A closer look at WNT/CTNNB1 signaling

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CHAPTER 1

Aim and outline of the thesis
DISCOVERY OF THE EVOLUTIONARY CONSERVED WNT/CTNNB1 PATHWAY

In this PhD thesis we will dive into the world of WNT/CTNNB1 signaling. Parts of this ancient signaling cascade first appear in *Dictyostelium discoideum*, better known as slime mold (reviewed in Holstein, 2012). Although it is not an animal, this social amoeba forms complex multicellular structures for which it relies on its primitive form of beta-catenin (CTNNB1): also known as aardvark (reviewed in Hardwood, 2008). If you continue to read the rest of my thesis, which is focused around the role of CTNNB1 in mammalian WNT/CTNNB1 signaling, you might wonder what the relevance of the slime mold is. Simply put, it highlights the fundamental relation between multicellularity and the control of CTNNB1 and thereby the high importance of understanding this particular pathway when studying complex organisms such as humans.

It has been roughly 40 years since the true metazoan WNT/CTNNB1 pathway was discovered independently in *Drosophila melanogaster*, the fruit fly, and in *Mus musculus*, the house mouse. WNTs are secreted proteins that activate downstream signaling that is essential in determining cell fate. The name ‘WNT’ reflects its separate discoveries in two species (Nusse et al. 1991). The ‘W’ is derived from the *Drosophila melanogaster* gene, which is called *wingless* due to malformation of the wings upon its mutation (Sharma and Chopra 1976), and the ‘NT’ is derived from the *int-1* gene that was discovered in an insertional mutagenesis screen, where proviral-insertion mediated overexpression induced the formation of mammary tumors in mice (Nusse and Varmus 1982). The latter highlights that WNT/CTNNB1 signaling is not only involved in normal development of multicellular animals, but also that its deregulation can cause serious diseases, such as cancer. Therefore, a detailed understanding of the WNT/CTNNB1 pathway will help us not only understand its fundamental role in tissue development and maintenance, but might also lead to improved treatments in diseases where this pathway is disrupted.

MECHANISM OF THE WNT/CTNNB1 PATHWAY AND RESEARCH METHODS

The following chapter will go into extensive detail on our current understanding of the molecular mechanism of WNT/CTNNB1 signaling. Here, I will briefly introduce some key signaling steps to substantiate the scope of this thesis. The central event in WNT/CTNNB1 signaling is the accumulation of CTNNB1. Conserved from *Dictyostelium* to mammals, CTNNB1 has continued to serve two main roles, namely maintaining cell adhesion and regulating cell fate (Grimson et al. 2000; Valenta et al. 2012). In metazoan WNT/CTNNB1 signaling, its main effector function is to drive gene transcription together with TCF/LEF
transcription factors, which ultimately leads to the cellular programs that drive important developmental and homeostatic processes in multicellular animals. In absence of WNT, a CTNNB1 destruction complex ensures the degradation of CTNNB1, thereby keeping its levels low. Binding of WNT protein to its receptors inhibits this complex, leading to higher levels of CTNNB1 in the cell and expression of target genes.

**FIGURE 1:** (adapted from Chapter 3, Figure 1A) Cartoon depiction of WNT/CTNNB1 signaling. Without WNTs present (left, “OFF”), a destruction complex consisting of AXIN, APC, CSNK1A1 and GSK3 captures and phosphorylates cytoplasmic CTNNB1, leading to its subsequent proteasomal degradation, resulting in low levels of CTNNB1 in the cytoplasm and nucleus. When WNT proteins bind their FZD and LRP receptors (right, “ON”) DVL inhibits the destruction complex and CTNNB1 accumulates in the cytoplasm and translocates to the nucleus. There it promotes the transcription of target genes, such as AXIN2, as a co-activator of TCF/LEF transcription factors.

Much of what we know about WNT/CTNNB1 signaling today, we owe to rigorous genetic analyses and detailed biochemical studies. One limitation of these methods, however, is that they often don’t capture the dynamic nature or heterogeneity of signaling events. In addition, many traditional read outs for WNT/CTNNB1 signaling have been binary. For example, nuclear accumulation of CTNNB1 that is visible on immunohistochemistry is widely used to determine whether cells have active WNT/CTNNB1 signaling. These types of readouts could make it seem that WNT is only active in very limited settings. However, there is increasing evidence that small changes in this pathway are biologically relevant in homeostasis and disease. To observe more subtle effects of WNT/CTNNB1 signaling, more dynamic, sensitive and quantitative studies are needed.
AIM AND OUTLINE

The aim of my PhD research was to use novel approaches to gain a better understanding of the dynamics of endogenous WNT/CTNNB1 signaling. **Chapter 2** provides an extensive overview of our current understanding of the molecular mechanisms of WNT/CTNNB1 signaling – with a specific focus on the CTNNB1 destruction complex – and of how this knowledge can be applied for the development of anti-cancer treatments. In **Chapter 3**, we use CRISPR/Cas9-mediated gene editing to fluorescently label CTNNB1 in mammalian HAP1 cells, to measure its molecular dynamics with imaged based techniques at physiological levels. We then use these quantitative data to build a new, minimal computational model of WNT/CTNNB1 signaling, which reveals that in addition to destruction complex inhibition, two additional nodes regulate WNT/CTNNB1 signaling outcome, namely nuclear/cytoplasmic shuttling and nuclear retention. In **Chapter 4**, we characterize a novel mouse model which also expresses fluorescent CTNNB1 at the endogenous level. In addition to showing its viability and functionality, we demonstrate that the molecular dynamics in primary mouse cells match our previous observations in HAP1 cells. Moreover, CTNNB1 increased only few-fold even in cell populations that are traditionally considered to have ‘high’ WNT signaling, such as the intestinal crypt. Together Chapter 3 and 4 highlight that WNT/CTNNB1 signaling is tightly regulated and that small changes in CTNNB1 levels are biologically significant. In **Chapter 5**, we perform insertional mutagenesis screens to uncover regulators of WNT/CTNNB1 signaling. By combining the HAP1 cells with endogenously tagged CTNNB1 from Chapter 3 with an established transcriptional reporter for WNT/CTNNB1 signaling, we separate regulators that act at the level of CTNNB1 stability or transcriptional activity. We follow up on SETD1B, a histone modifier which we find to regulate WNT/CTNNB1 transcriptional output. **Chapter 6** discusses the most important results of the former chapters and the relevance of small and dynamic changes in WNT/CTNNB1 signaling and signal transduction in general. In addition, we also highlight the challenges and possibilities that come with measuring these processes, which we further underline with several strategies we started to develop, discussed in the addendum **Chapter 7**.
BIBLIOGRAPHY


