Beta Catenin: An Essential Player in Both Cell-Cell Adhesion and Wnt Transcriptional Regulation

Jim Heffernan, Ben Hierlmeier, Katie Strobel and Devan Van Lanen-Wanek
Faculty Advisor: Michelle Harris , Ph.D. Research Mentor: Jeff Hardin, Ph.D.
University of Wisconsin-Madison

ABSTRACT

β-catenin is a multi-functional protein involved in two essential cellular pathways: cell-cell adhesion and transcriptional regulation[8]. β-catenin contains 12 armadillo repeats capped by a C-helix. An amino acid important for β-catenin's electrostatic interactions with ligands is Lys-435 known as the "charged button"[1]. Given its diverse functions, β-catenin has diverse binding partners. β-catenin's role in cell-cell adhesion is essential in the early stages of embryogenesis, and defective β-catenin results in inviable embryos[7]. This association is mediated by binding to E-cadherin. β-catenin also mediates events regulated by the Wnt pathway, through its binding of Tcf/Lef family transcription factors via its charged button domain. Wnt signaling is an important regulator of diverse events, including differentiation during embryonic development, and regulated proliferation. Its misregulation by APC, a protein utilized in the Wnt pathway involved in marking β-catenin for degradation, is an important event in colon cancer[5]. Although several binding affinities of β-catenin have been described, several questions remain. In particular, the function of the C-helix is poorly understood. Further studies could help illuminate the role of the C helix, using various biochemical assays and *in vivo* analyses in genetic model systems. A physical model would enhance the study of β-catenin's interaction with its binding partners. An online tutorial would also serve as a valuable teaching tool to illustrate key aspects of the structure of β-catenin that constrain and facilitate its interactions with its binding partners.

β-CATENIN'S ROLES

- β-catenin exists in two distinct populations[8]:
  - •Membrane associated pool binds to cadherins and α-catenin in the adherens junction
  - •Cytoplasmic and nuclear pool that acts in regulating transcription and only accumulates in response to the Wnt growth factor signal
- In the absence of a Wnt signal or mutation in the pathway, β-catenin is targeted for degradation by a large protein complex involving APC, GSK3β, and axin.
  - •The complex phosphorylates β-catenin near the N-terminus and the protein is then recognized and degraded by an ubiquitin-protease system.
  - •If β-catenin is not marked for degradation it associates with Tcf in the nucleus and promotes transcription of genes relating to cell growth and proliferation.
- The binding interactions with Tcf, APC, and E-cadherin take place in a similar manner, in the positively charged groove formed by the armadillo repeats.
  - •One interaction common to the ligands occurs at a "charged button" comprised of a positively charged lysine residue on the β-catenin and a negatively charged residue on the ligand[1].
- •Disruption of either pathway has deleterious implications
  - •Failure to form adherens junctions during embryogenesis leads to an inviable embryo[6].
  - •Failure to regulate the Wnt pathway can lead to uncontrolled cellular proliferation and thus cancer, most commonly manifested when APC mutations lead to colon cancer[5].
- Most higher organism have single β-catenin protein which performs both functions; however, *C. elegans* has one for adherens junctions (HMP-2) and one for transcription (WRM-1)[7].
  - •The most striking structural difference between these two is the presence of the "C-terminal helix".

FUTURE DIRECTIONS

- Many higher organisms have multiple genes coding for different forms of β-catenin with specialized functions.
- Our collaborating researcher is interested in looking at β-catenin forms in *C. elegans* and the correlation between structure and function in formation of adherens junctions during embryogenesis.
  - • In this system, BAR-1 functions as part of the Wnt pathway and its structure has a "C-terminal helix" in addition to its twelve armadillo repeats.
  - •In contrast, HMP-2 functions in the formation of the adherens junctions and its structure does not contain the C-terminal helix.
- Future studies would seek to determine if the C-helix is the structural component that allows BAR-1 to act in transcriptional regulation with Tcf, given that HMP-2 is not sufficient in this pathway.
  - •This would be done by creating a hybrid HMP-2 with the extra helix attached at the C-terminus, then running assays for activity of this mutant protein in Wnt signaling.
  - • This would lead to a greater understanding of how structure and function are correlated in different forms of β-catenin in *C. elegans*, and open the doors for similar studies of the varied forms in higher organisms.

The CREST Program is funded by grant #1022793 from NSF-CCLI.

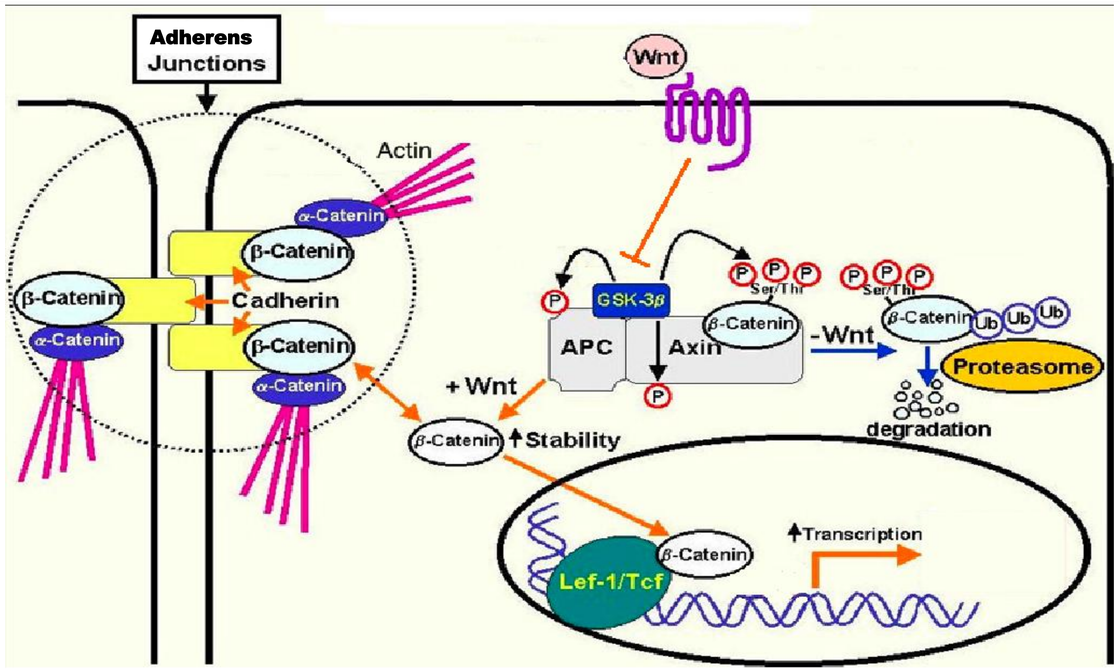


Figure 1. A schematic diagram of the two major cellular pathways that involve β-catenin, adherens junctions on the left and transcriptional signaling via the Wnt pathway on the right. Figure was modified from reference 4.

METHODOLOGY

CRYSTALLOGRAPHY

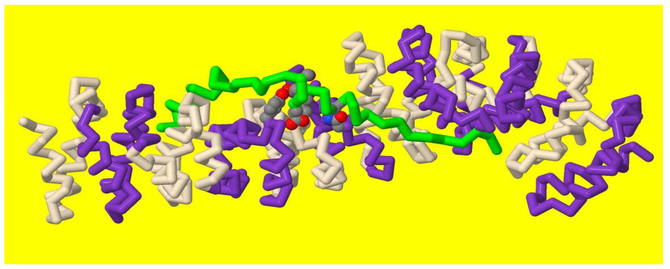
- Full length β-catenin armadillo repeat domain and C. helix have been purified in *E. coli*[2], zebrafish, and vertebrates[3].
- Xing et al (2008)[3] crystallized human β-catenin fragment with the repeats and the C terminal domain solving the structure by molecular replacement technique.
  - Can get the helix C in the C-terminal domain and the alpha helix in the N terminus but the other parts in both regions appear to be difficult to crystallize.

COIMMUNOPRECIPITATIONS

- Graham *et al* (2001)[1] used coimmunoprecipitations to ensure Tcf-4 bound to the B-catenin .
- •Also tested if the N-terminus of Tcf-4 was necessary for binding by immunoprecipitating both wild type and mutant Tcf-4 with the β-catenin.

SEQUENCE ALIGNMENTS

- Ligand binding regions on β-catenin were aligned comparing E-cadherin, APC, and Tcf-3 using CLUSTAL-W[2] finding similar binding area.



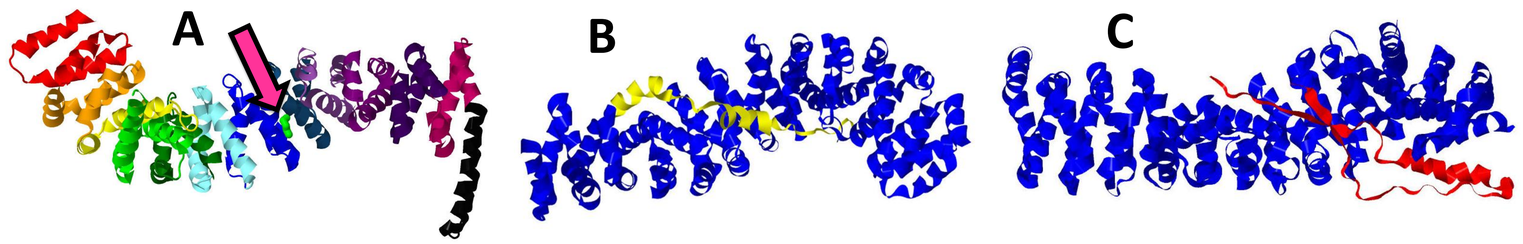


Figure 2 (above). Our physical model of β-catenin bound with Tcf-4. Alternating armadillo repeats are in purple and blanched almond, Tcf-4 is in lime, and relevant amino acid side chains to ligand binding are highlighted using the CPK color scheme.

Figure 3. Various β-catenin structures: **A.** The full length zebrafish β-catenin (2Z6G) with each armadillo repeat in a different color, C-helix in black, and Lys charged button side chain in lime(indicated by the pink arrow). **B.** Co-crystal (1JDH) of β-catenin (blue) with Tcf4 (yellow). **C.** Co-crystal (1I7X) of β-catenin (blue) with E-cadherin (red). Note the overlap in binding sites between the ligands in B and C.

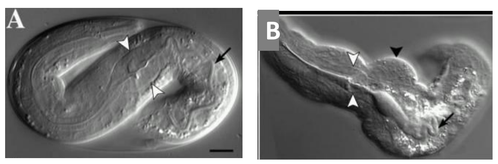


Figure 4. A. Wild type fully elongated *C. elegans* embryo . B. *hmp-2* mutant embryo phenotype.[9]

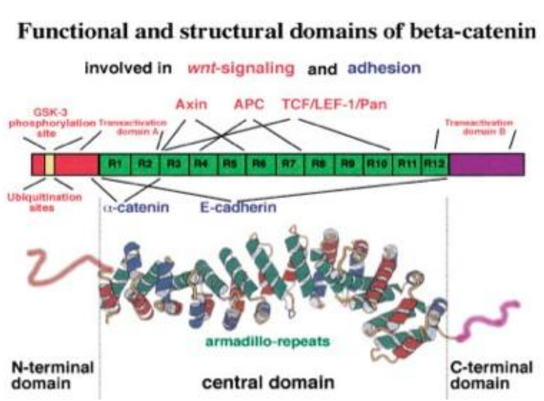


Figure 5 (right). A linear representation of the β-catenin gene with a corresponding structural representation, detailing important binding sites and relevant structural details.[10]

WORKS REFERENCED

1. Graham, Thomas; Ferkey, Denise; Mao, Feng; Kimelman, David; and Xu, Wenqing (2001). Tcf4 can specifically recognize β-catenin using alt. conformations. Natural Structural Biology 8(12): 1048-1052.
2. Huber, Andrew, and Weis, William (2001). The structure of the β-catenin/E-cadherin complex and the molecular basis of diverse ligand recognition by β-catenin. Cell 105: 391-402.
3. Xing, Yi; Takemaru, Ken-Ichi; Liu, Jing; Berndt, Jason; Zheng, Jie; Moon, Randall; and Xu, Wenqing (2008). Crystal Structure of a Full-Length β-Catenin. Structure 16: 478-487.
4. Hoover, B. A. (2005). Beta-catenin Mediated Wnt Signaling as a Marker for Characterization of Human Bone Marrow-Derived Connective Tissue Progenitor Cells, *The Journal of Young Investigators*, 12 (5) retrieved 21 April 2011 from http://www.jyi.org/research/re.php?id=190
5. Katharine Eklof Spink, Sofiya G.Fridman, William I.Weis. (2001). Molecular mechanisms of b-catenin recognition by adenomatous polyposis coli revealed by the structure of an APCεb-catenin complex . The EMBO Journal 20(22): 6203-6212.
6. Ronen Zaidel-Bar, Michael J. Joyce, Allison M. Lynch, Kristen Witte, Anjon Audhya, and Jeff Hardin. (2008). The F-BAR domain of SRGP-1 facilitates cell–cell adhesion during C. elegans morphogenesis. Journal of Cell Biology 191(4): 761-769.
7. Adam V. Kwiatkowskia, Stephanie L. Maidenb, Sabine Pokuttac, Hee-Jung Choic, Jacqueline M. Benjamina, Allison M. Lynchd, W. James Nelsona, William I. Weisc, and Jeff Hardin. (2010). In vitro and in vivo reconstitution of the cadherin–catenin–actin complex from Caenorhabditis elegans. PNAS 107(33): 14591-14596.
8. Hendrik C. Korswagen, Michael A. Herman, Hans C. Clevers. (2000). Distinct b-catenins mediate adhesion and signalling functions in C. elegans. Nature 406: 527-532.
9. Costa, M; Raich, W; Agbunag, C; Leung, B; Hardin, J; and Priess, JR (1998). A putative catenin-cadherin system mediates morphogenesis of the Caenorhabditis elegans embryo. J Cell Biology 141(1): 297-308.
10. Schneider, S; Finnerty, J; Martindale, M (2003). Protein evolution: structure-function relationships of the oncogene beta-catenin in the evolution of multicellular animals. J Exp Zool B Mol Dev Evol 295(1): 25-44.