

# Gone with the Wnt: Role of GSK-3

Team Members: Jenna Greene, Matthew Griesbach, Grant Hoppel, Thomas Hougard, Daniel Lawniczak, Pierce Lindell, Samantha Miller, Rachel Reiter, Grant Sadowski, Morgan Stachowski, Nicholas Tolson

Advisor: Stephen Plum

Kettle Moraine High School, Wales WI

Mentor: Anil Challa, PhD

## Summary

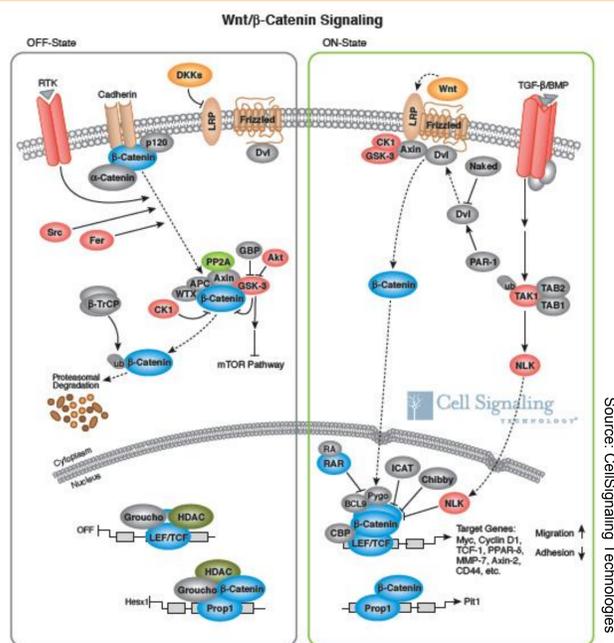
The Wnt/ $\beta$ -catenin pathway is a crucial cell signaling mechanism regulating gene expression throughout embryonic development and adult life. Inhibition of the pathway can have both desirable and undesirable effects. For example, inhibition during embryogenesis can lead to developmental defects, while later in life inhibiting this pathway provides a promising method of treating certain types of cancers and neurological disorders like Alzheimer's disease and bipolar disorder. One avenue of inhibition is the Glycogen Synthase Kinase 3 (GSK-3). Inhibition of GSK-3 during embryogenesis results in developmental defects due to altered expression of genes related to cell proliferation and cell death. Lithium chloride, a simple salt, and Phosphoaminophosphonic Acid-Adenylate Ester (AMP-PNP or ANP), a nucleotide, are two effective inhibitors of GSK-3. Both of these molecules inhibit GSK-3 function by interacting with two different sites of the protein tertiary structure. By studying how these and other molecules structurally interact with GSK-3, it is possible to not only inhibit the Wnt pathway but also treat various diseases.

## Background

### Cell Signaling in Development and Disease

Eukaryotic multicellular organisms utilize several biochemical signaling mechanisms to facilitate cell-to-cell communication and enable cells to interpret their extracellular environment. The Wnt/ $\beta$ -catenin signaling pathway is evolutionarily conserved and has been very well studied during cell proliferation and differentiation. The Wnt pathway plays an important role in orientation and specialization of embryos. In the absence of a secreted protein ligand (Wnt), cells are in an OFF state, and the signaling gets turned ON in the presence of the ligand. Both the OFF and ON states have biological effects in normal development of an embryo and physiology of cells. But inhibition of signaling or aberrant activation of the pathway can have deleterious consequences.

Glycogen Synthase Kinase-3 (GSK-3) is a serine/threonine protein kinase, which plays a key role in Wnt/ $\beta$ -catenin signaling during embryonic development. In addition, it has also been shown to play a role in a number of diseases like inflammation, cancer, Alzheimer's disease and bipolar disorder.



Source: CellSignaling Technologies

## Inhibition of Glycogen Synthase Kinase-3

### Lithium Chloride (LiCl)

Lithium inhibits GSK-3 both directly and indirectly. Interaction between magnesium and GSK-3 allows GSK-3 to phosphorylate many substrates. Magnesium ions are important for the binding of ATP (substrate) in the active site of GSK-3. In the presence of lithium, magnesium is unable to interact with the active site of GSK-3. The competitive inhibition between lithium and magnesium shows the direct inhibition of GSK-3 by lithium. In an alternate, indirect way, lithium increases the inhibitory phosphorylation of a critical serine residue in GSK-3, causing its inactivation.

Previous studies using 300 mM LiCl solution showed severe defects of both developing frog and fish embryos. We tested the effect of lower concentrations of LiCl (30 to 300 fold lower than previously studied concentrations) on early embryonic development of zebrafish.

### Experimental Set up

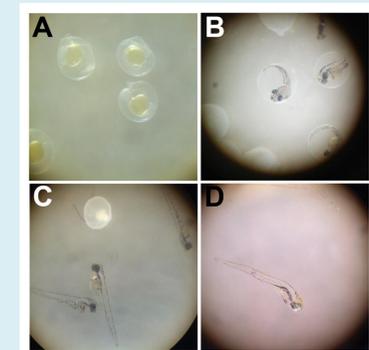
Treatment at	Final concentration of Lithium Chloride			
	0 mM	1 mM	3 mM	10 mM
4 hpf	Control	No visible phenotype	No visible phenotype	Visible Phenotype
10 hpf	Control	No visible phenotype	No visible phenotype	Visible Phenotype

Embryos were treated with LiCl at mid-gastrulation stage and observed for the next three days. While 1 and 3 mM LiCl solutions did not show any visible phenotypes, embryos treated with 10 mM LiCl showed the following phenotypes at different frequencies:

1. Slower growth compared to control (No LiCl).
2. Enlarged eyes during at early stages.
3. Reduced melanin pigmentation.

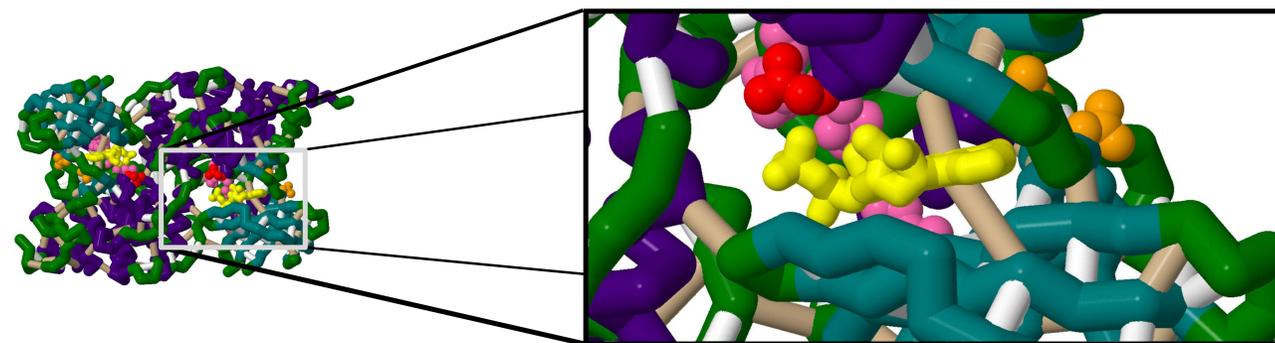
Further studies with experimental replicates have to be carefully done to confirm these observations.

### Results



Embryos treated with 10 mM LiCl. Panel A shows 18-20 hour old embryos; panels B and C show 42-48 hour old embryos; panel D shows 3+ day old embryo. (Images taken with cell phone cameras under a binocular dissection microscope).

## Structure of GSK-3 $\beta$ -ANP complex



GSK-3 is made of two mostly symmetrical subunits. The inside of the protein contains numerous  $\alpha$ -helices, while the ends form  $\beta$ -sheets, with one sheet rolling up to form a  $\beta$ -barrel. The natural substrate, adenosine triphosphate (ATP), binds near the ends of the protein between the barrel and the sheet, with specific amino acids enabling the attachment of particular subgroups of the ATP molecule. The nitrogen base (adenine) is held in place by Asp133 and Val135 (colored orange), the sugar attaches to Ala83 (red), and the phosphates are attached to Lys85, Glu97, Asp181, Lys183, Asn186, and Asp200 (pink) with the help of conserved magnesium ions and waters that are not being shown. ANP is shown in yellow.

## 1PYX Model

The 1PYX model was created by crystallizing GSK-3 with adenylyl imidodiphosphate (AMP-PNP), otherwise known as ANP. ANP is non-hydrolyzable analog of ATP and acts as a competitive inhibitor of GSK-3 and other ATP dependent systems/enzymes. Because of its structural similarity to ATP, ANP can efficiently bind to the active/catalytic site of GSK-3. While ATP is comprised of three phosphates in the tail attached with oxygens (phospho diester bonds), the  $\beta$ - and  $\gamma$ -phosphates of ANP are attached together with a nitrogen (imidodiphosphate bond). That way, although ANP is capable of binding to GSK-3, it cannot be hydrolyzed and used in the transfer of the terminal ( $\gamma$ ) phosphate. As a result GSK-3 is made incapable of inhibiting beta-catenin, allowing beta-catenin to go on and activate transcription factors.

### Selected References:

1. [http://en.wikipedia.org/wiki/Glycogen\\_synthase\\_kinase\\_3](http://en.wikipedia.org/wiki/Glycogen_synthase_kinase_3)
2. Jope RS. (2003) *Trends in Pharmacological Sciences*, 24 (9): 441-443.
3. Bertrand JA et al., (2003) *J Mol Biol*, 333(2):393-407.