

Sijen et al.  
Stuart Kim  
(203 words)

Goal: To use worm RdRP to increase the sensitivity of RNAi in mammalian cells.

Paper: This paper showed that RdRP creates longer antisense RNA using siRNA as a primer on mRNAs. The longer dsRNA is cleaved by DICER to generate more siRNA. This mechanism amplifies the RNAi response in worms. Mammals do not have an RdRP.

Approach. I will express the worm RdRP in mammalian tissue culture cells from a mammalian promoter. If the RdRP makes more antisense RNA, then mammalian DICER should chop up the dsRNA to increase the level of siRNA. This will amplify the RNAi response in the cell line, and make a super-sensitive mammalian cell line for use in RNAi screens. To test how well RNAi works:

1. I will first use a GFP reporter and dsGFP RNA. If the worm RdRP amplifies the response, I will see that there is less GFP in the transformed cells than the control.
2. I will then use transitive RNAi to see if antisense RNA made from a primary transcript can interfere with expression from a secondary GFP transcript.

Significance. If this works, I will have made a mammalian RNAi that is ultra-sensitive to RNAi, and could be used for mammalian screens.

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(233 words)

Hypothesis: The RNAi response in *C.elegans* is particularly robust because individuals take up nucleic acids at a high rate when feeding, and there is evolutionary pressure to protect against mutagenic or genome-parasitic effects of the foreign nucleic acid.

Approach: I will grow *C. elegans* wild type, *rde-1*, and *rrf-1* on *E. coli* that produces a “resurrected” vertebrate transposon, *Sleeping Beauty*, and assay the extent to which this transposon has been converted into heritable DNA, either as episomes or integrated into germline DNA. I will grow the strains for 0, 10, 30, and 100 generations, and then starve them; experiments will be run in quintuplicate. Then I will isolate DNA and generate 30 million sequencing reads from each population of worms. Then I will count the number of reads that match *Sleeping Beauty*. If *rde-1* (and perhaps also *rrf-1*) mutants show a statistically significantly larger number of *Sleeping Beauty* reads, my hypothesis is plausible, and it definitively shows that wild type *rde-1* protects against foreign nucleic acids.

Caveats: The assay (number of sequencing reads from *Sleeping Beauty*) is quantitative, and therefore uptake of the foreign DNA into the germline will have to occur at a sufficiently high rate for there to be statistically significant differences between the strains. Also, the statistical analysis has to account for reads coming from the same insertion of *Sleeping Beauty*, and should not necessarily treat each sequencing read as independent.