REVIEW

RUNNING HEAD: β-Catenin: A competitive interaction platform

The Double-Edged Nature of β-Catenin: From Multicellular Innovation to Cancer Vulnerability

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ABSTRACT

β-catenin embodies a fundamental paradox of multicellular life. The same molecular system that enabled the emergence of animal multicellularity by coupling cell–cell adhesion to gene regulation also creates a vulnerability that can drive cancer when misregulated. As a central regulator of cell physiology, β-catenin integrates cell–cell adhesion, mechanotransduction, and gene expression to coordinate tissue architecture with transcriptional programs controlling proliferation, differentiation, and homeostasis.

Phylogenomic analyses indicate that bona fide β-catenins form a metazoan-specific monophyletic clade derived from an ancestral armadillo-repeat scaffold. This conserved superhelical structure generates a single interaction groove that mediates mutually exclusive binding to E-cadherin, adenomatous polyposis coli (APC), and T-cell factor/lymphoid enhancer factor (TCF/LEF) transcription factors.

While this architecture enabled early metazoans to coordinate adhesion, signaling, and morphogenesis, it also introduced an intrinsic regulatory vulnerability. Mutations that disrupt β-catenin degradation stabilize the protein, uncoupling Wnt signaling from its normal regulatory constraints and driving persistent proliferative transcriptional programs. In parallel, emerging structural and biophysical studies reveal conformational plasticity and mechanosensitive properties that enable dynamic partitioning between adhesive and signaling pools.

Disruption of these regulatory layers promotes tumor progression, metastasis, immune evasion, and therapy resistance, positioning β-catenin as both a central oncogenic node and a challenging therapeutic target. In this review, we integrate evolutionary, structural, and mechanobiological perspectives to illustrate how β-catenin exemplifies the double-edged nature of biological innovation, an ancient protein that enabled multicellular organization yet whose dysregulation underlies fundamental mechanisms of human cancer.

Keywords: β-catenin; cancer; evolution; mechanotransduction; Wnt-signaling.

Introduction

The discovery of β-catenin represents a landmark convergence between developmental genetics and cell biology, as it was independently identified in studies of cell adhesion [1] and developmental genetics in Drosophila [2]. Initial biochemical studies identified a 102 kDa protein that consistently co-precipitated with E-cadherin. This discovery first identified β-catenin as a key cadherin associated protein, suggesting its integral role in the adhesion complex, essential for linking E-cadherin to the actin cytoskeleton and maintaining epithelial integrity [1,3]. In contemporary unrelated studies, genetic screens in the fruit fly Drosophila melanogaster identified a gene named Armadillo. Mutations in armadillo resulted in a striking embryonic phenotype [2,4] resembling that of mutants for Wingless (Wg), a key segment polarity gene involved in the embryonic development of the body axis. This genetic link suggested that Armadillo and Wingless could operate within the same developmental pathway [5] Epistasis analysis showed that the wingless mutation masks the effects of Armadillo loss, indicating that Armadillo acts downstream of the Wingless signal. Molecular and genetic studies further demonstrated that Armadillo indeed encodes a cytoplasmic protein whose stability is regulated by Wingless, allowing it to mediate the transcriptional response required for proper segment polarity [6].

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The connection between cell adhesion and signalling became clear in 1991, when Gumbiner's group purified and sequenced a 92-kDa E-cadherin–associated protein (β-catenin) from Xenopus laevis epithelial cells. In a pivotal finding, sequence analysis revealed about 70% identity with Drosophila Armadillo and 63% with mammalian plakoglobin, providing the first direct evidence that these proteins are evolutionary homologs [7,8].

Molecular Structure of β-catenin and its interacting partners

Structure and ARM domain as a competitive binding groove

The ability of β-catenin to perform its distinct tasks is fundamentally rooted in its unique protein structure. β-catenin presents a single primary interface for a wide array of binding partners, thus acting as a molecular competitive interaction platform (Figure 1). The β-catenin protein, consisting of 781 amino acid residues in humans, comprises three distinct regions: a flexible N-terminal domain (NTD), a flexible C-terminal domain (CTD), and a rigid central region (residues 141–664) composed of 12 tandem copies of a sequence motif known as the Armadillo (ARM) repeat (R1–12) (Figure 1A-B). Each ARM repeat forms a three-helix motif of 42 residues that assembles into a right-handed superhelix with a positively charged groove, providing a stable scaffold for binding partners[9]. The NTD and CTD have not been crystallised yet and they appear structurally flexible; however, recent structural modeling suggests that the NTD contains an α-helical element harboring a vinculin-binding site [10]. Adjacent to the final ARM repeat, near the CTD, lies a conserved alpha-helix known as Helix C, which plays a critical role in allosterically regulating partner access to the binding groove.

A critical consequence of β-catenin's architecture is that its single, highly conserved binding groove constitutes a site of intense molecular competition (Figure 1B). Biochemical and crystallographic studies have shown that many key binding partners contain highly similar binding motifs for β-catenin, leading them to compete for the same positively charged groove and preventing their simultaneous engagement. In particular, this exclusivity applies to key β-catenin interacting molecules: E-cadherin (the primary partner in adherens junctions), APC (the primary partner in the destruction complex), and TCF/LEF transcription factors (the primary partner associated to the Wnt signalling in the nucleus) [11]. These interactors bind to a core site that includes ARM repeats R3–R9, where they form salt bridges with two critical residues, Lys312 and Lys435. This electrostatic complementarity underlies the specificity of β-catenin interactions. Modifications of charged residues in the TCF/LEF and FOXO β-catenin-binding regions disrupt these interactions, highlighting the importance of negative charges in partner recognition [12]. Phosphorylation of these partners further increases their affinity for the binding groove [11], indicating that thermodynamic factors contribute to β-catenin partner selection. Other ARM repeats also contribute by reinforcing these interactions. This structural overlap, supported by co-immunoprecipitation assays in Neuro2A cells, renders these interactions mutually exclusive [13].

Conformational states and partner selectivity

This unique, contested binding interface requires tight regulation, as cellular outcomes depend on partner selection by β-catenin, emerging from the interplay between the thermodynamics of its biochemical interactions and its distinct structural conformations [11,14]. In its "open" conformation, the central ARM-repeat binding groove is fully accessible (R3-R9), enabling interactions with both cadherins at the membrane and TCF/LEF transcription factors in the nucleus, depending on subcellular context [9]. Alternatively, β-catenin can adopt a "closed" back-folded conformation in which Helix C from the CTD

folds onto the central groove. Biochemical evidence supports the idea that monomeric, back-folded β-catenin is preferentially transcriptionally active and engages TCF/ LEF [14,15].

It has been shown that, in addition to monomeric interactions, the formation of nuclear condensates is important for β-catenin transcriptional function and cell-type specificity [16]. Recent studies further indicate that these condensates are driven by multivalent intermolecular interactions involving β-catenin intrinsically disordered terminal regions and ARM repeats. Condensate formation is thought to be governed by homotypic intermolecular interactions between β-catenin IDR terminal regions and ARM repeats [12]. Disruption of these interactions using FOXO4- and TCF-derived peptides has been shown to impair condensate formation and transcriptional activity, supporting their functional relevance. At the plasma membrane, β-catenin forms a dimer with α-catenin that supports cadherin binding thereby channelling β-catenin toward adhesion rather than signalling. β-Catenin activity can also be fully suppressed by the inhibitor ICAT, which blocks its interaction with TCF without disrupting β-catenin/cadherin interactions [17–19].

Together, these conformational states (open, closed/back-folded, dimeric, and ICAT-bound) along with post-translational modifications, nuclear condensates and mutually exclusive partner interactions outline a sophisticated regulatory system that biases β-catenin toward adhesive roles at the membrane or transcriptional functions in the nucleus. Furthermore, recent studies point to the important role of the disordered terminus domains of β-catenin in the formation of new cell-cell adhesions via clustering of the cadherin-catenin complex [20].

The Canonical Wnt Pathway: A Toggle for Protein Stability

The interaction of β-catenin with the canonical Wnt signaling pathway is fundamental to metazoan developmental regulation and cell-fate determination. In the absence of Wnt ligands, cytosolic β-catenin is constitutively degraded by a complex composed of Axin, APC, GSK3β (glycogen synthase kinase 3 beta), and CK1α (casein kinase 1 alpha) [21]. This complex maintains extremely low cytoplasmic β-catenin levels and prevents ectopic transcriptional activation. Degradation begins with a precise sequence of phosphorylation events (Figure 1C): CK1α first primes β-catenin by phosphorylating S45, enabling GSK3β to sequentially phosphorylate T41, S37, and S33 [22]. Phosphorylation at Ser33 and Ser37 creates a recognition site for β-TrCP (beta-transducin repeat-containing protein), an E3 ubiquitin ligase that catalyzes β-catenin ubiquitination and subsequent proteasomal degradation [23].

In addition to phosphorylation and ubiquitination, β-catenin is subject to O-linked β-N-acetylglucosaminylation (O-GlcNAcylation) a post-translational modification that further fine-tune its signaling output. In particular, O-GlcNAcylation has been extensively described as a regulatory mechanism linking cellular metabolic state to β-catenin function [24–26]. This modification can occur on serine and threonine residues that overlap with phosphorylation sites and O-GlcNAcylation of T41 on β-catenin has been shown to directly compete with phosphorylation, influencing β-catenin stabilization [26]. Emerging evidence further indicates that O-GlcNAcylation can modulate β-catenin ubiquitination and degradation, highlighting a multilayered regulatory crosstalk between metabolic signaling and proteostatic control, highly relevant for cancer progression and therapeutic resistance [27]

Axin scaffolds the kinases and substrate, dramatically increasing phosphorylation efficiency [28], while APC contributes by antagonizing dephosphorylation [29]. GSK3β also phosphorylates Axin, further enhancing its activity in β-catenin turnover [30]. Importantly, degradation is restricted to the cytosolic pool and does not affect β-catenin bound to cadherins at adherens junctions [31].

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Wnt pathway activation

Upon activation of the Wnt pathway (Figure 2), secreted Wnt proteins bind to a receptor complex consisting of Frizzled (Fz, a seven-pass transmembrane receptor) and LRP5/6 (low-density lipoprotein receptor-related proteins 5 and 6), initiating a cascade that inhibits the degradation machinery [21]. The cytoplasmic protein Dishevelled (Dvl) is activated and interacts with Axin, likely inducing a conformational rearrangement that prevents effective assembly of Axin with GSK3β and β-catenin [32]. With GSK3β activity suppressed through a mechanism that remains incompletely resolved at the molecular level, β-catenin phosphorylation is blocked, ubiquitination ceases, and newly synthesized β-catenin becomes stabilized. As cytoplasmic β-catenin accumulates, it translocates into the nucleus, where it binds TCF/LEF transcription factors to activate downstream Wnt target genes involved in cell proliferation, differentiation, polarity, and survival [33].

The developmental significance of this pathway is underscored by genetic studies in Drosophila: mutations in Armadillo (Arm, the β-catenin ortholog) mimic loss-of-function mutations in Wingless (Wg, the Wnt homolog), producing identical defects in segmental patterning [34], establishing β-catenin as the essential downstream effector of Wnt signaling. Aberrant Wnt pathway activation, whether through mutations in APC, Axin, β-catenin phosphorylation sites, or dysregulated GSK3β activity, results in pathological β-catenin stabilization and is strongly implicated in disease, particularly cancer [35].

The nucleocytoplasmic shuttling of β-catenin is a highly regulated process, pivotal for its dual role in cell signaling and gene transcription. Importantly, nuclear accumulation of β-catenin is not determined solely by active transport mechanisms, but by the size and regulation of its available cytoplasmic pool. The pool is shaped by protein stability, degradation, and context-dependent interactions with binding partners. Despite lacking a canonical nuclear localization or export sequence (NLS or NES), β-catenin can access the nucleus through multiple mechanisms [36]. The first is a Ran-GTP-dependent mechanism, facilitated by chaperones such as FOXM1 and LEF-1, which leverages the importin α/β complex [37]. Alternatively, β-catenin can translocate through a Ran-independent mechanism, exploiting its structural similarity to importin-β to directly interact with nucleoporins such as Nup93, allowing autonomous translocation [37].

These observations suggest that nuclear entry is supported by multiple partially redundant pathways and is likely permissive rather than strictly rate-limiting, although the relative contribution of each mechanism remains an area of active investigation [37]. Nuclear export remains less characterized, but it is thought to involve classical CRM1 (Chromosomal Region Maintenance 1, also known as exportin 1)-mediated pathways, (relying on leucine-rich nuclear export signals and the Ran-GTP gradient) as well as additional CRM1-independent context-specific mechanisms, often involving β-catenin-associated proteins that shuttle between compartments[37]. Overall, this context-specific shuttling highlights β-catenin's adaptability in responding to diverse cellular signals.

Cell-cell Adhesion: structural element for mechanotransduction in tensional homeostasis

β-Catenin's functional output at adherens junctions (AJs) arises from its dual role as a structural linker and a mechanosensitive protein. In its classical role, β-catenin, within the cadhesome network [38], associates with the cytoplasmic tail of E-cadherin associated with the membrane, to create a physical connection with the actin cytoskeleton via α-catenin [10,39,40]. This linkage is vital for maintaining tissue architecture, integrity, and intercellular adhesion [41]. Beyond its well-known role as cadherin–α-catenin connector, β-catenin can also participate in alternative force-bearing configurations at AJs [42]. In a recent study, it was

demonstrated that when α-catenin is absent or reduced, β-catenin can directly bind non-autoinhibited vinculin via a conserved N-terminal vinculin-binding segment, linking the cadherin–catenin complex to the actin cytoskeleton [10]. This β-catenin–vinculin interaction sustains physiologically relevant forces in the range identified by single-molecule measurements (7-16 pN) and is sufficient to preserve junctional integrity and force transmission. Although of lower affinity than the canonical α-catenin–vinculin interaction, this linkage underscores the capacity of β-catenin to dynamically redistribute between distinct mechanical and signaling pools in response to force (Figure 3).

β-Catenin's mechanical properties are central to its role at AJs. Single-molecule force spectroscopy and molecular dynamics analyses have revealed that its ARM repeats region exhibits low mechanostability, unfolding in multiple alternative ways when force is applied. This compliance, shaped by its unstructured terminal regions, results in a rugged energy landscape, making β-catenin a force-sensitive element rather than a rigid linker [43]. The forces required to unfold β-catenin (~40–50 pN) overlap with those reported for cadherin–cadherin adhesion (~30–60 pN) but remain well below the mechanical stability of cadherin ectodomains (>150–300 pN). Molecular dynamics simulations further reveal that mechanical load can remodel the β-catenin–cadherin complex, as binding of the cadherin cytoplasmic tail stabilizes the ARM domain and channels unfolding along specific pathways. Notably, force-induced conformational changes expose regulatory tyrosine residues, such as Y489, linking junctional tension β-catenin phosphorylation, and protein turnover [43].

Cadherin-Mediated Buffering

Cadherin complex can antagonize β-catenin's signaling potential through at least two mechanisms. First, stoichiometric sequestration: because β-catenin binds phosphorylated cadherins with dramatically higher affinity (~570-fold greater than for nuclear transcription factors enabling sequestration β-catenin at the membrane and limit its nuclear accumulation [15,43,44]. Structural and biochemical studies demonstrate that phosphorylation of specific serine and threonine residues in the cadherin cytoplasmic tail not only stabilizes its association with β-catenin [15,45]. Second, cadherin binding modulates the turnover of cytosolic β-catenin by the degradation machinery promoting a junction-localized phosphodestruction complex that suppresses Wnt signaling [46]. However, the relationship between cadherin-mediated adhesion and β-catenin signaling is highly context-dependent. In this context, cadherins are best viewed as components of a dynamic buffering system that modulates the effective cytoplasmic pool of β-catenin, setting signaling thresholds rather than acting as simple constitutive inhibitors or passive reservoirs.

Together, these findings support a model in which cell–cell adhesion operates as an integrated mechanotransductive system, with β-catenin linking mechanical forces to biochemical regulation.

Broader Roles in Cellular Homeostasis

Relationship with Hippo pathway

Beyond its well-established functions in Wnt signaling and cadherin-mediated adhesion, β-catenin operates as a central competitive interaction platform within broader cellular regulatory networks that coordinate tissue growth, mechanical responses, and dynamic cell-state transitions. One such example is its indirect but essential role in regulating the Hippo signaling pathway, a mechanosensing cascade that restricts tissue expansion by phosphorylating and inhibiting the transcriptional co-activator Yes-associated protein (YAP) [47,48]. Mature E-cadherin/β-catenin/α-catenin complexes at adherens junctions suppress nuclear YAP accumulation, thereby restraining proliferation in densely packed tissues [49–52]. β-

Catenin contributes to this regulation by anchoring α-catenin at adherens junctions, where α-catenin directly engages Hippo pathway components such as Large Tumor Suppressor (LATS) kinases and Ajuba LIM protein (AJUB) to suppress YAP/transcriptional co-activator with PDZ-binding motif (TAZ) activity [51,53–56].[51,56]. Thus, β-catenin's adhesive function indirectly but essentially modulates YAP/TAZ localization, linking junctional mechanics to tissue homeostasis.

Cytoskeletal remodeling and EMT

In parallel, β-catenin integrates mechanical signals into cytoskeletal remodeling, further underscoring its role in maintaining cellular homeostasis. As discussed in the previous paragraphs, the cadherin–catenin complex connects to the actin cytoskeleton through a dynamic network of proteins, involving adaptors such as cortactin and vinculin, both of which fine-tune junctional strength and mechanotransduction [57,58]. Cortactin promotes actin polymerization at junctions via Arp2/3 recruitment, while vinculin acts as a molecular clutch and reinforces mechanical anchorage in response to force and phosphorylation [40,59]. Phosphorylation of cortactin at S298 by protein kinase D1 (PKD1) modulates this balance, compromising vinculin's engagement with β-catenin and impairing junctional mechanostability [60]. This tunable molecular switch allows cells to adjust actin connectivity in response to tension, highlighting β-catenin's role in adapting cytoskeletal dynamics to mechanical stimuli.

In addition to this indirect role within adherens junction associated mechanotransductive complexes, emerging evidence suggests that β-catenin itself may exhibit direct mechanosensitive properties. Force-dependent phosphorylation events, such as at Y654, modulate β-catenin binding affinity and its interaction with cadherins [61]. Furthermore, cytoskeletal tension influences serine/threonine (S/T) phosphorylation states in β-catenin and associated junctional components, linking actomyosin contractility to phosphorylation-dependent regulation [62]. This complementary mode of mechanosensitivity reinforces β-catenin's role as a central mediator of mechanochemical coupling at cell–cell junctions. The significance of these mechanosensitive properties becomes particularly evident during processes such as epithelial-mesenchymal transition (EMT). Here, transcriptional repressors like Snail family transcriptional repressor 1 (SNAIL) and Snail family transcriptional repressor 2 (SLUG) downregulate E-cadherin, releasing β-catenin from the membrane and increasing its cytosolic availability [63,64]. This not only activates Wnt signaling but also establishes a positive feedback loop, as SNAIL is a direct β-catenin/TCF transcriptional target [65,66].

Dissociation of the E-cadherin–β-catenin–α-catenin complex from the membrane can also be induced by phosphorylation of key tyrosine residues in β-catenin. Particularly, phosphorylation of tyrosine 654 (Y654) in the last armadillo repeat by the kinases c-src or EGFR leads to the loss of E-cadherin-binding, and cytoplasmatic accumulation β-catenin. Since the last armadillo repeat is not required for LEF/TCF binding, Y654 phosphorylation does not interfere with β-catenin interaction with LEF/TCF after its nuclear translocation [67–69]. Additionally, the phosphorylation of tyrosine 142 (Y142) in the first armadillo repeat by the kinases Fer or Fyn disrupts and precludes the interaction of β-catenin with α-catenin, while favoring binding to the nuclear co-factor BCL9-2. This phosphorylation promotes a nuclear complex formation containing β-catenin, BCL9-2 and LEF/TCF proteins, activating Wnt signaling pathway [67,70]. By reinforcing EMT commitment, β-catenin drives migratory and invasive behavior [71]. positioning it as a cornerstone in cellular homeostasis[72].

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Structural Evolution of β-Catenin as the Foundation for Adhesion–Signaling Integration

From ancient scaffold to metazoan innovation

The multifunctional nature of β-catenin derives from its highly conserved ARM repeats domain, which forms an elongated, adaptable binding groove capable of engaging diverse interaction partners. As shown in Figure 4, this modular architecture first appeared in early metazoans, where the coordination of adhesion and transcription became indispensable for the emergence of multicellular life [73]. Rather than representing a *de novo* innovation, β-catenin likely emerged as a metazoan-specific functional specialization of a more ancient armadillo-repeat protein architecture. The ARM protein superfamily, to which β-catenin belongs, represents an ancient and structurally versatile group of proteins found across eukaryotes. These proteins are involved in fundamental cellular processes such as cell adhesion, signal transduction, and cytoskeletal organization. Their functional versatility arises from the repeated ARM domain, which mediates a wide range of protein–protein interactions. Although β-catenin itself appears to be metazoan-specific, phylogenetic analyses indicate that the broader ARM proteins superfamily shares a common premetazoan ancestor, with roughly one-third of its members already detectable in pre-metazoan lineages such as choanoflagellates and filastereans [74,75]. This reflects a deep eukaryotic ancestry, with early diversification giving rise to distinct functional branches leading to the emergence of the ancestral forms of the δ- (p120-) catenin and β-catenin subfamilies [34,75]. Phylogenetic analyses of Arm-repeat proteins by Gul and colleagues indeed indicate that the metazoan catenins did not arise from a single ancestral "proto-catenin" [74]. Instead, β-catenin and the δ-catenin family seems to have originated from distinct pre-metazoan Arm proteins present in unicellular holozoans. Phylogenetic evidence indicates that delta2 or ARVCF (armadillo repeat protein deleted in velo-cardio-facial syndrome) represents the ancestral member, from which δ-catenin likely arose at the origin of vertebrates, introducing new regulatory mechanisms for cadherin-based adhesion, an innovation that may have facilitated complex morphogenetic processes such as neural tube formation and epithelial–mesenchymal transitions [76]. Reconstructing the evolutionary trajectory of β-catenin requires careful consideration of its phylogenetic boundaries. While eukaryotic ARM-repeat proteins such as Aardvark, Vac8p, and Arabidillo share structural similarities with β-catenin, including a conserved core of 12 ARM repeats, these resemblances reflect convergent evolution rather than direct homology [73,77]. True homology requires evidence of shared ancestry rather than convergent structural features, and ARM repeats are particularly prone to independent evolution due to their short, modular nature (~40 amino acids). Phylogenetic analyses consistently fail to recover these non-metazoan proteins within the well-supported β-catenin clade, and their functional contexts differ substantially, lacking the dual roles in cadherin-mediated adhesion and Wnt signaling that define canonical β-catenin [73,77]. Together, these evidences suggest that *bona fide* β-catenin arose specifically within the metazoan lineage through the diversification of an ancestral ARM protein.

Phylogenomic evidence and early metazoan diversification

Comprehensive phylogenomic analyses using databases such as PhylomeDB [78], Ensembl [79], and OrthoFinder [80] consistently place all *bona fide* β-catenins within a well-supported, metazoan-exclusive monophyletic clade, implying descent from a single ancestral gene present in the last common ancestor of animals [75,77]. This conclusion is reinforced by presence–absence data: β-catenin family members are not detected in the newly sequenced single-celled eukaryotic protist Capsaspora owczarzaki[81] while in the

pre-metazoan choanoflagellate Salpingoeca rosetta it has been identified ARM6 with fewer armadillo repeats and little conservation of the functional motifs observed in cnidarian and poriferan, with the exception of a few phosphorylation sites [82]. In contrast, true β-catenin is present in early-diverging metazoans, including all non-bilaterians phylum such as Ctenophora (Mnemiopsis leidyi), Porifera (Oscarella), Placozoa (Trichoplax adhaerens) and Cnidaria [83,84]. Notably, the Trichoplax genome encodes not only β-catenin but also components of the Wnt/β-catenin pathway, along with focal adhesion kinase, paxillin, and talin, suggesting that its dual functions in adhesion and signaling were established early in metazoan evolution [85].

ARM protein's phylogenetic analysis suggests a common holozoan precursor between β-catenin, Armc6, Apc, Kifap3 and Armc2, followed by an early metazoan adoption and co-evolution [74].. This metazoan origin is further supported by the absence of β-catenin in non-animal eukaryotes and its conserved presence in early-branching metazoans, where it already exhibited the dual roles in Wnt signaling and cadherin-mediated adhesion that define its function in modern organisms [82,86].

Structural bioinformatics provide a complementary perspective on β-catenin's evolutionary roots. While sequence-based phylogenetics firmly anchors β-catenin within Metazoa, quantitative comparisons of AlphaFold-generated structures reveal significant fold similarity between human β-catenin and yeast Vac8p (Table 1). The Template Modeling (TM)-scores > 0.5 and Root Mean Square Deviation (RMSD) values between 4.05 to 4.78 Å, metrics indicative of a shared ancestral fold despite low sequence identity [87–89]. The high TM-score (0.67, Table 1) obtained using jCE-CP provides quantitative evidence of a shared ancestral fold between the two proteins. Like β-catenin, Vac8 is composed of 12 ARM domains and interacts with diverse protein partners. This shared structural motif likely underlies their binding versatility[90]. However,this similarity does not imply direct evolutionary descent. Instead, it represents a case of structural conservation coupled with functional divergence from an ancestral ARM-repeat scaffold. Such motif likely emerged during early eukaryotic evolution and adapted to distinct cellular functions in different lineages [90].

Lineage-Specific Diversification and Functional Specialization

The idea of this ancestral ARM scaffold and convergent evolution is further supported by Aardvark structure-function as shown in studies in D. discoideum. This β-catenin-like protein has an important role in cell polarity and tissue organization during stalk development, interacting with an α-catenin/vinculin homolog in a system lacking detectable homologues of classical cadherins [91]. In addition, Aardvark structure does not include both terminal regions seen in β-catenin, which are typically involved in interactions with Wnt pathway components, absent in D. discoideum [92]. Together, these observations support the view that catenin-mediated cytoskeletal organization and polarity functions predate cadherin-based adhesion and canonical Wnt signaling. Following its emergence, β-catenin underwent lineage-specific functional diversification, shaped by increasing organismal complexity. In early-branching metazoans such as Spongillidae and Oscarellidae (Ephydatia muelleri and Oscarella spp.), β-catenin exhibits an ancestral, multifunctional role, associating not only with cell–cell junctions but also with cell–substrate adhesions, suggesting that its adhesive function was initially broader and later specialized in more derived lineages [83,93]. In cnidarians, which represent a more derived but still early-diverging metazoan phylum, β-catenin is essential for both Wnt signaling and the regulation of epithelial integrity, playing a critical role in axis formation and tissue patterning during development [82,86,94].

The evolution of the vertebrate canonical Wnt/β-catenin pathway indicates that it did not arise as a novel developmental signaling system, but rather formed around a much older cellular adhesion and polarity

module. Proteins that regulate β-catenin stability, especially the kinase GSK3, have been detected in unicellular organisms where they still control cellular polarity rather than embryonic development observed in higher metazoa. In Porifera (sponges, throught to be one of oldest metazoan clades still living), β-catenin still primarily localizes to adherens-like junctions and participates in epithelial organization, despite Wnt ligands and Frizzled receptors not having evolved. Placozoa (represented by the well-studied Trichoplax) and later in Ctenophora (comb jellies eg. *Mnemiopsis leidyi*), the components of the canonical pathway (Dishevelled, Axin, TCF, and GSK3-mediated β-catenin degradation) become assembled into a regulatory cascade, but its developmental role remains limited.

Potentially only with radial symmetrical body plan as seen in Cnidaria (Coelenterata) does Wnt/β-catenin signaling clearly function as a morphogenetic system controlling the oral–aboral body axis. Evolutionarily, Wnt signaling therefore represents the recruitment of a pre-existing cadherin/β-catenin adhesion complex into a ligand-regulated transcriptional pathway: Wnt inhibition of GSK3 stabilizes β-catenin, allowing it to leave junctions, accumulate in the nucleus, and regulate gene expression. We can speculate that the key innovation leading to more complex metazoa, bilateria and beyond was not the evolutionary arrival at the of β-catenin protein, but extracellular control over its localization and availability, enabling multicellular pattern formation and, ultimately, complex animal body plans. After the Cnidaria, the line towards vertebrates acquired the bilaterian body plan and suffered the evolutionary branching of deutrosomes and protostomes.

In nematodes, such as Caenorhabditis elegans, a later-branching protostome lineage within Bilateria, the ancestral gene duplicated and diversified into three paralogs that underwent subfunctionalization, each specializing in a subset of the ancestral functions related to either adhesion or transcriptional activation [95]. Within Insecta (Phylum Arthropoda), exemplified by *Drosophilamelanogaster*, a single armadillo gene was retained under strong purifying selection, preserving both functions within a pleiotropic protein [34,75].

In vertebrates, a duplication event, likely occurring during the early Cambrian genome expansion, gave rise to β-catenin and plakoglobin (γ-catenin), with the latter acquiring a specialized role in desmosomes, thereby enhancing mechanical resilience in vertebrate tissues [74,96].

Having traced the diversification of β-catenin across metazoan lineages, it is important to consider the methodological basis of these evolutionary inferences. Current models rely on Bayesian phylogenetic reconstruction, genomic databases, and structural alignments, each with inherent limitations. In this framework, confidence in key nodes, such as the monophyly of metazoan β-catenins, is assessed through posterior probabilities. The sea anemone Nematostella vectensis (phylum Cnidaria) serves as a key outgroup, providing polarity to the evolutionary tree and anchoring the reconstruction of ancestral relationships. Further progress in elucidating catenin evolution will depend on several complementary research directions.

The evolution of β-catenin illustrates a fundamental principle: the hierarchical construction of molecular complexity through the repurposing of ancient structural frameworks. This diversification provided the foundation for the adhesion and signaling systems that define animal multicellularity, with subsequent duplications, such as the split between β-catenin and plakoglobin in vertebrates, further expanding functional diversity [13,97]. The history of β-catenin emerges as a paradigm for how gene duplication, functional specialization, and the co-option of ancient scaffolds drive the evolution of molecular systems that underpin the diversity of life [77,83].

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From Evolutionary Innovation to Oncogenic Vulnerability

The evolutionary emergence of β-catenin as a dual-function protein, linking mechanical adhesion and transcriptional regulation, was pivotal to animal multicellularity. However, this innovation also created a molecular vulnerability: disruption of β-catenin's regulatory balance can decouple growth control from adhesion, a failure central to the development of many human cancers [13,33,98,99]. Rather than simply adding functions, this innovation imposed a structural constraint: multiple essential cellular processes, such adhesion, mechanotransduction and transcription, became coupled through a single molecular interface. This creates an intrinsic trade-off, whereby the same interaction surface must be dynamically partitioned among mutually exclusive partners making the system inherently vulnerable: small perturbations in β-catenin stability, binding affinity, or localization can disproportionately shift this balance, leading to inappropriate activation of transcriptional programs.

In many tumors, mutations in either the tumor suppressor APC or in β-catenin itself prevent its degradation, resulting in cytoplasmic accumulation and nuclear translocation. In the nucleus, β-catenin forms constitutive complexes with TCF/LEF transcription factors, driving transcription of oncogenes such as c-myc, cyclin D1, and c-jun [13,100,101]. Approximately 80% of human colorectal cancers are initiated by APC mutations, whereas tumors without APC defects frequently harbor activating β-catenin mutations, particularly at N-terminal phosphorylation sites critical for proteasomal degradation. Similar alterations are observed in melanoma, hepatocellular carcinoma, ovarian and endometrial cancers, medulloblastomas, pilomatricomas, and prostate cancers [13,101,102]

Functional studies confirm the oncogenic potential of β-catenin. Deletion of its N-terminal domain converts β-catenin into a strong oncogene *in vitro* [103], and increased expression of β-catenin wt or its mutations induces intestinal adenomas [104–106] and benign skin tumors resembling human pilomatricomas [107,108]. Conversely, expression of conductin (Axin2), which assembles the β-catenin degradation complex, suppresses Wnt signaling and inhibits tumorigenesis [109]. Beyond APC and conductin, β-catenin stability is fine-tuned by a network of specialized E3 ubiquitin ligases (such as β-TrCP [110]) and by deubiquitinases (such as USP4 and USP7 [111,112]). In addition to proteasomal degradation, β-catenin levels can be modulated by autophagy. Under conditions of metabolic stress or nutrient deprivation, cytoplasmic β-catenin can be selectively sequestered into autophagosomes and degraded by autolysosomes, providing an alternative mechanism to limit Wnt signaling and prevent inappropriate activation of oncogenic transcriptional programs [101,113].

Tumor progression and metastasis

The adhesive function of β-catenin is equally critical in tumor progression. The E-cadherin–β-catenin–α-catenin complex links the actin cytoskeleton to neighboring cells, preserving epithelial cohesion. In many carcinomas, cadherin-based adhesion is disrupted by transcriptional repression or CDH1 mutations, resulting in dedifferentiation and enhanced invasiveness. Restoration of E-cadherin can block invasiveness *in vitro* and prevent metastasis *in vivo*[114,115]. Loss of E-cadherin may alter the distribution of β-catenin between membrane-associated and cytoplasmic pools [101]; however, it has been observed that this does not necessarily correlate with an upregulation of β-catenin nuclear signalling, suggesting a compensation ability of the Axin/APC degradation complex under normal conditions and an uncoupling of the signalling and structural functions [116,117]. Notably, in Xenopus embryos, β-catenin signaling can occur independently of adhesion, suggesting functional separation of the pools under certain contexts [101]

β-catenin can act as an alternative connector when α-catenin is downregulated, mutated, or absent. While α-catenin contributes to cell–cell cohesion, its role in tumor progression is context-dependent: its loss can

promote invasiveness by weakening adhesion, whereas in some metastatic settings cells may retain cohesive migration even in its absence. In particular, as mentioned above, it was recently demonstrated that β-catenin can associate with vinculin in the absence of α-catenin, preserving intercellular cohesion and potentially enabling collective migration in invasive metastatic cells[10]. These observations align with earlier reports indicating that α-catenin is not always essential for coordinated migration of invasive cancer cells [118], highlighting the adaptability of adhesion networks during tumor dissemination. Furthermore, experimental evidence indicates that loss of E-cadherin can enhance local invasion while impairing subsequent steps of metastasis, including tumor cell survival and metastatic outgrowth [119]. This paradox suggests that invasiveness alone does not ensure metastatic success, as cell–cell cohesion is also required for survival during dissemination and colonization.

As we have described earlier, although β-catenin lacks a canonical NLS, its nuclear accumulation is tightly regulated. Beyond its role in proliferation, nuclear β-catenin also contributes to the maintenance of stemness in both normal and malignant cells, supporting tumor initiation, therapy resistance, and metastatic potential [101,120].

Immune Evasion

Moreover, β-catenin signalling can modulate the tumor microenvironment by suppressing T-cell responses, thereby facilitating immune evasion and influencing the effectiveness of immunotherapy [121]. Consistent with this, aberrant activation of β-catenin signalling has been associated with the establishment of a non-inflamed tumor microenvironment [122]. Mechanistically, β-catenin activates the transcriptional repressor ATF3 (activating transcription factor 3), which suppresses the production of the chemokine CCL4, ultimately weakening the priming and infiltration of cytotoxic T-cells into the tumor [121]. As a result, β-catenin-driven immune exclusion is linked to reduced responsiveness to immune checkpoint therapies. In this context, β-catenin–driven immune exclusion has been associated with reduced responsiveness to immune checkpoint blockade [121–124].

Therapeutic Opportunities and Challenges

Therapeutically, β-catenin is an appealing target, with nuclear β-catenin staining serving as a diagnostic and prognostic marker. However, targeting β-catenin presents challenges, including the risk of disrupting its essential adhesive functions with potential off-target effects. Notably, accumulating evidence suggests that this functional separation may be more tractable than initially anticipated. Indeed, several small molecules that interfere with β-catenin transcriptional activity, such as inhibitors of β-catenin/TCF or β-catenin/co-activator (e.g., CBP/p300) interactions, appear to have minimal impact on its adhesive function [125]. These advances shift the central challenge from functional selectivity to tissue specificity. β-catenin signaling is essential for homeostasis in multiple adult tissues, including the intestinal epithelium and bone, raising significant concerns about systemic toxicity. Thus, a major unmet need in the field is the development of strategies that enable tumor-restricted modulation of β-catenin activity. [98,100,126,127] (see Table 2).

Relationship with chemotherapy resistance

Intriguingly, some studies have shown that sustained activation of β-catenin is associated with resistance to chemotherapy [105]. Wnt/β-catenin activation is associated with poor response and resistance to sorafenib in hepatocellular carcinoma [128]. Accordingly, pharmacological β-catenin inhibition sensitizes tumors to sorafenib, showing therapeutic synergy in vitro and in murine models (Table 3) [129,130]. More broadly, these findings are consistent with a role for Wnt/β-catenin signaling in sustaining therapy-

resistant tumor cell populations. Pharmacological targeting of β-catenin transcriptional activity, most notably through CBP/β-catenin antagonists such as ICG-001 and its clinical derivative PRI-724, has emerged as a strategy to enhance therapeutic responses and counteract resistance to both cytotoxic and targeted agents [129,131].

In prostate cancer, resistance to abiraterone has been linked to activation of canonical Wnt/β-catenin signaling, as revealed by transcriptomic analyses of resistant tumors. Increased androgen receptor (AR) and β-catenin expression sustain AR-dependent transcription despite androgen deprivation. Combined inhibition of AR signaling and β-catenin activity (e.g., with ICG-001) disrupts this crosstalk, suppresses stemness programs, and restores drug sensitivity, highlighting β-catenin co-targeting as a strategy to overcome resistance [132].

In breast cancer, Wnt/β-catenin activation is associated with chemoresistance, stem cell features, and enhanced survival following cytotoxic challenge in triple-negative models. Pathway inhibition resensitizes cells to platinum agents such as carboplatin[133]. However, it is not only the role of β-catenin in the Wnt pathway that is relevant in cancer. For example, in oral squamous cell carcinoma, it has been shown that the loss of β-catenin at the cell membrane, together with that of E-cadherin, is an early event that correlates with late clinical stage and lymph node metastasis [134]. On the other hand, in epithelial ovarian cancer, strong membranous expression of β-catenin, together with E-cadherin and Wnt ligands, is associated with resistance to chemotherapy and poorer progression-free survival [135]. This suggests that alterations of adherens junctions and the relocation of β-catenin contribute to early invasion and cancer progression.

Conclusions and Future directions

The evolutionary journey of β-catenin, from its pivotal role in enabling metazoan multicellularity to its central function in oncogenesis, exemplifies the double-edged nature of biological innovation. As a competitive interaction platform coupling adhesion (mechanics) with transcriptional control (signaling), β-catenin's deregulation illustrates how mechanisms maintaining tissue organization can be hijacked to drive cancer progression, metastasis and therapy resistance.

β-Catenin had cellular functions prior to its incorporation into the canonical Wnt pathway. Consistent with this, tumors tend not to activate Wnt signaling by mutating Wnt ligands or Frizzled receptors, which remain subject to extracellular regulation and feedback from the tissue niche, but instead preferentially target intracellular control points, particularly the β-catenin destruction complex. Cancer-promoting mutations in exon 3 of the β-catenin gene, as well as in APC and AXIN, disrupt the phosphorylation-dependent degradation process that normally requires CK1 and GSK3β activity, leading to Wnt-independent nuclear accumulation and transcriptional activation.

This duality exposes a key vulnerability but also highlights important gaps in our understanding. In particular, how β-catenin integrates mechanical, adhesive, and transcriptional inputs across cellular contexts remains to be fully defined. The regulation of its intrinsically disordered terminal regions, including their role in nuclear condensates and conformational dynamics, is still incompletely understood, as is the molecular basis of mechanotransduction, such as tension-dependent phosphorylation and partner switching. Similarly, the precise control of GSK3β activity and β-catenin nuclear shuttling remains unresolved. From an evolutionary perspective, resolving when these regulatory features emerged will require the integration of phylogenetic and functional analyses.

In cancer, β-catenin signaling promotes tumor growth, survival, and therapy resistance, in part through the maintenance of cancer stem cell populations, while emerging evidence indicates that post-

translational modifications, including crosstalk between O-GlcNAcylation and ubiquitination, add further regulatory complexity in resistant states. Together, these insights position β-catenin as both a source of fragility and a therapeutic opportunity. By dissecting its multilayered regulation, from degradation pathways to nuclear dynamics and microenvironmental crosstalk, it may be possible to exploit this vulnerability. A central challenge moving forward will be to translate this knowledge into tumor-specific strategies that preserve its essential physiological functions, effectively transforming β-catenin from an evolutionary liability into a strategic Achilles' heel in cancer.

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During the preparation of this work the authors used Le Chat (Minstral) in order to improve language and readability of the manuscript. After using this tool, the author reviewed and edited the content as needed and take full responsibility for the content of the publication. Figures 2, 3 and 5 have been generated with the help of Scispace AI.

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FIGURE LEGENDS

Figure 1: Human b-catenin structure and interacting partners. A) 3D structure for UniProt #P35222, obtained from EMBL-EBI AlphaFold Protein Structure Database (alphafold.ebi.ac.uk)[136] and corresponding to Catenin b-1 from Homo sapiens. AlphaFold produces a per-residue model confidence score (pLDDT) between 0 and 100 (as indicated in the legend). Some regions below 50 pLDDT may be unstructured in physiological conditions. B) Reported interacting partners of b-catenin by experimental evidence. In black are the cadherin-mediated adhesion related partners and in green the Wnt signalling related ones. The list is not exhaustive and is only highlighting the proteins described in the current Review. C) Phosphorylation-dependent functional summary. Phosphorylation sites are shown with varying levels of experimental support, with some (e.g., S552, S675) requiring further validation.

Figure 2: The role of β-catenin in the canonical Wnt signaling pathway. On the LEFT panel, the Wnt signal is ON (or in Active state), activated by Wnt ligands, thus the Destructive complex is inhibited and β-catenin accumulates in the cytoplasm. On the RIGHT panel, the Wnt signal is OFF (or in a default state) and the Destructive complex is ACTIVE, thus β-catenin is tagged (ubiquitinated) and sent for degradation. This figure was developed using Scispace AI.

Figure 3: The interaction of β-catenin with E-cadherin for cell-cell adhesion stabilization. On the LEFT panel, classical model for β-catenin interaction at adherens junctions. On the RIGHT panel, alternative mechanism by which in absence of α-catenin, β-catenin directly interacts with vinculin [10]. This figure was developed using Scispace AI.

Figure 4: Evolutionary routes of β-catenin related genes. The flow map shows proposed evolutionary pathways. Metazoan and non-metazoan ARM proteins would have evolved from a common scaffold. Pre-metazoan ARM6 in Choanoflagellates has been identified as a β-catenin-like protein, with fewer armadillo repeats. The phylogenetic tree traces β-catenin's emergence in early metazoans, such as sponges and Trichoplax, where it exhibits dual adhesive and Wnt signaling functions. In pre-metazoan evolution, a diversification event led to the generation of δ- and β-catenin subfamilies precursors. Although, the exact moment is unknown, it is expected to have occurred in early holozoan or pre-holozoan history. The β-catenin ancestor followed distinct evolutionary paths. Gene duplication and subfunctionalization in nematodes produced 3 paralogs that specialized in different ancestral functions. Strong selection conserved only 1 gene in organisms like Drosophila. Moreover, in the vertebrate lineage, a new gene duplication event gave rise to plakoglobin (γ-catenin) which later acquired new specialized functions.

TABLES

Alignment Method	TM-score	RMSD	Aligned residues	Identity
jFATCAT (rigid)	0.64	4.78	471	16%
JCE (rigid)	0.63	4.05	441	15%
TM-align (rigid)	0.63	4.68	339	15%
jFATCAT (flexible)	0.57	2.83	500	17%
jCE-CP (flexible)	0.67	4.05	441	15%

Table 1: Structural comparison of yeast Vac8 and human β-catenin. Analysis of the protein fold consistently reveals a structural similarity between both ARM proteins. The comparison is shown between the high confidence or above pLDDT regions of Vac8 (AF_AFP39968F1) and β-catenin (AF_AFP35222F1). While

having a low sequence identity, RMSD values lower than 5 Å and TM scores greater than 0.5 indicate a significant global folding similarity. The aligned residues and corresponding sequence identity is displayed for each method.

Region	Key Sites	Typical Cancer Mutations	Effect
N-terminal regulatory	D32, S33, G34, I35, H36, S37, S45, T41, S45	D32G/N/Y/V/H/A, S33C/Y/F/P/A/T/L, G34R/E/V, I35S, H36P, S37F/C/A/P/Y, T41A/I/N, S45F/P/Y/C, deletions	Stabilization & uncontrolled Wnt signalling
Armadillo repeats	K335, W383, N387	K335I/T mutations, W303C/G/R, N387K/I/Y mutations	Pathogenic activation of signalling potential
C-terminal tail	(no common cancer mutations reported)	*Rare/non-hotspot in cancer*	Mostly transcription regulation role

Table2: Summary of Mutation and Phosphorylation Map in Cancer. Residues and substitution information are included as per cBioPortal.

Inhibitor name	Mode of action
ICAT	Blocks β-catenin /TCF interaction (Daniels & Weis, 2002; Graham et al., 2002; Tago et al., 2000)
ICG-001	Disrupts AR/β-catenin cooperation (Wang et al., 2023)
Bay 43-9006/ Sorafenib	Inhibits tyrosine kinase (Wang et al., 2023)
NCS 241240/ Carboplatin	Inhibits DNA synthesis (Tomas et al., 2026)
PRI-724	Inhibits binding protein CREB/ β-catenin (National Center for Biotechnology Information, 2026)
SM08502	Reduce β-catenin production (Tam et al., 2020)
MSAB	Degrades β-catenin (Hwang et al., 2016)
CWP232291	Inhibits Wnt/ β-catenin signaling pathway (Lee et al., 2020)

Table 3: Summary of b-catenin inhibitors. Inhibitors and their mode of action as indicated in the text.

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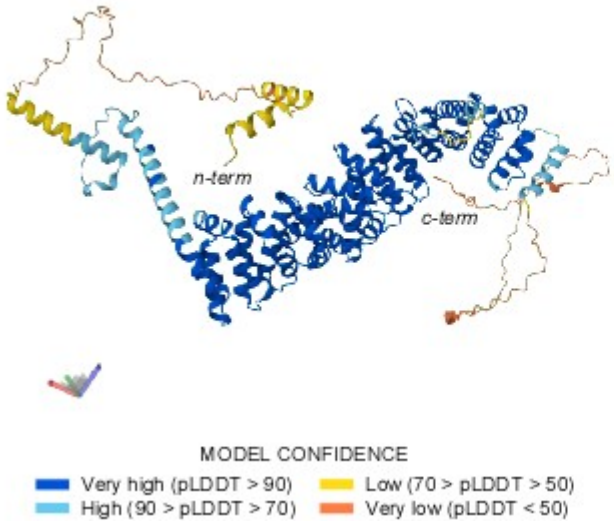
A



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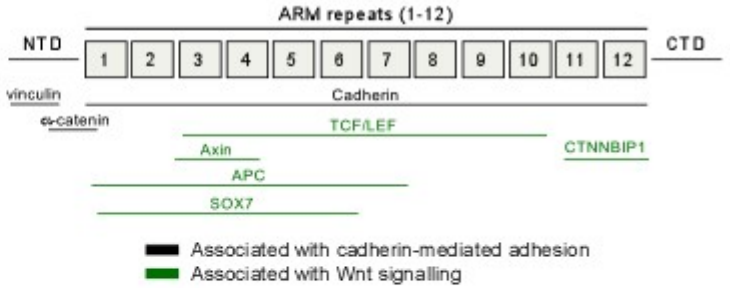
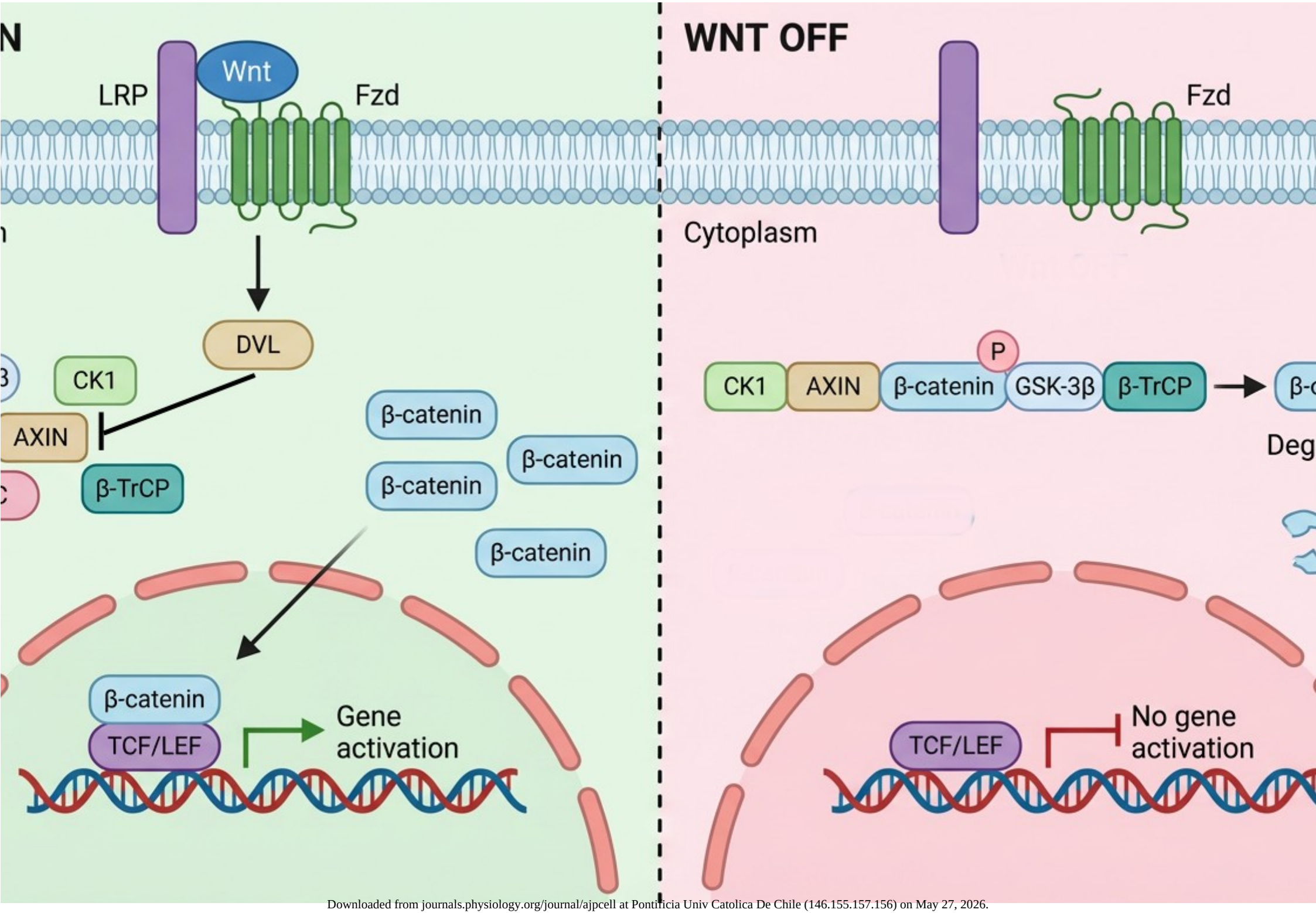
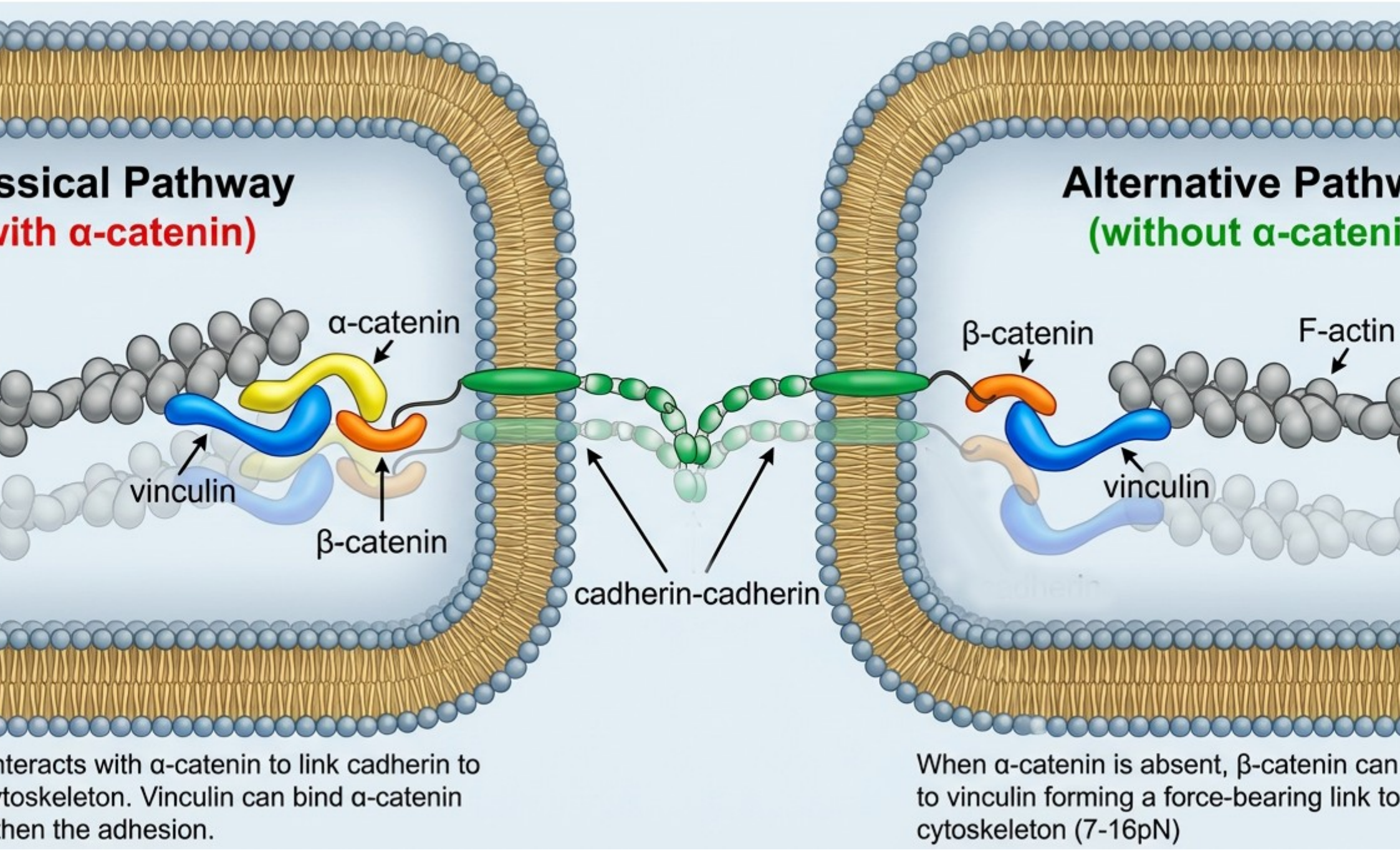
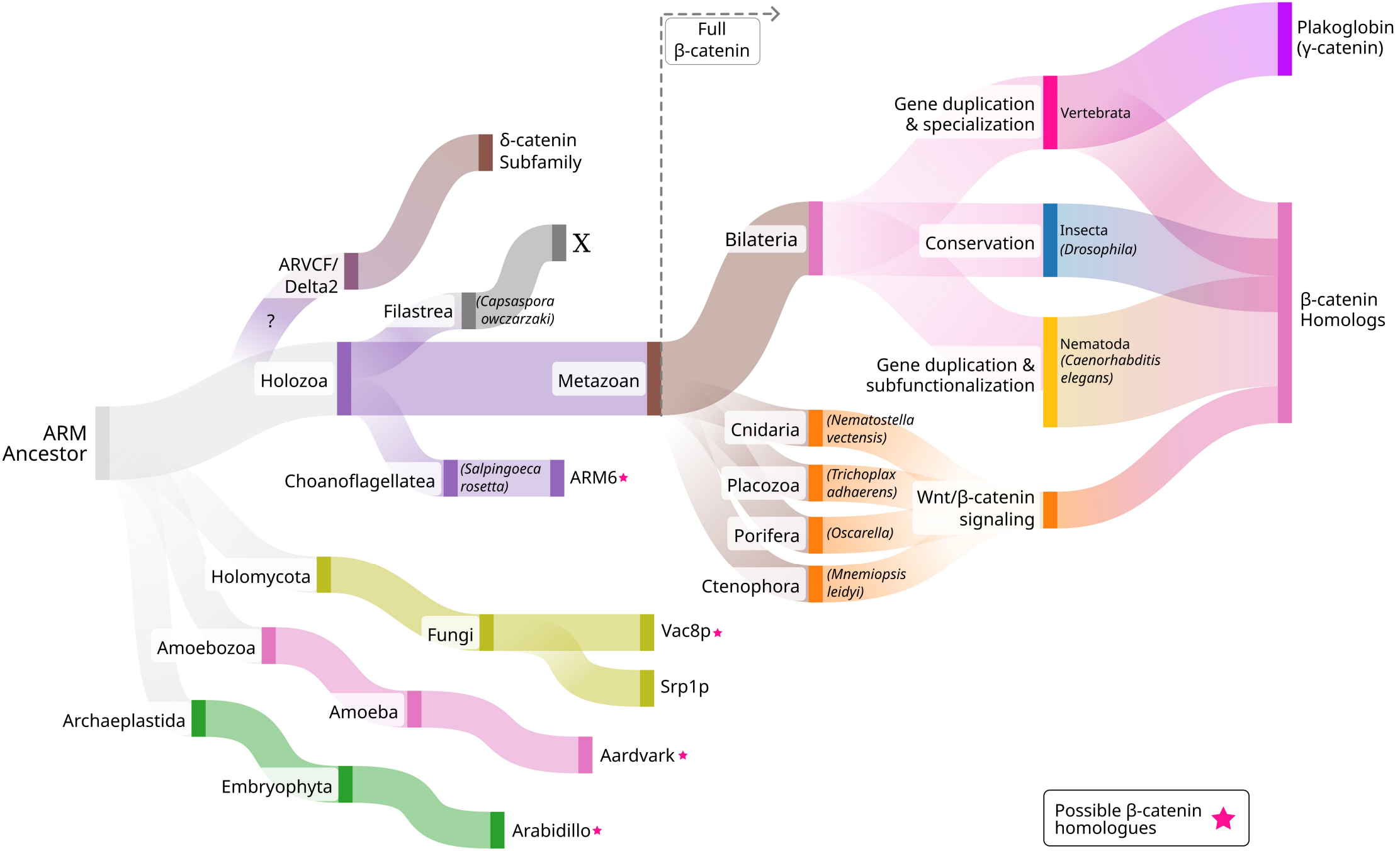


C

Phosphorylation site	Position	Kinase	Main Outcome
Thr41, Ser37, Ser33	N-terminal region	GSK-3ß	Ubiquitination & degradation
Ser45	N-terminal region	CK1a	Priming for degradation
Tyr142	Arm Repeat 1	Src/Fyn	Adherens junction destabilization
Ser552	Arm Repeat 10	Akt	Nuclear accumulation
Tyr654	Arm Repeat 12	Src/RTKs	Loss of adhesion, increase signaling
Ser675	C-terminal region	PKA	Transcriptional activation







β-Catenin: A competitive interaction platform