Title

A Novel Approach For Stable, Cell-Type Restricted Knockdown of Gene Expression in C.ELEGANS

Author

Kathryn N Maher, *University of Massachusetts - Amherst*

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First Advisor

Daniel L Chase

Second Advisor

Gerold B. Downes

Third Advisor

Jerrold S. Meyer

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Abstract

Removal of protein activity by genetic mutation or pharmacological inhibition has been used extensively to understand the normal function of a protein. However, null mutations eliminate gene function in all cells and pharmacological agents can diffuse through tissues to have similar global effects that can obscure the physiological function of a protein. This is a particular problem when studying proteins that function in many cell types or that have different cell-specific activities. The most direct strategy to study the function of a protein is to reduce or eliminate its activity only in specific cell types, rather than in all cells of an organism. The idea of targeting gene knockdown to specific cell types or to individual cells is not new and many strategies aim to do just this. However, these strategies result in variable knockdown efficiencies and can have silencing effects in neighboring cells and therefore knockdown is never cell-specific.

We developed a novel method to knock down the expression of any gene and to restrict this knockdown to specific cell types in *C. elegans.* In this method we replaced endogenous genes with single copy integrated transgenes containing an engineered sequence tag that introduces premature stop codons (PTCs) into transgene mRNA. This tag causes the natural stop codon to be recognized as a PTC by the host's nonsense-mediated decay (NMD) machinery and does not disrupt gene function. In NMD-competent animals, a PTC-containing transgene is degraded and in NMD-defective animals, a PTC-containing transgene is expressed. Therefore, the expression of PTC-containing transgenes can be controlled by cell-specific activation of NMD. Using this technique, we replaced two endogenous genes with PTC-containing transgenes and directed degradation of their mRNA to specific cell types by restoring NMD activity in these cells. The single copy transgenes were expressed at levels comparable to the endogenous genes and were knocked down to ~10% of endogenous by NMD, resulting in both global and cell-specific null-like phenotypes. This knockdown strategy can be used to cell-specifically knock down essentially any gene in the *C. elegans* genome and should provide new insights into understanding protein function.

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