* 1993). All three cytosolic isoforms are detected in association with axin, and phosphorylation of Ser^{45} in β-catenin *in vitro* was confirmed for CK1δ (Amit *et al.,* 2002). RNAi experiments in mammalian cells as well as *Drosophila* however suggested that CK1α is the primary kinase responsible for β-catenin Ser^{45} phosphorylation in living cells (Liu *et al.,* 2002). Besides their role in Wnt/β-catenin signalling, CK1 kinases are involved in various cellular processes, including membrane transport, cytoskeleton maintenance, DNA repair and nuclear localization (Cruciat, 2014).

In mammalian cells, two different genes encode for GSK3 isoforms **GSK3α** and **GSK3β** (Woodgett, 1990). While GSK3α displays a more extended N-terminus as compared with GSK3β, both kinases appear to function redundantly in the destruction complex (Doble *et al.,* 2007) and thus will be termed GSK3 throughout this review. Of note, only a small fraction (5–10%) of the total cytosolic pool of GSK3 is bound to axin and dedicated to β-catenin destruction (Lee *et al.,* 2003; Ng *et al.,* 2009; Kaidanovich-Beilin and Woodgett, 2011). Such compartmentalization of kinase activity allows GSK3 to control many cellular activities, including glycogen biosynthesis, microtubule stability, cell-cycle control and the regulation of inflammatory pathways (Ding *et al.,* 2000; Frame and Cohen, 2001).

Axin is the primary coordinator of destruction complex activity

Axin brings together all core components of the destruction complex and is thus regarded as its main organizer. Axin carries folded, structured domains at both its termini that are interconnected by a large, intrinsically disordered central region (Spink *et al.,* 2000; Noutsou *et al.,* 2011) (Figure 2A). The N-terminal axin regulators of G-protein signalling (RGS) domain displays homology to the RGS protein family and provides a primary binding site for APC, the second scaffold of the destruction complex (Zeng *et al.,* 1997; Behrens *et al.,* 1998; Kishida *et al.,* 1998; Spink *et al.,* 2000). Details of the axin–APC interaction are discussed below.

The C-terminally located DIX domain of axin (or DAX for DIX-of-axin) exhibits an ubiquitin-like fold and can self-polymerize in a head-to-tail manner, nucleating the formation of DIX domain filaments *in vitro* that merge into higher-order fibres (Fagotto *et al.,* 1999; Kishida *et al.,* 1999; Schwarz-Romond *et al.,* 2007a; Fiedler *et al.,* 2011). In cells, axin DIX-mediated self-interactions drive the assembly of highly dynamic, spherically shaped cytosolic puncta (Fagotto *et al.,* 1999; Schwarz-Romond *et al.,* 2007a; Fiedler *et al.,* 2011). While these studies generally rely on axin overexpression, endogenous axin puncta can be observed in conditions where its degradation is inhibited, indicating concentration-dependent effects (de la Roche *et al.,* 2014). The high local concentrations of axin in these puncta are deemed to mediate enhanced avidity for low-affinity binding partners, promoting assembly of the β-catenin destruction complex (Schwarz-Romond *et al.,* 2007a; Bienz, 2014). As DIX–DIX interactions are relatively weak (mid-micromolar range), efficient axin multimerization probably depends on additional intermolecular interactions with partner proteins, such as APC (Lee *et al.,* 2003; Pronobis *et al.,* 2015). Besides self-polymerization, the DIX domain can also mediate heterotypic interactions with the DIX-containing proteins Dvl and Ccd1 (Kishida *et al.,* 1999; Julius *et al.,* 2000; Shiomi *et al.,* 2003; Liu *et al.,* 2011). These Wnt-induced interactions

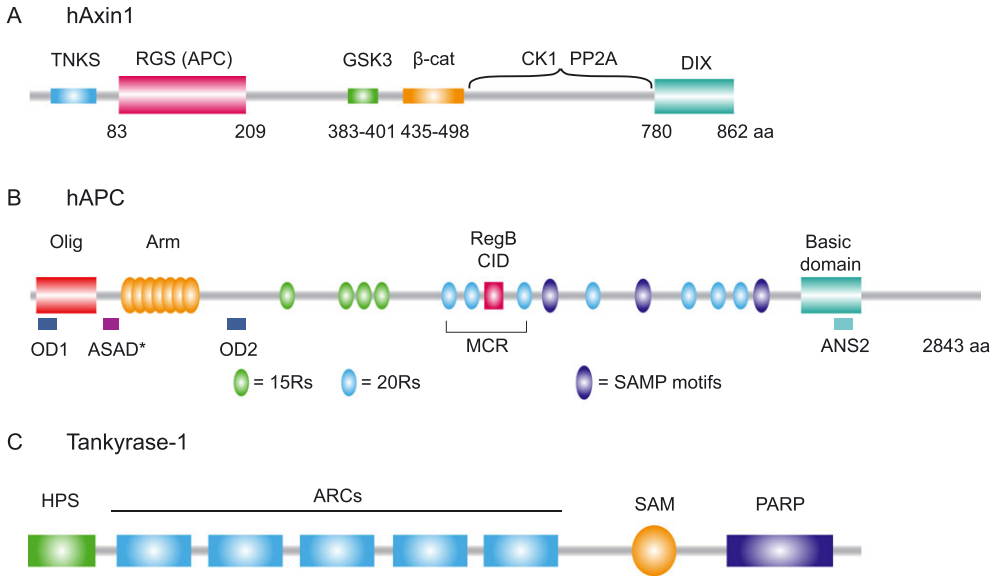

Figure 2

Structural organization of axin, APC and tankyrase (TNKS). (A) Human axin carries two structured domains, indicated as the N-terminal RGS domain and C-terminal DIX domain. The N-terminal region contains a TNKS binding motif. The central intrinsically disordered region of axin contains binding motifs for GSK3, β-catenin (β-cat), CK1 and PP2A. (B) Human APC contains multiple domains including the oligomerization domain (Olig, red), Armadillo repeat domain (Arm, yellow), Region B (RegB, pink) or CID and the basic domain (aqua blue). The β-catenin binding 15-mer repeats (15Rs; green) and 20-mer repeats (20Rs; blue) and axin binding SAMP motifs (purple) are indicated. Cancer mutations in APC frequently truncate the APC protein in the mutational cluster region (MCR). Self-oligomerization of APC is facilitated by N-terminal OD1 and OD2 and C-terminal ANS2. *Drosophila* APC can self-polymerize *via* the ASAD domain that shows sequence conservation in human APC, shown here as ASAD*. (C) Human TNKS1 contains five Ankyrin repeat clusters (ARCs; blue), a polymerization domain (SAM, yellow) and a C-terminal catalytic PARP (purple) domain. The N-terminus contains an HPS domain (green), a homopolymeric run of histidine, proline and serine of which the function is unknown.

interfere with destruction complex activity, thereby regulating pathway activation.

The intrinsically disordered central region of axin harbours short linear binding segments for the kinases CK1α, GSK3, their substrate β-catenin as well as protein phosphatase 2A (PP2A) and protein phosphatase 1 (PP1) (Figure 2A) (discussed below) (Ikeda *et al.,* 1998, 2000; Hsu *et al.,* 1999; Yamamoto *et al.,* 1999, 2001; Rubinfeld *et al.,* 2001; Liu *et al.,* 2002; Luo *et al.,* 2007). By bringing the kinases and their substrate in close proximity, axin strongly accelerates their chemical interactions (Ikeda *et al.,* 1998; Kikuchi, 1999; Rubinfeld *et al.,* 2001; Liu *et al.,* 2002; Dajani *et al.,* 2003; Ha *et al.,* 2004; Noutsou *et al.,* 2011; Xue *et al.,* 2013). Crystal structures of axin–β-catenin and axin–GSK3 complexes show that the disordered axin segments involved turn into helices upon binding (Dajani *et al.,* 2003; Xing *et al.,* 2003). Moreover, the GSK3 catalytic domain and flexible N-terminus of β-catenin remain available for enzyme–substrate interactions in the bound state. The structure of the CK1α–axin complex has not yet been resolved, probably due to the fact that CK1α interacts with two well-separated regions in the disordered axin central domain (Zhang *et al.,* 2002; Sobrado *et al.,* 2005). The interaction mode is predicted to involve loop formation of axin segments, which might further enhance colocalization of proteins in the complex (Xue *et al.,* 2013).

Thus, axin coordinates the assembly of a multiprotein complex that brings APC, CK1, GSK3 and β-catenin in close proximity to facilitate the capturing, phosphorylation and subsequent degradation of β-catenin. Notably, axin variants in which individual binding domains for APC, GSK3 or β-catenin are deleted retained a significant level of tumour suppressor activity when tested for their ability to rescue *Drosophila* axin null mutations *in vivo* (Oosterveen *et al.,* 2007; Peterson-Nedry *et al.,* 2008). Moreover, while a double deletion of the RGS- and β-catenin binding domains was deleterious, heteroallelic coexpression of the individual deletion mutants showed functional complementation (Peterson-Nedry *et al.,* 2008). These findings support a model in which multiple direct and indirect interactions between components redundantly cooperate to enhance robustness of the destruction complex. These redundancy features of the axin complex critically depend on interactions with APC (Pronobis *et al.,* 2017).

Axin2/conductin-mediated feedback promotes β-catenin destruction in Wnt-stimulated cells

Both vertebrate and nematode genomes carry an axin homologous gene, called axin2 or conductin. Both axin and axin2 proteins share key sequence elements, show similar structural organization and are functionally related (Behrens *et al.,* 1998; Fagotto *et al.,* 1999; Chia and Costantini, 2005). However, axin is constitutively expressed, while axin2 is a direct Wnt target gene that is up-regulated after pathway activation (Jho *et al.,* 2002; Lustig *et al.,* 2002). These findings have

implicated axin2 as an important negative feedback regulator of Wnt signalling, by increasing cellular destruction complex concentrations. Interestingly, the activity of axin and axin2 might not be fully redundant, since overexpression of axin2 was unable to compensate for knockdown of axin in skeletal muscle satellite cells (Figeac and Zammit, 2015). Moreover, Wnt pathway activation only seems to drive a modest increase of axin2 levels relative to axin, suggesting that quantitative expression differences do not explain its feedback role. Instead, the interaction of axin2 with Dvl is markedly reduced as compared with axin and, consequently, its role in β-catenin degradation is relatively insensitive to Dvl-mediated interference (Bernkopf *et al.,* 2015). Thus, this diminished sensitivity of axin2 for inhibition by upstream signalling provides an elegant explanation for the effective restoration of destruction complex activity by axin2 (Bernkopf *et al.,* 2015). The importance of axin2-mediated feedback is further illustrated by the clear association between axin2 germline variants with increased cancer risk (Liu *et al.,* 2014; Aristizabal-Pachon *et al.,* 2015; Rosales-Reynoso *et al.,* 2016; Bahl *et al.,* 2017) and the occurrence of somatic axin2 frameshift mutations in various types of cancer (Mazzoni and Fearon, 2014; Li *et al.,* 2015).

Essential role of APC in the destruction complex

The second critical scaffold for destruction complex activity is the large 310 kD protein APC. Mammals carry two *APC* genes, named *APC* (2843 aa) and the slightly shortened *APC2* (2303 aa). The APC N-terminus contains an oligomerization domain and an armadillo repeat (Arm) domain (Figure 2B). The Arm domain binds a number of cytoskeletal regulators that have not been linked to β-catenin destruction, as well as B56, an essential regulator subunit of PP2A (Seeling *et al.,* 1999; Kawasaki *et al.,* 2000; Jimbo *et al.,* 2002; Watanabe *et al.,* 2004; Breitman *et al.,* 2008). The remainder of the protein, spanning the entire region between the Arm domain and the C-terminus, is predicted to be unstructured (Li and Nathke, 2005; Liu *et al.,* 2006; Minde *et al.,* 2013). This region of APC harbours a number of short axin and β-catenin binding motifs as well as regulatory regions essential for β-catenin proteolysis, as discussed below. At its very C-terminus, APC carries a basic domain that promotes actin assembly (Okada *et al.,* 2010) and a microtubule interaction region, both of which are dispensable for β-catenin degradation (Smits *et al.,* 1999; McCartney and Nathke, 2008; Pronobis *et al.,* 2017). Overall, regulatory interactions of APC with the cytoskeleton are thought to mediate alternative roles of APC in spindle formation, kinetochore attachment, microtubule stability as well as the regulation of cell motility and polarity (Nathke, 2006; Okada *et al.,* 2010).

The multiple independent β-catenin binding motifs in the APC unstructured central region comprise four homologous 15 amino acid repeats (15Rs) and seven 20 amino acid repeats (20Rs) (Rubinfeld *et al.,* 1997a; Eklof Spink *et al.,* 2001). Three short Ser-Ala-Met-Pro (SAMP)-containing repeats are located, interspersed between the third 20R motif and basic domain, which mediate the interaction with axin (Behrens *et al.,* 1998; Spink *et al.,* 2000). The affinity of the 20Rs for β-catenin is strongly enhanced (about 300-fold) by CK1- and GSK3-mediated phosphorylation (Rubinfeld *et al.,* 1996; Ha *et al.,* 2004; Liu *et al.,* 2006). Presumably, phosphorylation occurs within the destruction complex when APC is brought in close proximity to axin-bound kinases (Figure 3C) (Ikeda *et al.,* 2000; Rubinfeld *et al.,* 2001). Notably, the β-catenin binding surface of phosphorylated 20R overlaps with that of axin, indicative of a competitive interaction (Xing *et al.,* 2003; 2004; Ha *et al.,* 2004). These data led to a cyclic model in which phosphorylated β-catenin is transferred from axin to high affinity phosphorylated 20Rs in APC, after which APC facilitates the delivery of β-catenin to the E3 ligase β-TrCP (Figure 3C–E). In this model, PP2A-mediated dephosphorylation of APC resets the system for binding and processing a new β-catenin (Kimelman and Xu, 2006; Xu and Kimelman, 2007). This attractive model however requires precise timing of APC phosphorylation and dephosphorylation, which is considered an unlikely feature due to the random collisions that mediate interactions between unstructured protein segments in the complex, as discussed previously (Stamos and Weis, 2013; Xue *et al.,* 2013). In an alternative model, high and low affinity binding sites on APC offer a wide dynamic range for efficient sequestration of β-catenin in the Wnt off (low β-catenin) and Wnt on (high β-catenin) state (Figure 3) (Ha *et al.,* 2004). However, in this model the question of when and how β-catenin is transferred from APC to axin to undergo phosphorylation remains unexplained, leaving room for future investigation.

Another functionally important APC region comprises the 20R repeat 2 (20R2), which does not interact with β-catenin (Liu *et al.,* 2006; Kohler *et al.,* 2008), and an adjacent conserved sequence called the 'catenin inhibitory domain' (CID) or region B (Figure 2B) (Kohler *et al.,* 2009). Based on results obtained with various truncated APC fragments, the 20R2-CID region was determined to be essential for β-catenin ubiquitination, independent of β-catenin binding activity (Kohler *et al.,* 2009; Roberts *et al.,* 2011). Mechanistically, the 20R2-CID region was proposed to mediate the association with β-TrCP, protect β-catenin from PP2A-mediated dephosphorylation and modulate the interaction of axin and APC, as discussed below (Su *et al.,* 2008; Pronobis *et al.,* 2015) (Figure 3D). The nature of the underlying protein–protein interactions required for these 20R2-CID-mediated activities remains unclear, but might involve a functional interaction of the CID domain with α-catenin, as proposed recently (Choi *et al.,* 2013).

Self-polymerization of human APC is mediated *via* its N-terminal oligomerization domain (OD) 1, OD2 and C-terminal ANS2 domains, but these interactions are not deemed relevant for Wnt pathway regulation (Figure 2B) (Li *et al.,* 2008; Okada *et al.,* 2010). In contrast, a recent study described an N-terminal coil in *Drosophila* APC2, called the APC-self-associating domain (ASAD), that increased the size of cytosolic axin–APC puncta and promoted destruction complex efficiency in both *Drosophila* S2 and SW480 cells (Kunttas-Tatli *et al.,* 2014). While the predicted coil structure of the ASAD domain appears conserved in all Bilateria APC

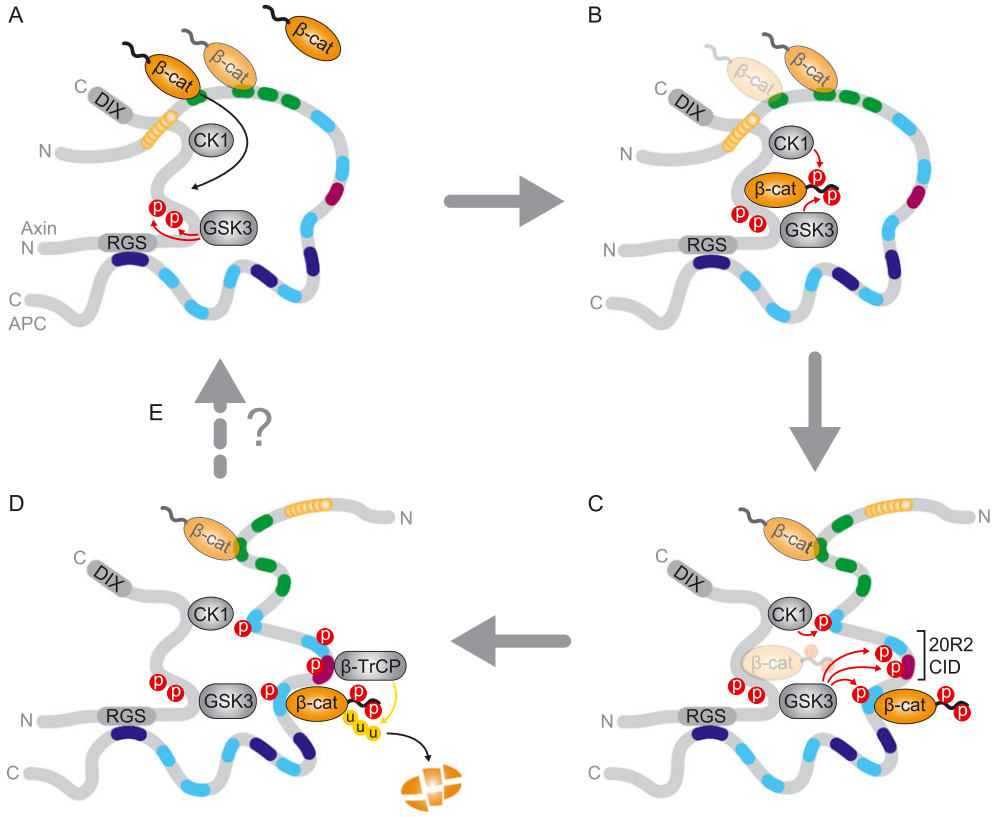

Figure 3

Schematic summary of destruction complex assembly and the molecular steps involved in β-cat degradation. For APC, Arm domain (yellow), CID domain (pink), 15R (green), 20R (light blue) and SAMP repeats (dark blue) are indicated. (A) Interactions between axin and APC are stabilized *via* multiple binding sites as well as *via* self-oligomerization (not shown for clarity). Due to redundancy in β-cat binding sites, β-cat substrate might enter the complex either *via* APC or axin binding. The initial capturing of free β-cat from the cytosol by β-cat binding motifs in APC (15R repeats, green) is shown. Axin-bound kinases induce phosphorylate the axin central region to keep the protein in an open conformation that allows for efficient β-cat binding and processing. (B) β-catenin captured by non-phosphorylated, low-affinity binding sites in APC is transferred to axin, followed by CK1- and GSK3-mediated phosphorylation. (C) Phosphorylation of APC 20R repeats in the complex creates high affinity β-cat binding sites that enables phosphorylated β-cat to transfer from axin to APC. Phosphorylation of the 20R2-CID region induces a rearrangement in the complex that leads to the release of the APC Arm repeats from axin. (D) APC shields β-cat from phosphatases and presents phosphorylated β-cat to β-TrCP, followed by ubiquitination and proteasomal degradation. (E) After handing over β-cat for proteasomal degradation the destruction complex might be recycled for another round of β-cat destruction. This step possibly involves dephosphorylation by destruction complex-associated phosphatases.

proteins (Figure 2B) (Kunttas-Tatli *et al.*, 2014), its role in mammalian Wnt pathway regulation remains to be established.

The axin–APC interaction is highly dynamic

Key interactions between axin and APC are mediated *via* binding of axin RGS to the SAMP repeat motifs of APC (Figure 3A) (Behrens *et al.*, 1998; Kishida *et al.*, 1998; Spink *et al.*, 2000). However, the role of SAMPs in regulating destruction complex activity might be more complex than initially anticipated as individual SAMP repeats display differential axin binding affinities and are strongly regulated by phosphorylation (Kunttas-Tatli *et al.*, 2015). These findings suggest that the SAMP repeats possibly mediate functionally distinct yet cooperative roles. Notwithstanding the mechanism, the importance of the SAMP repeat region for β-catenin proteolysis is evidently shown by APC cancer truncations that have lost all SAMPs and exhibit strong oncogenic effects (Smits *et al.*, 1999; Kohler *et al.*, 2009; Roberts *et al.*, 2011). Surprisingly, however, a *Drosophila* APC2 variant lacking all SAMPs displayed residual APC–axin binding activity, revealing the existence of alternative interaction sites (Roberts *et al.*, 2011). Indeed, Peifer and colleagues uncovered a second interaction mode in which the Arm domain of *Drosophila* APC2 binds the central region of axin (Figure 3A) (Pronobis *et al.*, 2015). This interaction is highly dynamic and regulated by GSK3-mediated APC phosphorylation of the 20R2-CID region. In the proposed model, APC2 employs multiple interactions with axin to promote multimerization, thereby increasing the size and stability of the destruction complex. In subsequent steps, GSK3 phosphorylates β-catenin as well

as parts of APC, including the 20R2-CID region. Next, phosphorylated 20R2-CID induces the release of the weak interaction of APC–Arm with axin, opening up the complex and allowing the transfer of phospho-β-catenin to β-TrCP (Figure 3C) (Pronobis *et al.,* 2015). This model introduces a number of novel regulatory steps and provides an attractive explanation for misregulation by APC cancer truncations by hypophosphorylation or loss of the 20R2-CID region. However, substantial validation will be required to explain the proposed phosphorylation-induced rearrangements in APC–axin interactions within the complex as well as the consequences for interactions with the ubiquitin machinery.

Due to the presence of multiple binding sites for a single partner as well as overlap in self-oligomerization capacity, axin and APC appear to partially share redundant functions inside the destruction complex. To identify the essential parts of both scaffold proteins, a recent study compiled a minimal destruction complex by using only five essential regions of axin and APC (Pronobis *et al.,* 2017). For APC, these regions included the self-associating ASAD domain, Arm repeats and the 20R2-CID region. These APC regions were coupled to the axin C-terminus containing the β-catenin binding domain and DIX domain. The artificial scaffold protein formed cytosolic puncta and allowed full restoration of β-catenin destruction in APC-mutant SW480 cells (Pronobis *et al.,* 2017). While these results are highly informative, it should be noted that these experiments relied on overexpression and were performed in the presence of endogenous wild-type axin as well as truncated APC, both of which could contribute to the formation and activity of the destruction complex. One related and unresolved issue concerns the question of how kinases are recruited to this artificial complex. If and how the minimal complex is susceptible to inhibition by Wnt signals also remains a matter for future investigation.

Regulation by dephosphorylation

PP2A and PP1 both associate with the destruction complex, suggesting they affect a balanced regulation *via* phosphorylation and dephosphorylation, Their precise modes of interaction and functional roles however remain to be clarified (Hsu *et al.,* 1999; Seeling *et al.,* 1999; Ratcliffe *et al.,* 2000; Yamamoto *et al.,* 2001; Luo *et al.,* 2007). PP1 interacts with axin (Luo *et al.,* 2007), while PP2A was reported to bind both axin and APC (Hsu *et al.,* 1999; Seeling *et al.,* 1999; Yamamoto *et al.,* 2001). Of note, PP2A binding to axin might be indirect, involving the heat shock protein 70 (HSP70) family member HSP105 (Yu *et al.,* 2015). PP2A is composed of a core catalytic subunit (PPP2CA), a structural subunit (PR65/A) and variable regulatory B subunits (Janssens and Goris, 2001). A consistent finding across numerous studies is that PP2A dephosphorylates β-catenin to prevent its ubiquitination and support the Wnt activation pathway (Su *et al.,* 2008; Zhang *et al.,* 2009; Yu *et al.,* 2015). In the first model, PP2A-mediated dephosphorylation of the APC 20R region was proposed to mark the end of a destruction complex cycle, allowing phosphorylated β-catenin to leave the complex and so initiate a new round of β-catenin modifications (Xu and Kimelman, 2007). However, Weis and colleagues were unable to dephosphorylate the APC 20R region when bound to β-catenin using the catalytic domain of PP1 in an *in vitro* setting (Ha *et al.,* 2004). Besides, this assumption is also in conflict with the recently proposed role of the 20R2-CID region, for which phosphorylation appears to be required to release axin and transfer β-catenin to β-TrCP (Pronobis *et al.,* 2015). The precise role of heterotrimeric PP2A inside the destruction complex thus awaits further experimental validation.

The phosphatase PP1 was reported to promote Wnt signalling *via* intramolecular autoinhibition of axin (Kim *et al.,* 2013). In the suggested model, destruction complex activity strongly depends on the phosphorylation status of axin. In the absence of Wnt, GSK3 phosphorylates axin at Ser^{497} and Ser^{500}, which retains the protein in an active, 'open' state that allows for β-catenin binding and processing. Wnt-mediated receptor activation leads to the recruitment of axin (Mao *et al.,* 2001; Cliffe *et al.,* 2003; Tolwinski *et al.,* 2003; Tamai *et al.,* 2004; Zeng *et al.,* 2005; Bilic *et al.,* 2007; Schwarz-Romond *et al.,* 2007b; MacDonald *et al.,* 2008; Fiedler *et al.,* 2011) and subsequent inhibition of GSK3 *via* pseudosubstrate interactions with the phosphorylated LRP6 cytosolic tail (Cselenyi *et al.,* 2008; Piao *et al.,* 2008; Wu *et al.,* 2009; Kim *et al.,* 2013; Stamos *et al.,* 2014). These steps initiate PP1-dependent axin dephosphorylation, after which the scaffold undergoes a conformational switch. Mechanistically, dephosphorylation of axin promotes an intramolecular interaction between the β-catenin binding domain and the DIX domain, inducing the protein to adopt an inactive, 'closed' conformation and its release into the cytosol (Kim *et al.,* 2013). The resulting drop in destruction complex activity allows the stabilization of β-catenin and pathway activation. When intracellular levels of β-catenin rise above a critical concentration, β-catenin binding might compete with the autoinhibitory interaction, restoring assembly of the axin-based destruction complex to avoid excessive accumulation. Together, these findings highlight the critical importance of axin phosphorylation in the regulation of β-catenin turnover. Further studies are needed to elucidate the contribution of other axin phosphorylation sites, shed light on the structural details of different axin conformational states and analyse the consequences for assembly with other binding partners, including APC. Moreover, the question of how the different axin conformational changes depend on axin multimerization deserves further investigation.

Regulation by poly-ADP-ribosylation

Over recent years, a major regulatory pathway has emerged that potentiates cellular responses to Wnt *via* poly-ADP-ribosylation (PARylation)-mediated destabilization of axin. The enzymes responsible are tankyrase (TNKS) 1 and 2, members of the PARP family (Smith *et al.,* 1998). TNKS binds the axin N-terminus *via* its large ankyrin repeat cluster (ARC) domain after which the C-terminal PARP domain catalyses the modification of axin by poly-ADP-ribose chains (Huang *et al.,* 2009) (Figure 2C). Next, PARylated axin is recognized and ubiquitinated by the E3 ligase RNF146, which targets axin for proteasomal degradation (Callow *et al.,* 2011; Zhang

et al., 2011). Decreased axin levels presumably compromise the activity of the destruction complex, leading to enhanced activation of the Wnt pathway. Thus, TNKS 1 and 2 were identified as positive regulators of the Wnt signalling pathway.

Structural approaches revealed molecular requirements for TNKS-mediated regulation of Wnt signalling. The axin–TNKS crystal structure divulged two TNKS-binding motifs in axin, each of which binds to a different ARC domain within the TNKS protein (Figure 2A, C) (Morrone *et al.,* 2012). Notably, the sequence of the second binding motif is considerably different from the agreed TNKS-binding sequence, and TNKS binding to this region was not detected by standard biochemical protein interaction methods, possibly due to a weaker affinity (Croy *et al.,* 2016). Furthermore, despite the presence of five ARC repeats, the structural properties of these domains limit the interactions with axin only to specific ARC combinations within one TNKS molecule (Eisemann *et al.,* 2016). The overall multiplicity of intermolecular binding sites as well as the polymerizing properties of the TNKS SAM domain promote assembly of higher order complexes that allow for efficient targeting of axin for ADP-ribosylation and degradation (Figure 2C) (Mariotti *et al.,* 2016).

Interestingly, an additional role of TNKS-mediated PARylation of axin in promoting Wnt pathway activation was recently reported. In this study, Wnt stimulation resulted in a rapid increase in the pool of PARylated axin in both *Drosophila* and human cells (Yang *et al.,* 2016). Mechanistically, PARylation induced the recruitment of axin to the plasma membrane *via* an enhanced interaction with phosphorylated LRP6, thereby promoting Wnt signalling (Yang *et al.,* 2016). These findings raise a number of important questions that deserve further investigation, including how Wnt signals alter TNKS activity towards axin, which protein domains promote the interaction of PARylated axin with LRP6 and what are the molecular consequences for signalosome assembly.

Impact of cancer mutations on destruction complex activity

Mutational inactivation of destruction complex activity is a prevalent occurrence in cancer. The most prominent example involves mutations in *APC* that are found in 80–90% of both inherited and sporadic colorectal cancers (CRC) (Clements *et al.,* 2003; Polakis, 2007; Kandoth *et al.,* 2013; Brannon *et al.,* 2014). Loss of function of both alleles induces inappropriate activation of β-catenin-mediated transcription in individual cells, leading to the growth of adenomas or polyps (Polakis, 2007; Polakis, 2012). Additional mutations in genes like *KRAS*, *TP53* and *SMAD4* are required subsequently to induce these polyps to progress toward malignancy (Kinzler and Vogelstein, 1996; Conlin *et al.,* 2005; Drost *et al.,* 2015; Matano *et al.,* 2015; Fumagalli *et al.,* 2017; Melo *et al.,* 2017). Unlike other tumour suppressors, APC mutants CRCs do not carry homozygous null mutations but usually keep at least one allele encoding a truncated APC protein. Truncations are generated through frameshift mutations that occur in the so-called mutational cluster region, generating shortened APC proteins that preserve the Arm domain and some of the 20Rs while lacking all of the SAMP repeats (Figure 2B) (Beroud and Soussi, 1996; Kohler *et al.,* 2008). Notably, truncated APC cancer variants retain a residual ability to target β-catenin for degradation (Albuquerque *et al.,* 2002; McCartney *et al.,* 2006; Gaspar *et al.,* 2009; Voloshanenko *et al.,* 2013). These findings have led to the 'just-right' hypothesis in which low levels of destruction activity are retained by tumour cells to prevent apoptosis induced by excessive β-catenin-mediated signalling (Albuquerque *et al.,* 2002). Mechanistically, the weak suppressor activity of truncated APC might be mediated *via* cytoplasmic retention of β-catenin (Roberts *et al.,* 2011), weak interactions with axin through the recently described interactions between APC Arm repeats and the axin central domain as well as the residual ubiquitination-promoting activity of the 20R2-CID region (Voloshanenko *et al.,* 2013; Pronobis *et al.,* 2015). Strikingly, the invasive growth of malignant APC depleted, *KRAS* and *TP53* mutant CRC cells could be reversed by restoring the expression of APC, which triggered differentiation and re-establishment of tissue homeostasis (Dow *et al.,* 2015). These findings provide strong support for the continuation of the intense search for Wnt pathway inhibitors as potential therapeutics for CRC.

Another well-known class of mutations leading to uncontrolled Wnt pathway activity comprise activating mutations in β-catenin. These mutations occur in about 5% of CRC patients and are mutually exclusive with APC mutations (Luchtenborg *et al.,* 2005; Thorstensen *et al.,* 2005) [cBioportal.org (Cerami *et al.,* 2012; Gao *et al.,* 2013)]. Moreover, in contrast to APC mutations, β-catenin mutations are found in many other types of cancer types, including hepatocellular carcinoma, endometrioid ovarian cancer and medulloblastoma (Rubinfeld *et al.,* 1997b; Bell, 2005; Polakis, 2007). Oncogenic β-catenin mutations predominantly hit the phosphorylation sites in the flexible N-terminus, masking recognition sites for destruction complex-mediated phosphorylation, thereby preventing β-catenin proteolysis.

Mutations in *AXIN1* also associate with a diverse set of human tumours, including hepatocellular carcinoma, medulloblastoma and colorectal carcinoma (Salahshor and Woodgett, 2005). Missense mutations are prevalent within the *AXIN1* mutational spectrum, but mechanistic information on associated tumourigenic roles is largely lacking. Recently, missense mutations in the axin N-terminal RGS domain were shown to disrupt Wnt signalling and promote tumour growth *in vivo* by an unprecedented molecular mechanism (Anvarian *et al.,* 2016). Relevant point mutations in cancer destabilized the structure of the axin RGS domain, driving the formation of soluble, small-sized axin oligomers. Non-aggregating unstructured regions of axin were found to protrude from the oligomer as 'molecular tentacles' that aberrantly engage key regulators. Collectively, the altered interactions of the mutant axin rewired its signalling network to activate β-catenin-mediated transcription. Of note, blocking aggregation partially restored the tumour suppressor activity of the mutant protein, providing a potential new avenue in the search for Wnt pathway inhibitors.

Targeting the destruction complex in cancer

Due to its key role in the regulation of β-catenin activity, the destruction complex provides an attractive target for therapeutic manipulation. Over recent years, a number of small molecules were identified that enhance the activity of the destruction complex, and have potential as anti-cancer drugs.

Inhibitors of TNKS are a major class of novel destruction complex regulators that were first discovered in 2009 to potently inhibit Wnt/β-catenin signalling in APC-mutant cancer cells (Huang *et al.,* 2009). The small molecule XAV939 has been found to bind and inhibit the catalytic activity of TNKS, leading to stabilization of axin and the subsequent down-regulation of β-catenin levels (Karlberg *et al.,* 2010; Kirby *et al.,* 2012). Following this initial finding, numerous studies have applied alternative screening approaches, which have led to the identification of additional TNKS inhibitors with distinct structural properties, further highlighting the potential of this therapeutic approach. Notably, cells treated with the TNKS inhibitors XAV939 and G007-LK display a rapid induction of enlarged cytosolic puncta called degradasomes, to which all components of the endogenous β-catenin complex are recruited, including phosphorylated β-catenin and β-TrCP (Thorvaldsen *et al.,* 2015). Subsequent studies revealed that treatment with TNKS inhibitors actually promotes and stabilizes TNKS–axin interactions, further boosting oligomerization and the assembly of functional destruction complexes (Martino-Echarri *et al.,* 2016). Furthermore, close examination revealed that treatment of SW480 CRC cells with a TNKS inhibitor strongly and selectively increases the levels of axin2, indicating that degradasome formation largely depends on axin2 stabilization in these cells (Thorvaldsen *et al.,* 2017). Of note, SW480 cells are APC mutant and display constitutive Wnt pathway activation, leading to permanent expression of target genes including axin2. In line with these findings, a recent study demonstrated that CRC cancer cells with short truncated APC variants lacking all seven 20Rs were dependent on high β-catenin levels and responded best to TNKS inhibitors. These results suggest that short APC truncations might provide a biomarker for TNKS inhibitor sensitivity (Tanaka *et al.,* 2017). Despite these promising results, prolonged Wnt stimulation may render cells unresponsive to treatment with TNKS inhibitors and thus potentially put constraints on their use in clinical applications (de la Roche *et al.,* 2014).

Another class of compounds reported to regulate destruction complex activity targets alterations in kinase activity. One example is pyrvinium, a small molecule that binds and activates the kinase CK1α, thus promoting β-catenin phosphorylation and proteolysis (Thorne *et al.,* 2010). In subsequent work, pyrvinium was reported to inhibit the proliferation of lung cancer cells *in vitro* at a dose below 10 nM (Zhang *et al.,* 2015). Another small molecule, Wnt inhibitor is KYA1797 that was shown to bind directly to the axin RGS domain. Through its activation of GSK3, it promotes destruction complex-mediated β-catenin phosphorylation and degradation (Cha *et al.,* 2016). However, further investigations are required to determine the suitability of these inhibitors as future anti-cancer drugs.

Concluding remarks

Even though it is now 22 years since its discovery, a unifying theory of the inner workings of the β-catenin destruction complex has not been accomplished. Emerging evidence shows that numerous molecular activities are shared between axin and APC, securing the robustness and adaptability of destruction complex activity under different cellular conditions. Although it is clear that both scaffolds co-operate, the exact role of APC remains poorly defined. Progress is expected to come from high resolution structural information on the intra- and intermolecular interactions at the core of the complex, although flexible protein segments and dynamic interactions complicate this endeavour. Recent studies have also emphasized the importance of post-translational modifications in the regulation of destruction complex activity. A precise balance between phosphorylation, ADP-ribosylation, ubiquitination and other possible modifications presumably regulate intra- and intermolecular interactions within the complex. An increased understanding of the timing and order in which these modifications take place will be important to resolve outstanding mechanistic issues. Finally, current knowledge is largely based on studies in which components of the destruction complex are overexpressed, which alters the relative ratio of protein concentrations in the cell that are deemed important for precise pathway regulation. Novel technologies such as CRISPR/Cas9 genome editing are likely to provide the appropriate tools to modify and analyse destruction complex components at their endogenous levels in the cell. In addition, recently emerged organoid technologies provide a controllable environment where different cell types form and grow in organized structures similar to complex tissues (Clevers, 2016). Combining organoid culture with endogenous genome editing thus provides advanced test systems for concepts in Wnt pathway regulation as well as the evaluation of newly generated therapeutic compounds.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.,* 2016), and are permanently archived in the Concise Guide to PHAMRMACOLOGY 2015/16 (Alexander *et al.,* 2015a,b).

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Conflict of interest

The authors declare no conflicts of interest.

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