Screen For Negative Regulators of RNA Interference

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The newly discovered RNA interference (RNAi) mechanism has potential to become the primary method for gene knockout making it valuable in the field of genetic research. However, the pathway is poorly understood and until we have a better grasp of its mechanism, the applicability is greatly decreased. My research was involved with identifying genes that would negatively regulate this pathway. METHODS: RNAi is initiated by the introduction of double stranded RNA, of a specific gene, into an animal, in this case C. elegans. This double stranded RNA serves as a signal for degradation of the organism's own RNA for that gene. My research focused on identifying genes which would negatively regulate this mechanism; the deletion of these genes would allow RNA interference to work well even with small amounts of double stranded RNA as a signal. C. elegans are ideal for this type of experiment because after the introduction of double stranded RNA, the progeny can be checked for phenotype the next day. We desired to set up a screen for worms with improved RNAi because of a mutation in the genes that would negatively regulate the pathway. We know that worms injected with a gene called unc-29 lose a certain neuro-receptor causing them to move more slowly than wildtype worms. The drug levamisole is lethal to wildtype started by mutenigizing a large number of worms to knock out the RNAi negative regulator gene. Next we fed the worms double stranded unc-29 RNA. Feeding worms double stranded RNA is usually less effective method because only a small amount of RNA is delivered. However, if the negative regulator has been knocked out there will be a stronger phenotype. Thus RNAi feeding of unc-29 will work only on those worms whose negative regulator genes have been knocked out and will be resistant to the levamisole. Once mutants were isolated the gene would then be cloned.

RESULTS: Previously, RNAi feeding was performed on plates with worms. Our experiment required us to look at a large number of worms, more than could be examined on plates. Thus we developed a protocol for performing RNAi feeding in liquid culture allowing us to observe huge numbers of worms. Next, a protocol for screening with levamisole was established. Both dosage and timing were important in getting the desired reaction from the worms in liquid culture. Finally, we created vectors carrying unc-29 for both feeding and injecting into worms. Neither feeding or injecting unc-29 RNAi worked. It is not understood why unc-29 was resistant to RNAi, again lack of knowledge of the mechanism makes it difficult to speculate. Two new