

**Stony Brook University
The Graduate School**

Doctoral Defense Announcement

Abstract

Differential mediation of the Wnt canonical pathway by mammalian
Dishevelleds-1, -2, and -3

By

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In the *Drosophila*, a single copy of Dishevelled (Dsh) is found. In higher organisms, three isoforms of Dishevelled (Dvl1, Dvl2, and Dvl3) are present. In the fly, Dsh functions in the Wnt-sensitive stabilization of β -catenin and activation of the Lef/Tcf-sensitive transcriptional response known as the Wnt “canonical” pathway. We explore the expression of Dvls in mammalian cells and provide an estimate of the relative abundance of each Dvl. In mouse F9 cells, Dvl2 constitutes more than 95% of the total pool, the sum of Dvl1 and Dvl3 constituting the remainder. Similarly, Dvl2 constitutes more than 80% of the Dvl total pool in mouse P19 and human HEK 293 cells. Results from siRNA-induced knock-down of individual Dvls demonstrated that activation of the canonical signaling pathway by Wnt3a was dependent upon the presence of Dvl1, Dvl2, and Dvl3, but to a variable extent. Conversely, the overexpression of individual Dvl was found to be capable of promoting Lef/Tcf-sensitive transcriptional activation in the absence of Wnt3a, i.e., overexpression of Dvl1, Dvl2, or Dvl3 is Wnt3a-mimetic. Graded suppression of individual Dvl by siRNA suggesting that canonical signaling was most sensitive to changes in the abundance of either Dvl3 or Dvl1. Changes in expression of Dvl2, the most abundant of the three isoforms, resulted in the least effect on canonical signaling. Dvl-based complexes were isolated by pull-downs from whole-cell extracts with isoform-specific antibodies and found to include all three Dvl isoforms. Rescue experiments were conducted in which depletion of individual Dvl suppresses Wnt3a activation of the canonical pathway and the ability of a Dvl isoform to rescue the response evaluated. Rescue of Wnt3a-stimulated transcriptional activation occurred only by the expression of the very same Dvl isoform depleted by the siRNA. Thus, Dvls appear to function cooperatively as well as uniquely with respect to mediation of Wnt3a-stimulated canonical signaling. Dvl3 plays the most obvious role, whereas the most abundant (Dvl2) plays the least obvious role, suggesting that individual Dvl in mammals may operate as a network with some features in common and others rather unique.

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