Characterization of the systemic RNAi export pathway

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Due to its specificity and ability to robustly knock-down genes, RNA interference (RNAi) has significant therapeutic potential. However, this potential is yet unreached due to an inability to effectively deliver silencing cues to target tissues. In *C. elegans*, RNAi silencing signals are efficiently exported, such that silencing cues exported from the intestine result in gene silencing throughout the entire worm. In this proposal, **I outline a strategy to characterize how silencing cues are exported *in vivo*.** By exploring how silencing signals are naturally exported, my research may assist researchers who wish to package silencing signals for therapy. My research has three specific aims: 1. Characterize and map the export mutant *qt132*. 2. Identify proteins involved in the systemic RNAi export pathway. 3. Determine the localization of *B0365.7*, a novel export mutant I identified, and its pattern of movement.

Background and motivation: In. *C. elegans* and other organisms, the import of silencing cues occurs through SID-1, a passive double-stranded RNA (dsRNA) channel [1]. It was previously thought that SID-1 was responsible for both import and export of dsRNA, however, it was recently shown that SID-1 is not necessary for silencing signal export [1]. The import of silencing signals is both highly conserved throughout evolution and developmentally essential: in both frogs and mice, for example, reducing SID-1 expression results in mortality [2]. Although it is clear that the import of silencing signals is critical, almost nothing is known about the export of silencing signals. Therefore, my research aims to characterize the export of silencing cues.

<u>Specific Aim 1</u>: Mapping of the export mutant *qt132*.

Prior to my research, no genes had been assigned a role in the export of RNAi silencing cues. To identify such genes, we performed a screen that identified two mutants, *qt131* and *qt132* [3]. I have mapped *qt131* to *B0365.7*, a previously uncharacterized dynein paralog. I am currently determining the precise location of *qt132*, using coarse mapping and whole-genome sequencing. Understanding what *qt132* encodes may provide insight into to how silencing export occurs, and may also be useful in understanding how B0365.7 transports silencing signals.

<u>Specific Aim 2</u>: Identify novel proteins involved in the systemic RNAi export pathway.

qt131 harbors a frameshift in the last exon of *B0365.7*, a dynein paralog. It is known that dynein associates with a variety of proteins, including adaptor proteins that help dynein bind to its cargo [4]. To determine what proteins associate with *qt131* and therefore possibly help export silencing signals, I will perform both a pull-down and a yeast two-hybrid screen.

Pull-down: I will introduce a tandem affinity purification (TAP) tagged version of B0365.7, and pull-down the protein from worm lysates. I will then send the eluate from the purification, along with eluate from worms without the TAP-tag, to Harvard's William Lane proteomics facility to determine what proteins bind to B0365.7.

Yeast two-hybrid screen: A complementary approach to the pull-down is a yeast two-hybrid screen. Only the last 70 base pairs of the 11-kb *B0365.7* gene are altered *qt131*. I hypothesize that the last exon of B0365.7 confers specificity of B0365.7's binding to protein partners. To determine what proteins bind to the last exon of *B0365.7*, I will perform a yeast two-hybrid screen.

Potential pitfalls and validation: Both the pull-down and yeast two-hybrid assays have false-positive issues. To combat this problem, I will focus most heavily on proteins that overlap

between the two assays. These would be strong candidates for interacting with B0365.7 and may also play an essential role in the export of RNAi signals. Using RNAi and genetics, I will determine if the proteins I find are essential for silencing signal export. If so, I would conclude that these proteins play some role in the systemic RNAi export pathway.

Specific Aim 3: Determine the localization and movement of B0365.7

Since B0365.7 is a predicted dynein paralog, we hypothesize that B0365.7 transports a cargo containing either dsRNA or proteins essential for export of silencing signals. Using microscopy, I will address the following two questions: **where does *B0365.7* localize within the cell and what is its pattern of movement?**

Given dynein's role as a minus-end directed motor protein, three simple hypotheses arise: 1. B0365.7 moves towards the periphery of the cell, towing a cargo responsible for systemic RNAi export. 2. B0365.7 moves towards the center of the cell, perhaps bringing RNAi silencing cues to the nucleus to be amplified or processed. 3. B0365.7 stays at the periphery of the cells, but "pulls" along a microtubule cable, moving cargo associated with the microtubule towards the periphery. I will test these hypotheses using microscopy. First, I will label B0365.7 with GFP. As described previously [5], I will track the movement of fluorescently labeled B0365.7, along microtubules, and will note if its processive movement is towards or away from the membrane of the cell. I will then compare this pattern of movement to the pattern of movement when we add the well-established dynein specific inhibitor, EHNA, which eliminates dynein motor function [4]. It is possible that in addition to moving in a minus-directed pattern, B0365.7 freely diffuses or is transported by other motor proteins. However, comparing these two patterns (+/- EHNA) will reveal B0365.7 motor-dependent movement, i.e., how B0365.7 moves under its own power.

Potential pitfalls: Fluorescence microscopy presents a variety of challenges, however, I will rely on my strong undergraduate background in quantitative fluorescence microscopy to overcome them. In addition, I will employ both the equipment and expertise of Harvard's outstanding Center for Biological Imaging to obtain reproducible and meaningful data.

It is possible that tagging B0365.7 with GFP inhibits its function; therefore, I will only proceed if my fluorescently-labeled B0365.7 is capable of rescuing the export phenotype of *qt131*. If I have issues with signal-to-noise, I will label B0365.7 with multiple copies of GFP. Finally, if tracking particles is difficult due to an excess of GFP particles, I can use photoactivatable-GFP and therefore only track a subset of particles.

Broader Impacts: RNAi has strong clinical potential. For example, knock-down of specific oncogenic genes could significantly retard the progress of cancer. However, a major challenge is how we should package silencing cues for uptake. My research will reveal how silencing cues are naturally exported and perhaps packaged, which may provide direction for further research into how to package silencing cues for therapy.

Finally, in conjunction with my involvement with the program Science in the News, an organization that educates the Cambridge public about science, I will broadly disseminate my research. In several of my lectures, I have already discussed my research and the potential of RNAi therapeutics. An NSF grant will allow me to continue to educate the public both through lectures about the role of silencing and through published articles to a general audience.

References: 1. Jose AM, Smith JJ, Hunter CP. *Proc. Natl. Acad. Sci. U S A.* 2008;106:2283–2288. 2. Pang, K. Personal Communication. 3. For more details, see previous research essay. 4. Kardon, J.R. and R.D. Vale. *Nat. Rev. Mol. Cell Biol.* 10:854-865. 5. S Ma and R.L Chisholm. *J. Cell Sci.* 2002; 156:1453–1460.