

A functional Kinases and phosphatases screen identifies a role for Tao-1 in microtubule regulation

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As cells differentiate during animal development they undergo a series of changes in form that help to shape the developing embryo. They then take up a wide variety of tissue-specific forms, such as elaborately branched hippocampal neurons, which are designed to perform specific functions in the adult animal. Loss of differentiated tissue-specific cell shape is also one of the basic pathological features of disease states such as cancer. These facts make understanding cell shape a fundamental problem in biology and biomedical sciences.

The morphological diversity of different cells is decided largely by differential gene expression and by the tissue specific effects of extracellular signals. These cell signalling pathways act by altering the dynamics of the actin and tubulin-based cytoskeletons to alter cell shape (Pires-daSilva and Sommer 2003). This is often achieved by the action of protein kinases and phosphatases, which alter the phosphorylation state and activity of different cytoskeletal regulators.

In this study of cell morphogenesis, we will therefore focus on the functional analysis of the Kinases and Phosphatases in *Drosophila*. The goal of this project is to use a powerful genetic tool, high-throughput RNAi screening to dig out the novel genes involved in cell shape regulation and delineate the signalling cascades by which cells control their shapes. To do this, we have designed and built parallel dsRNA libraries, based on different oligos, targeting all *Drosophila* Kinases and Phosphatases. These RNAi screening plates were then used to screen for morphological phenotypes in totally 7 cell lines from 3 different tissues: embryo, neuronal and wing discs.

Finally, 16 hits have been confirmed using our two parallel libraries. Some hits, for example minibrain (mnb), have phenotypes only in certain cell lines, whereas other hits, such as Tao-1, have phenotypes in all cell types studied. We decided to follow Tao-1 to explore its molecular and biological function because it showed the strongest phenotype in all cell lines screened. Compared to the Human homologues of Tao-1, only one isoform of Tao-1 in *Drosophila* has been confirmed by RNAi and multi-peptide antibody. Full length Tao-1 was cloned from a *Drosophila* cDNA library. Subsequently, 4 mutations have been made based on Tao-1's functional domain analysis results in order to carry out a structure function analysis. So far, we have addressed the localisation of Tao-1 in SR+ cell during both interphase and mitotic stage using GFP fusion Tao-1 and antibodies, and cell biological experiments show Tao-1 plays an important role in microtubule network organization, mitotic spindle formation and chromosome alignment. In addition, a cell proliferation study also showed a corresponding result. Based on all these results, a hypothesis about Tao-1's biological function has been built. Additional cell biological and biochemical experiments are planned to test the hypothesis in the following months.