Characterizing the function of Casein Kinase 2 in early sea urchin development

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Abstract

Embryonic development is a critical life event. The asymmetric organization of this embryo and activation of the Wnt signaling pathway is an important determinant for early development. One identified protein involved in the Wnt pathway is Disheveled (Dvl), which localizes at the vegetal pole of unfertilized eggs and early embryos where it activates the Wnt pathway. Casein kinase II (CK2) is a prominent protein kinase involved in development and is an important regulator of Wnt signaling. CK2 has been shown to interact with Dvl and mediate post-translational modification of Dvl by phosphorylation, but how CK2 phosphorylation of Dvl regulates its function within the Wnt pathway is not well known. The goal of this project is to characterize CK2 and understand how it interacts with Disheveled to regulate Wnt signaling during early development. For this project I conducted a phylogenetic analysis of the CK2 alpha and beta subunits to understand the structure of CK2 in sea urchins as compared to other metazoans. As an initial investigation of the functional roles of CK2 in early development, I attempted site-directed mutagenesis of a CK2 construct to create dominant-negative mutations of CK2. Using a construct made by a previous member of the lab containing a mutation for the CK2α ATP binding site, mRNA was injected into embryos, which when imaged demonstrated a range of phenotypes including partial anteriorization and a shortened gut. These results are preliminary data which aim to further the goal of understanding the mechanisms behind CK2's role in regulating development.

Background

Dvl is a key component of the Wnt signaling pathway

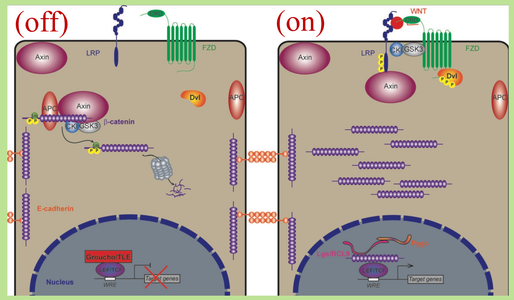

Figure 1. Canonical Wnt signaling depicting the activation of the Wnt pathway and nuclearization of β-catenin (Grainger & Willert 2018)

CK2α is expressed throughout embryos in early development

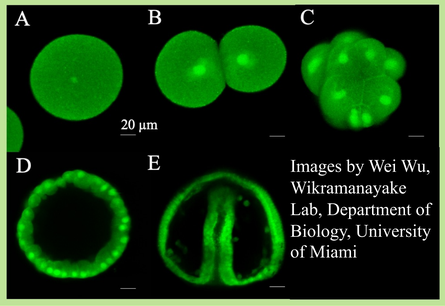

Figure 2.Visualization of SpCK2α::GFP at A) fertilized egg, B) 2-cell stage, C) 16-cell stage D) Blastula stage, E) Gastrula stage

Inhibition of CK2α leads to disrupted gut formation in sea urchin embryos

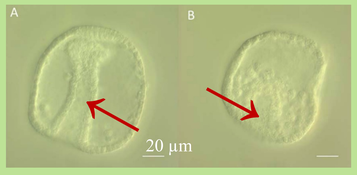

Figure 3. Drug inhibition of CK2α A) normal gastrula B) embryo exposed to 0.2 μM TBB; arrows indicate gut formation (images by Wei Wu, Wikramanayake Lab, Department of Biology, University of Miami)

Protocol for mRNA microinjections

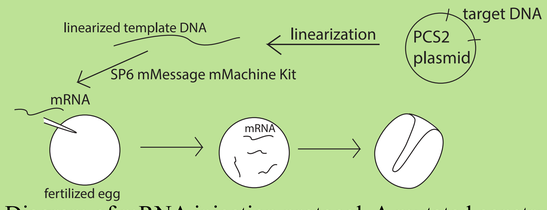

Figure 4. Diagram of mRNA injection protocol. A mutated construct for the ATP binding site of SP_CK2α (K66A) made previously in the lab was used to make mRNA for injection into *Lytechinus variegatus* embryos

The CK2 tetramer is generally conserved in sea urchins

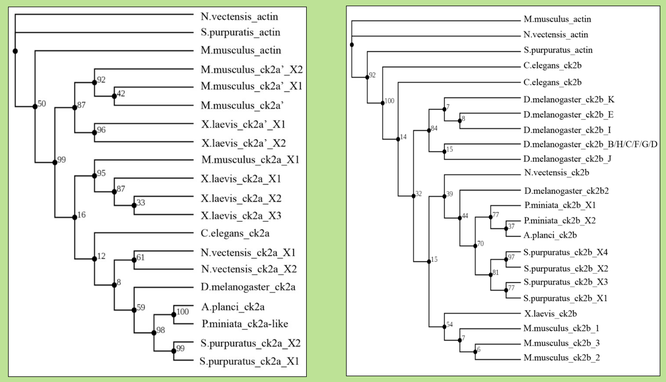

Figure 5. Phylogenetic analysis of CK2 separated by the A) catalytic (alpha/alpha') subunits, B) regulatory (beta) subunits

Mutation of the CK2α ATP binding site disrupts gut formation in sea urchin embryos

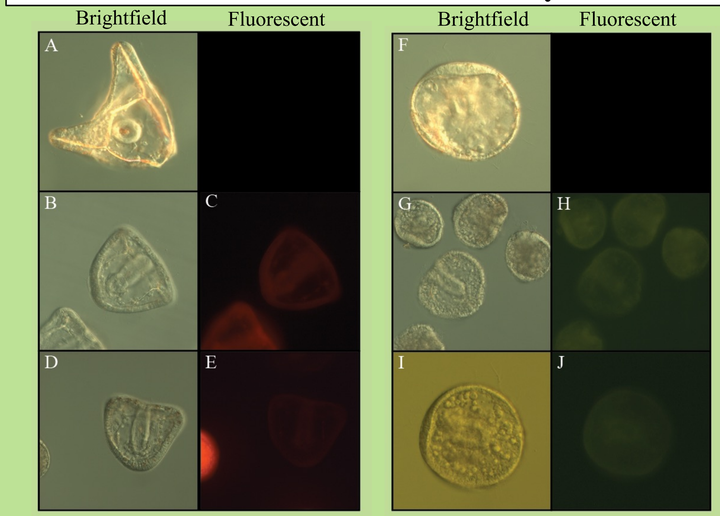

Figure 6. Effect of CK2 on *L. vareigatus* development A) normal pluteus stage embryos, B-E) late gastrula/early prism stage embryos after dextran injection, F-J) partially anteriorized embryos injected with mRNA coding for a mutation at the ATP binding site of CK2α (K66A)

Summary & Future Directions

1. CK2 is generally conserved, and sea urchins have both CK2α and CK2β
2. CK2α has an alpha prime lineage in vertebrates
3. Injection of dominant-negative mutation for the ATP binding site showed partial anteriorization, incomplete gut formation

Questions remaining:

- Competition between CK2αK66A vs endogenous CK2α?
- Other factors regulating Dvl?
- Recovery after knockdown?
- Injection dosage dependent effect?

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