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Review

The functional diversity of β-catenin: the interrelationship between its cell junction and nuclear distribution

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ABSTRACT

β-catenin is a multifunctional protein. β-catenin is a cell junctional protein that is found within adherens junctions, where its binding to the intracellular domain of cadherins helps link these complexes to the actin cytoskeleton. β-catenin is also a transcriptional enhancer protein, whose interaction and regulation of specific genes elaborate the transducing signals initiated by binding of Wnt proteins to their cell membrane receptors. One question about β-catenin that has yet to be fully answered concerns how or if the cell junctional and transcriptional functions of β-catenin are connected. This essay will summarize the current understanding of the dual roles that β-catenin plays, evaluate whether there is an interconnection and/or interrelationship between the diverse functions of this protein, and if the two functions of this regulatory protein are distinctly regulated. The question to be proposed is whether this information can collectively produce a unitary regulatory framework on how β-catenin regulates the overall biology of the cell and organism.

KEYWORDS: β-catenin, Wnts, signal transduction, transcriptional regulation, cell adhesion.

INTRODUCTION

β-catenin, which is sometimes referred to as CTNNB1, is a multifunctional protein that exercises regulatory functions at both the cell membrane and nucleus [1-3]. β-catenin is a component of adherens junctions, as it binds to cadherins, and forms part of a molecular chain that links cell-to-cell junctions to the actin cytoskeleton [4-6]. β-catenin is also a transcriptional regulator, where it serves as a component of the canonical Wnt signaling pathway, as it transmits the regulatory activity of Wnt proteins to the gene level [7-9]. This essay will provide an overview of β-catenin biology within the cell, but is not intended to be a detailed review of the complex molecular interactions that play a part in the functional activities of β-catenin. Rather, the intent here is to address an issue that is still perplexing, about whether and how the dual roles of β-catenin cooperatively regulate cellular biology.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at New York Medical College approved all animal protocols of this study. Bone marrow was harvested from 8–12 wk C57BL/6 mice, with MSCs obtained from the isolated tissue using standard procedures, as described in previous publications [10, 11]. The QCE6 cell line was derived from mesoderm tissue from a Japanese quail embryo and cultured as previously described [12, 13]. Recombinant Murine Wnt3a was obtained from PeproTech/ThermoFisher. Wnt1 was expressed in QCE6 cells by transfection with a full-length Wnt1 cDNA (kind gift of Randall Moon) inserted into the eukaryotic expression vector pcDNA3 (ThermoFisher), in the presence of LipofectAMINE (ThermoFisher), as we described in a previous publication [13]. Mouse anti-β-Catenin was obtained from BD Transduction Laboratories, and applied to cultures following fixation protocols

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as previously reported [10, 13]. DyLight 488-conjugated secondary antibody (Jackson ImmunoResearch) was used to detect primary antibody labeling of β-catenin, with cells counterstained with 4′,6′-diamidino-2-phenyindole (DAPI; Life Technologies) to identify nuclei.

RESULTS AND DISCUSSION

Cell junctional β-catenin

Adherens junctions are cell membrane associated structures that join neighboring cells together (Figure 1), and which help provide structural support for the encompassing tissue. Adherens junctions also link the cell membrane to the actin cytoskeleton, which supports the overall structural unity of individual cells and the larger tissue [5, 14, 15]. The key component of these structures are the cadherin transmembrane proteins [16, 17]. Cadherins exist as multiple isoforms, with the most prevalent expressed forms being E-cadherin and N-cadherin. As the extracellular face of the adherens junction, cadherins facilitate cell-to-cell adhesion by binding to its like counterpart on neighboring cells. Within the cell, cadherins are connected to the actin cytoskeleton *via* their linkage to β-catenin and α-catenin — which despite their names, are not related proteins [2, 18]. The designation of these two proteins as "catenins" reflect their joint discovery as cadherin-associated molecules [19].

β-catenin belongs to the Armadillo family of proteins [20], of which the founding member is the *Drosophila* protein Armadillo [21]. β-catenin is the vertebrate Armadillo ortholog [2]. Affiliation with this protein family is marked by their display of multiple armadillo repeat regions that consist of a ~42 amino acid triple α-helical-containing motif [20, 22]. β-catenin binds to both the cytoplasmic end of the cadherin transmembrane proteins and α-catenin [23, 24], which in turn hooks onto filamentous actin and thus enables the adherens junctions to be connected to the cytoskeleton [18]. α-catenin, which is a vinculin-related molecule does not actually refer to a single protein, but comprises multiple isoforms — such as, αE-catenin and αN-catenin — whose individual expression is determined according to specific tissue and cell types [18, 25].

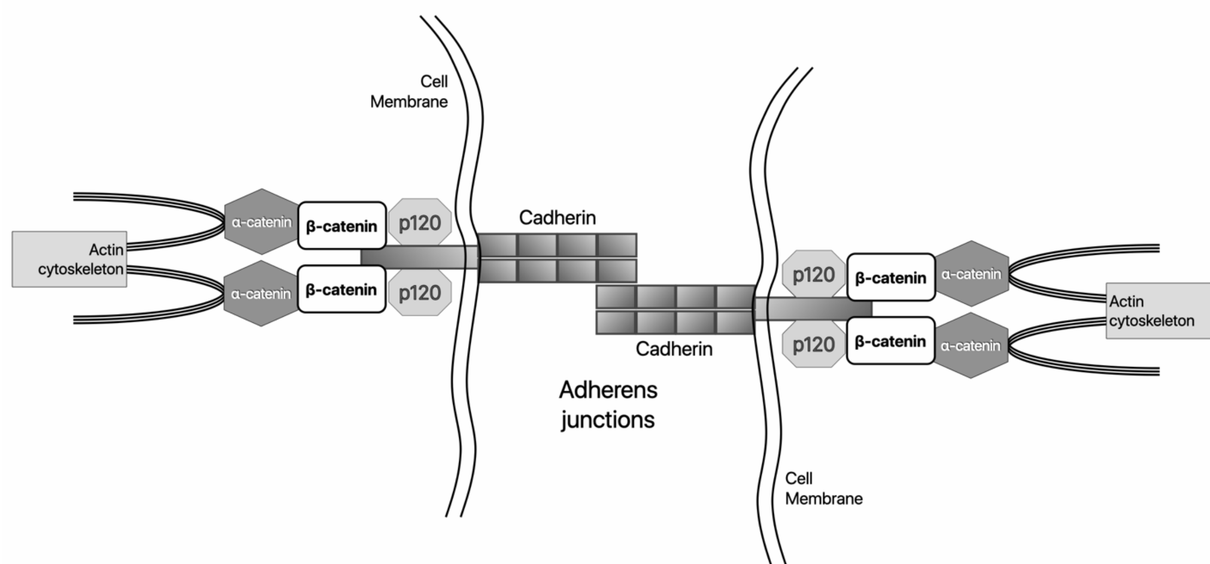

Figure 1. Schematic diagram of adherens junctions. Adjacent cells are anchored together *via* the homophilic binding to the extracellular domain of the cadherin family of transmembrane proteins. β-catenin binds to both the intracellular end of the cadherins and to α-catenin, with the latter protein hooked to filamentous actin. Thus, β-catenin serves as a bridge protein that allows the adherens junction to connect to the actin cytoskeleton. Despite the similarity of the names, α-catenin and β-catenin are not related proteins. However, β-catenin is related to p120, as both molecules are members of the Armadillo protein family. The p120 protein, which also binds to the cadherin cytoplasmic tail, supports the structural integrity of the adherens junction.

A second Armadillo family protein that is an essential component of adherens junctions is p120 catenin, which both helps assemble and supports the structural integrity of cadherin-catenin cell membrane complexes [26]. A third armadillo protein that contributes to cell junctional complexes is plakoglobin (also known as γ-cadherin). Plakoglobin is primarily associated with desmosomal junctions, although this molecule is occasionally exhibited in adherens junctions, where it may substitute for β-catenin [27].

Adherens junctions are essential components of epithelial cells where these cell membrane structures help knit together the cellular components of a tissue, promote the structural integrity of tissue, and transmit mechanical forces between cells [5, 14]. Mesenchymal cells also exhibit adherens junctions within actin-containing filopodia that extend out toward neighboring cells. Formation of adherens junctions is an important component of mesenchymal to epithelial transitions (MET). Accordingly, the dissociation of adherens junctions is a feature of epithelial to mesenchymal transformations (EMT) [28-30]. Adherens junctions also play a role in the regeneration and differentiation of stem cells, which cluster and form cell-cell contacts within niches of various tissues. Signals that promote the differentiation of stem cells will cause these cells to be released from their niches by disassembly of their cell junctions. Some of these cells may then reestablish adherens junctions when they give rise to differentiated cell types. Other cells, such as those that make up the blood will remain nonadherent [31-33].

Like many cellular structures, adherens junctions exhibit a dynamic life cycle. Generation of adherens junctions first begins in the endoplasmic reticulum with cadherin binding to β-catenin. The cadherin/β-catenin complex is then rapidly transported to the cell membrane where it joins to α-catenin, which afterwards becomes connected to the actin cytoskeleton. Thus, the function of β-catenin in adherens junctions is not a regulatory one. Instead, β-catenin facilitates the formation of the adherens junction and the movement of this nascent complex to the cell membrane. Moreover, β-catenin, *via* its linkage to α-catenin, serves as a bridge between cadherin cell membrane receptors and actin filaments within the cell [5, 6, 14, 34].

Tissue and cellular dynamics both determine the assembly and disassembly of adherens junctions. Changes in cell phenotype that occur during EMT, transitions from adherent to nonadherent phenotypes, cellular detachment during cell proliferation, and responses to mechanical stress can promote the disassembly and remodeling of adherens junctions. Even cells with a static phenotype undergo a process of junctional homeostasis where adherens junctions are recycled and reassembled regularly [35, 36]. *In vitro* studies with cell lines have indicated that the half-life of cell membrane-associated adherens junction proteins ranges from 10 to 13 hrs [37]. These structures are assembled, disassembled, recycled, and re-assembled at many points during an individual cell's lifespan. Some questions to consider include: what is the fate of β-catenin in this process and does the life cycle of the adherens junction impact β-catenin's availability for its role as a signal transduction molecule?

β-catenin as a second messenger in Wnt signal transduction

The function of β-catenin as a component of cell junctions has become overshadowed by its role as a transcriptional regulator that mediates Wnt signal transduction [9, 38, 39]. Wnts comprise a large family of secreted signaling proteins, ranging from 38-44 kD MW, that regulate pattern formation in the developing embryo, control stem cell behavior and fate, play significant roles in the homeostasis of various organs and tissues, and whose misexpression is associated with a variety of cancers [40-43]. The name 'Wnt' is an amalgam that pays homage to the two independent investigative paths that led to the discovery of these proteins [44]. Members of this molecular family were first identified in vertebrates by the characterization of genes that exhibited abnormally high levels of expression within mouse mammary tumors due to the proximity of mouse mammary tumor virus (MMTV) integration into the genome [45, 46]. Although the multiple genes that were upregulated by MMTV were not all genetically related, as a collection they were designated as "*int*" genes. Among the characterized *int* genes were a few molecules of previously unknown lineage, including the molecule assigned as *int1*, which was the first identified vertebrate Wnt. However, the first discovery of a *wn*t gene

occurred earlier from studies of genetic mutations that affect *Drosophila* development, although the novelty of the molecule that was encoded at this genetic locus was not recognized at the time. The *wingless* (*wg*) locus encodes for a segment polarity gene, which when mutated disrupts the normal anterior-to-posterior polarity of each segment within the *Drosophila* embryo [47, 48]. Later after the discovery of both *int1* and *Wg*, sequence analysis indicated that the two genes were orthologs. Because not all *int* genes were genetically related, the name Wnt was invented to group together molecules that shared sequence similarities with **W**ingless and I**nt**1. Thus, Int1 became Wnt1, and Irp (Int1 related protein) became Wnt2. To date, there are nineteen distinct *wnt* genes identified in the mouse and human genome [9]. In *Drosophila,* seven distinct *wnt* genes have been discovered including *wg*, and orthologs of *wnt5* and *wnt 7* [49, 50].

The first indicator that β-catenin was involved in Wnt signal transduction was established by *Drosophila* genetics. One of the genetic mutations that mimicked the *wg* mutant phenotype was *armadillo*, which was later discovered to be the *Drosophila* ortholog of β-catenin [51]. There were several other genes whose mutations also generated similar segment polarity disruptions as *wg*, such as *zeste-white 3 (zw3)* and *disheveled (dsh)* [52-54]. Because vertebrate β-catenin was initially characterized for its function in cell junctions, and its *Drosophila* ortholog Armadillo was demonstrated to be a second messenger of the *Drosophila* Wnt1 (i.e., Wg) pathway, much of the initial investigative focus on Wnt signaling tried to force fit cell junctional β-catenin as a signal transduction component of this pathway. That all changed with the near simultaneous reports by several laboratories that β-catenin is also exhibited in the nucleus and can act as a transcriptional regulator [55-57].

Nuclear β-catenin

The identification of β-catenin as a downstream mediator of Wnt signaling was the initial milestone in elucidating the mechanism of this signal transduction pathway. In addition to the localization of β-catenin to adherens junctions, it was soon discovered that cytoplasmic levels of β-catenin increase in response to Wnt signals [58]. The interpretation of this finding then and now, is that Wnt signaling is elaborated by its direct enhancement of the amount of cellular β-catenin. Still for a while, what that increased level of β-catenin did within the cell remained a mystery. Although it had been understood that β-catenin was acting as a second messenger for Wnt signals, there was a stumbling block in the study of this pathway as Wnt regulation of cellular phenotype and activity didn't seem to be correlated with what was then known about β-catenin's function. For example, one of the first functions in embryonic development ascribed to Wnts was in regulating the formation of the primary axis [59]. A classic assay for studying vertebrate axis formation involved grafting the organizer region from the dorsal side of a *Xenopus laevis* blastula-stage embryo into the ventral side of another similarly staged blastula - which results in an anterior duplication of the primary axis [60]. An identical axis duplication could be also obtained by injecting mRNA encoding Wnt1, Wnt3a, or Wnt8a into the ventral side of the frog blastula [61]. Injection of β-catenin mRNA at the same site would likewise promote secondary axis formation [62]. Yet, when the β-catenin sequence was altered to prevent its binding to cadherins, the injection of the mutated mRNA still induced a secondary axis [63]. Clearly, the mechanism by which β-catenin transduces Wnt signals did not involve its function in cell junctions. Thus, investigative efforts were begun to find additional binding partners for β-catenin in the attempt to ascertain previously unknown functional properties of this protein. Almost simultaneously, multiple laboratories reported that β-catenin binds to members of the lymphoid enhancer-binding factor/T cell factor (TCF/LEF) transcription factor family, and subsequently showed that in response to Wnt treatment, β-catenin can localize to the nucleus [55-57, 64, 65]. After these findings were published, many laboratories were able to show for many cell types, depending on culture conditions (e.g., exposure to Wnts), that β-catenin can be as readily detectable in the nucleus as it is at the cell membrane (Figure 2). Probably the reason that this was not noticed earlier was one of assumptions, as immunostaining for nuclear proteins requires distinct fixation conditions that may have not been previously used for visualizing a protein that was not expected to be located in the nucleus.

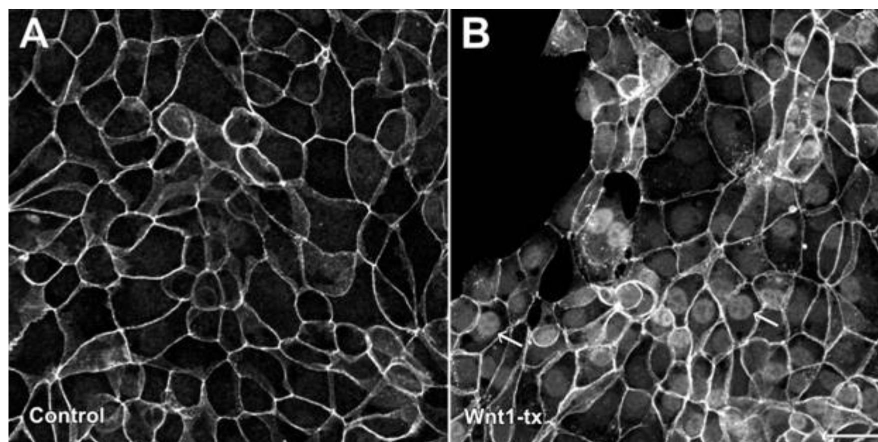

Figure 2. Cellular distribution of β-catenin in the absence or presence of Wnt1. The quail mesoderm cell line QCE6 was cultured without or with prior transfection with a mouse Wnt1 cDNA expression plasmid. After 5 days of incubation, cultures were immunolabeled for β-catenin. In both the (A) control and (B) Wnt1-transfected cultures, bright β-catenin staining was displayed at the cell periphery. However, only in the Wnt1 expressing cultures did β-catenin show antibody staining in the nuclei (arrows). Scale bar = 25 µm.

Wnt signal transduction

Before discussing β-catenin's role in Wnt signal transduction, it should be noted that there are many complexities to Wnt biology. Information in this specific section is mostly tangential to the focus of this essay on β-catenin, but is included here to direct readers who wish to explore the functional and mechanistic properties of Wnt proteins in greater depth. First among these many complications is the existence of multiple signal transduction pathways elicited by Wnt proteins. The Wnt signaling pathway involving β-catenin, which will be described in the following section, is designated as the canonical Wnt pathway [9, 66]. In addition, there are two characterized noncanonical Wnt pathways that do not involve β-catenin: the planar cell polarity and the Wnt/calcium pathways [67-69].

An additional level of complexity to Wnt signaling is due to the disparate functional activities that are displayed by members of this protein family. This heterogeneity in functional activities among Wnt proteins prompted these molecules to be classified into two separate groups [38, 70]. The Wnt1 or canonical Wnt group, which includes Wnt1, Wnt2, Wnt3, Wnt3a, Wnt8, and Wnt8a, appears to stimulate cellular responses exclusively by activating the canonical Wnt/β-catenin pathway. In contrast, the Wnt5A/noncanonical group that includes Wnt4, Wnt5A, and Wnt11, was defined by their propensity to trigger preferentially noncanonical Wnt pathways that do not involve β-catenin. The different pathways these disparate Wnt groups promote are thought to result from their relative affinities for different isoforms of the Wnt receptors, which are members of the Frizzled family of transmembrane proteins (defined in the next section). The differential activities of canonical and noncanonical Wnt proteins may also correlate with distinct accessory membrane proteins that interact with different Frizzled isoforms following Wnt ligand binding [38, 67]. Among the nineteen Wnt proteins that are encoded in the mammalian genome, the specific signaling groups most of these various Wnts fall into is ambiguous. Nor is it clear how definitive the demarcation is between the two Wnt groups. For example, the signaling properties of the Wnt5A group can switch from being noncanonical to canonical (i.e., β-catenin-mediated) pathway activators depending on the presence of specific Frizzled isoforms and/or accessory proteins [71, 72]. Thus, the molecular context within a cell matters (i.e., what specific Wnt pathway components are present in a receptive cell) whether a specific Wnt protein stimulates canonical or noncanonical pathways. That being said, at least for what has been reported for Wnt1, Wnt3a, and Wnt8, these canonical Wnts appear to transduce signal

exclusively *via* β-catenin, as there are no reported instances of their stimulation of noncanonical pathways. As the focus of this review is on β-catenin, the noncanonical Wnt pathways will not be discussed further in this essay. Information on noncanonical Wnt pathways can be obtained from the articles cited in this section plus several excellent published overviews listed on this topic.

Canonical Wnt signaling

The central participant of the canonical Wnt pathway is β-catenin, which transduces Wnt signals to the genome. An outline of the canonical Wnt pathway is shown in Figure 3, which highlights the principal molecular players of this signal transduction sequence. There are the Wnt cell membrane receptors which are the ten known members of the Frizzled family of transmembrane proteins. Also at the cell membrane are the low-density lipoprotein receptor-related protein (LRP) isoforms LRP5 and LRP6, which function as co-receptors for Wnt binding to the cell membrane [73, 74]. A key component in this pathway is the scaffolding protein Axin [58, 75], which coordinates the formation of a large molecular complex that includes a secondary scaffold protein, adenomatous polyposis coli (APC); the cytoplasmic phosphoprotein Dishevelled (abbreviated as either Dsh or Dvl), and the phosphotransferases casein kinase 1 (CK1) and glycogen synthase kinase 3β (GSK3β; the vertebrate ortholog of zw3) [9, 76]. These intracellular proteins comprise the β-catenin "Destruction Complex" that together determine β-catenin bioavailability within cells [9, 66].

When Wnts are absent, β-catenin is recruited to the Destruction Complex, where it becomes phosphorylated by the two kinases CK1 and GSK3β. This process marks β-catenin for ubiquitination and proteasomal degradation. However, when Wnts are present, its binding to the Frizzled and LRP5/LRP6 lure both Dsh and Axin to the cell membrane, which splinters the β-catenin Destruction Complex. Without an intact Destruction Complex, phosphorylation of β-catenin molecules no longer occurs, and forecloses its targeted degradation. β-catenin then is released into the cytoplasm, where its accumulation lays the foundation for its translocation to the nucleus. Nuclear β-catenin then regulates expression of Wnt target genes primarily *via* its interactions with members of the lymphoid enhancer-binding factor/T cell factor (TCF/LEF) DNA binding protein family [9, 66]. It is through this series of events, which are initiated by the canonical Wnt pathway, that various cellular changes can occur, such as alterations in cell lineage commitments, phenotype, differentiation, proliferative state, and functional activity.

The dual roles of β-catenin: what is the connection?

The above discussion outlined β-catenin's involvement in the formation/function of adherens junctions, as well its facilitating role in transducing Wnt signals to the nucleus. The issue that β-catenin investigators and Wnt biologists have grappled with is finding a definitive answer on how and whether there is interconnection between the two functional roles of β-catenin. There have been many interesting hypotheses proposed on the interconnectedness of β-catenin's role in cell adhesion and Wnt signal transduction. For example, a well-reasoned argument suggested that during evolution β-catenin acquired these two functions to "coordinate nuclear functions with cell-cell adhesion" [77]. It has been postulated that β-catenin acts as a pivot between cell adhesion and Wnt signaling, as if this protein can exhibit only one of its disparate activities at a given moment [78]. Along those lines, it has been suggested that adherens junction and nuclear binding partners compete with each other for a limited pool of cellular β-catenin, or the components of adherens junctions restrain canonical Wnt signaling by titrating the amount of available β-catenin [79, 80]. Others have argued that the two distinct roles of β-catenin allow this protein to act as a mechanosensor that regulates cell phenotype in response to tissue remodeling events [81, 82]. While these various hypotheses may all have validity, they still do not explain sufficiently how the cell membrane and nuclear venues of β-catenin are interconnected. To begin to address this question, let's review what is known about β-catenin expression in the cell to determine if a better understanding can be obtained on the global role this molecule plays in cell biology.

β-catenin can be exhibited in three different localities within the cell: the cell membrane, the nucleus, or cytoplasm. Cell membrane β-catenin is associated with cadherins within adherens

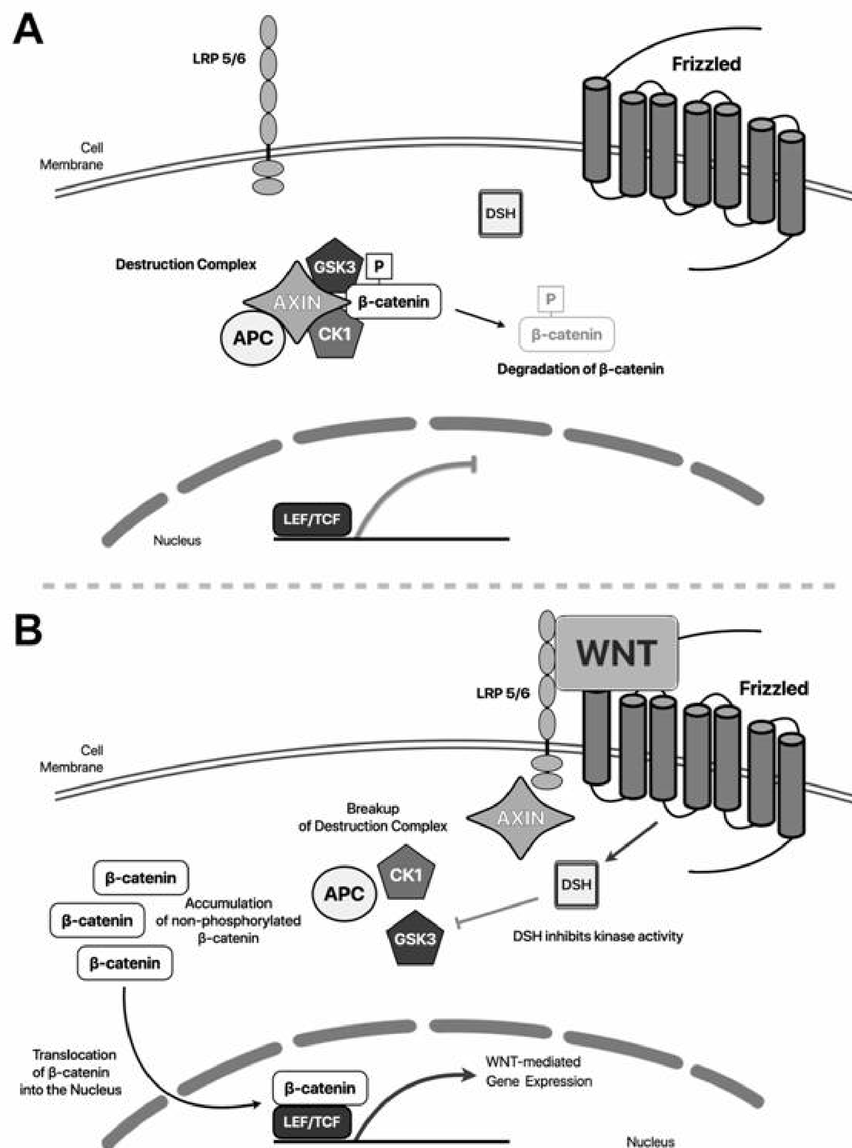

Figure 3. The canonical Wnt signal transduction pathway. (A) In the absence of Wnt binding to its cell membrane receptors, β-catenin is sequestered within a multiprotein cluster ("Destruction Complex") that includes Dishevelled (DSH), the two phosphotransferases casein kinase 1 (CK1) and glycogen synthase kinase 3β (GSK3β), and the scaffolding proteins Axin and APC. GSK3 and CK1 phosphorylation of β-catenin targets the latter protein for degradation by ubiquination. (B) Wnt binding to Frizzled and LRP5/6 transmembrane proteins recruits Axin to the cell membrane, which promotes the breakup of the Destruction Complex and transduces signal via DSH to inactivate GSK3β. The resulting inhibition of β-catenin's phosphorylation allows this protein to accumulate within the cytoplasm. Subsequently, β-catenin translocates to the nucleus and forms a transcriptional enhancer complex with LEF/TCF DNA binding proteins, which results in the up-regulation of Wnt target genes. Please refer to the text for references that provide a more in depth description of this signal transduction pathway.

junctions. Nuclear β-catenin is a transcriptional regulator that mediates Wnt signal transduction. β-catenin displayed within the cytoplasm doesn't have an equivalent discrete type of role, but whether or not it accumulates in this location determines whether this protein gets distributed elsewhere within the cell. There are several questions that need to be considered on the origin

of β-catenin within the cell. Does the β-catenin that is distributed to the cell membrane or nucleus represent two distinct pools of the protein, or is a single β-catenin pool diverted from one role to the other at different points in a cell's life span? Is β-catenin associated with either the cell membrane and nucleus derived from *de novo* synthesized and/or recycled protein?

Let's start with the synthesis of *de novo* β-catenin and examine how it is distributed afterward within the cell. When cells begin to establish cell-to-cell junctions— whether cells are undergoing mesenchymal to epithelial transitions, reestablishing junctional contacts following a spurt in cell proliferation, or during the normal homeostasis of adherens junctions that involves regeneration of existing junctions— the components of these newly formed junctions emanate primarily from newly synthesized protein [83, 84]. In contrast, Wnt enhancement of β-catenin levels in the cell results from the prevention of the latter protein's degradation and not to the direct stimulation of its *de novo* synthesis [9, 62]. That being said, it is possible that newly generated β-catenin induced by other stimuli could contribute to the nuclear distribution, when combined with Wnt signals that would enhance β-catenin levels by preventing its rapid turnover, and/or when cell junctional binding partners are not expressed at high enough levels to competitively prevent β-catenin association with nuclear partners. It should also be noted that although nuclear translocation of β-catenin is mostly associated with Wnt signaling, the relocation of β-catenin into the nucleus doesn't always require Wnt's involvement [85, 86]. For example, this latter scenario may come into play among *in vitro* cultures when cells plated at low density in the absence of Wnt form new cell junctions as they proceed to reach confluency. When cultures are still at subconfluent densities and starting to form cell-cell junctions, some nuclear staining β-catenin may be observed in addition to immunolabeling for this protein at the cell membrane (Figure 4A). However, by the time cells reach a confluent density, when cell junctions are fully exhibited along the entire cell periphery, β-catenin staining remains bright at all the cell borders, but is no longer apparent in the nuclei of Wnt-negative cultures (Figure 4B).

As discussed above, Wnt binding to the cell membrane disrupts the β-catenin destruction complex, thereby increasing β-catenin bioavailability within cells. β-catenin that is released from the destruction complex in response to Wnt signals is not customarily distributed to adherens junctions. In contrast, this Wnt-mediated freeing of β-catenin from proteosomal degradations allows this protein

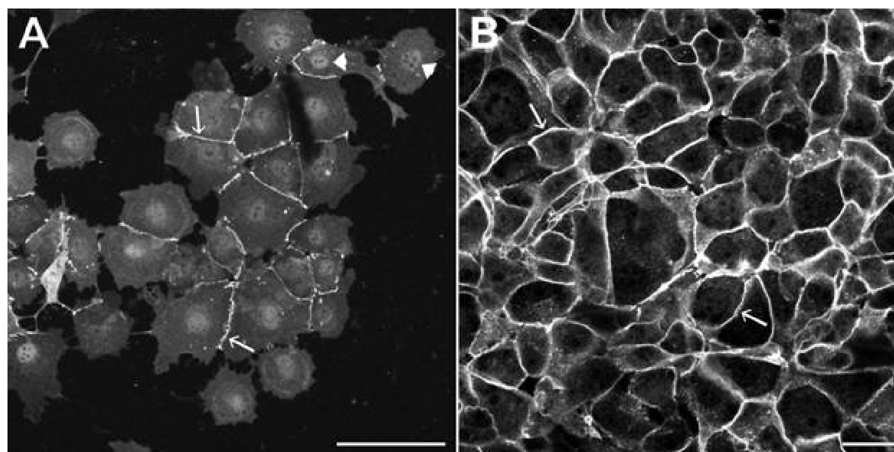

Figure 4. Cellular localization of β-catenin as a function of cell confluence. QCE6 cells were plated at low cell density and cultured for either (A) 1 day or (B) 5 days, in the absence of canonical Wnt exposure, prior to immunolabeling for β-catenin. (A) At low cell densities, β-catenin is exhibited both at the borders (arrows) of neighboring cells that are forming attachments, but also in the nuclei (arrowheads) of the still sparsely populated cells. (B) However, by the time the cultures reached confluency, β-catenin labeling only remained exhibited at the cell periphery (arrows), but was absent from nuclei. Scale bars = 25 µm.

to translocate into the nucleus, where it will exert transcriptional control of Wnt-target genes. So where is the supply of β-catenin associated with the destruction complex coming from? Does it come from a pool of newly synthesized β-catenin that Wnt saves from immediate destruction? It is conceivable that the low level *de novo* β-catenin that is generated for the homeostasis of adherens junctions could be amplified by a decreased turnover rate in response to Wnt signaling. Or is the bulk of the β-catenin that translocates to the nucleus primarily recycled from disassembled adherens junctions, when β-catenin becomes saved from degradation by canonical Wnt signaling? Well actually, a number of studies do support the latter supposition. For example, during epithelial-mesenchymal transitions, β-catenin released from the breakdown of adherens junctions has been shown to account for the pool of this protein that provides for Wnt-mediated gene regulation [87]. Release of stem cells from their niches following tissue damage also increases cytoplasmic levels of β-catenin after their release from disassembled cell junctions [88]. In the embryo, mechano-transducing events that promote turnover in adherens junctions also correspond to increased levels of nuclear β-catenin. Mechanical forces that are brought to bear on tissues by tumor growth can also disrupt cell junctions and lead to Wnt-mediated β-catenin signaling [81, 82].

Our work studying mesenchymal stem cells (MSCs) has indicated that treatments with canonical Wnts can promote the accumulation of cytoplasmic β-catenin of cells that undergo a transition from an adherent to nonadherent phenotype, and with that a change in the phenotypic potential [10]. As shown in Figure 5, cultures of mouse bone marrow MSCs will generate large numbers of nonadherent

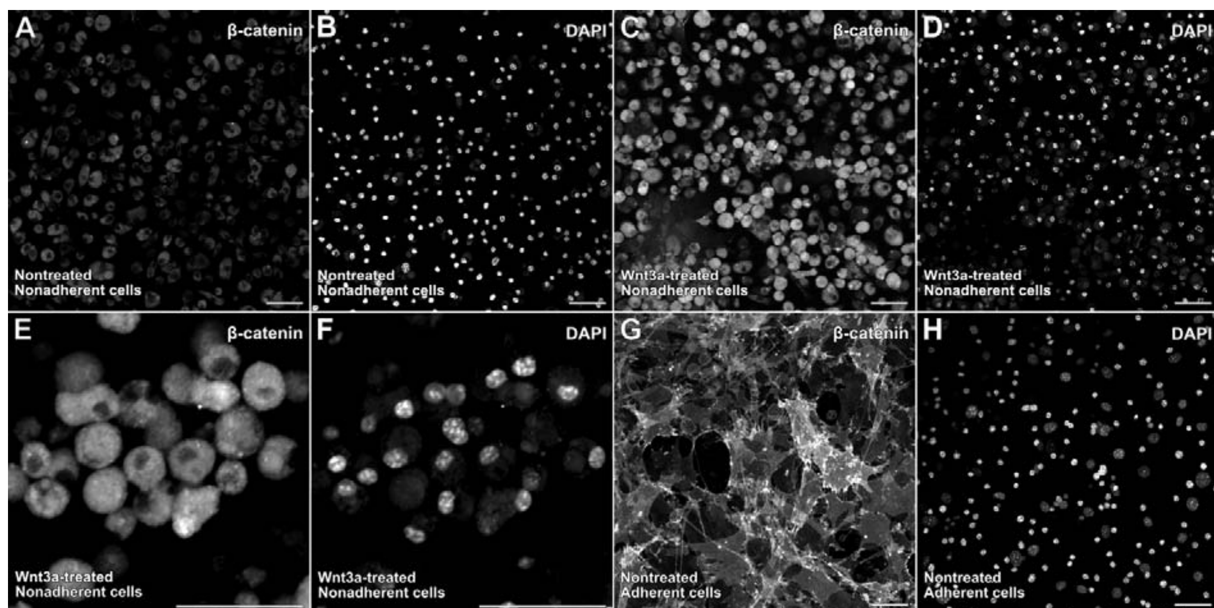

Figure 5. Wnt3a enhances cytoplasmic β-catenin accumulation in nonadherent cells. Cultures of mouse bone marrow MSCs were immunostained for β-catenin and nuclear counterstained with DAPI, which are shown side-by-side for individual cellular fields. Mouse bone marrow MSCs were grown to confluency under standard conditions to generate large numbers of nonadherent "phase bright" cells that exhibit a stem cell phenotype [10, 11]. These nonadherent cells were transferred to gelatin-coated dished for 2 days in (A, B) medium alone or (C-F) in the presence of Wnt3a protein. (A, B) While nontreated nonadherent MSC-derived cells only displayed a low level fluorescence after antibody labeling, the Wnt3a-treated cultures, shown at (C, D) low and (E, F) high magnification displayed many brightly stained β-catenin-positive cells, whose staining was localized to the cytoplasm. In contrast (G, H) the remaining adherent cell layer of nontreated MSC cultures showed β-catenin immunoreactivity that was exhibited solely at the cell membrane. Scale bars = 50 μm.

cells after reaching confluency. When these cultures are exposed to WNT3a, accumulation of cytoplasmic β-catenin will be exhibited by greater numbers of the nonadherent cells, which would provide a new source of β-catenin that is available for transmitting Wnt signals into the nucleus [10].

To recapitulate the points made above, Wnts do not appear to directly induce *de novo* β-catenin production, but increase the accumulation of this latter protein by preventing its degradation. β-catenin that accrues in the cytoplasm will then be available to move into the nucleus and exert transcriptional control over receptive gene targets. Available evidence indicates that the primary source of β-catenin that Wnt signals save from being destroyed is recycled adherens junctions. Recycling of adherens junctions does occur during normal homeostasis of these structures, and thus Wnts could then presumably rescue this β-catenin pool from destruction and allow for its distribution to the nucleus. But that supply of β-catenin would represent a relatively minor source for Wnt signal transduction. In contrast, the times when cells become optimally receptive to canonical Wnt signals are during events where cells exhibit a far greater breakdown of its adherens junctions — which for example, occurs during responses to mechanical stress, epithelial-mesenchymal transformations, transitions between adherent to nonadherent phenotypes, or part of a proliferative process when cells temporarily detach from their neighbors. It is during these episodes when Wnt signaling would be able to free up the highest amount of pre-existing β-catenin from the proverbial scrap heap and re-direct its function to its role as a transcriptional regulator. It is at these inflection points where Wnts would exert its greatest impact on cell and tissue biology. In other words, the major interrelatedness of cell membrane-associated and nuclear β-catenin is that the former source of this protein provides the substrate for the latter regulatory function of this protein at key moments during the lifespan of a cell, tissue, organ, or organism.

CONCLUSION

β-catenin performs two major functions in cells. As a cell membrane protein, β-catenin serves as a connection between cadherin cell adhesion molecules and the actin cytoskeleton. β-catenin is also a transcriptional regulator that transduces signal induced by Wnt binding to cells. By carrying out these two tasks, β-catenin helps define cell fate, potential, phenotype, function and proliferative state, which in turn impacts embryonic development, tissue remodeling and homeostasis. Aberrant expression or mutation of β-catenin may also cause organs to function abnormally and promote cancer formation [89-91]. The question that biologists have long grappled with is how the two principal functional roles of β-catenin are interrelated [92, 93]. Is there a cooperative relationship between the two pools of active β-catenin to adjust cell phenotype/function according to the needs of a tissue, organ, and organism? Is there a yin-yang where two opposing and complementing facets of β-catenin co-regulate cell function? Do nuclear and cell membrane-associated binding partners compete with one another for the cell's free β-catenin? While all those conditions may come into play at times, the main feature that characterizes the relationship between cell membrane and nuclear β-catenin is that one pool not only provides most of the supply for the other, but that the disassembly of β-catenin from cell junctions determines the critical moments in a cell's and tissue's existence when it optimally responds to Wnt signaling. Thus, we postulate that Wnts have their main impact by triggering cells at favorable times when there is the opportunity to free up high amounts of β-catenin for regulating Wnt target genes. It is precisely at those points in the life span of a cell, when adherens junctions are disrupted and recycled, that the timely exposure to Wnts will have the maximum impact in shifting cell fate, phenotype, and function.

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CONFLICTS OF INTEREST STATEMENT

The authors declare that there are no conflict of interests regarding the publication of this paper.

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