

## **Cell-to-Cell Spreading of RNA Interference in Mammalian Cells**

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RNA interference (RNAi) is an evolutionarily conserved mechanism for gene silencing in organisms ranging from fungi to mammals. In *C. elegans*, systemic RNAi is a phenomenon that the silencing effect of RNAi can be observed far from the initial site of dsRNA delivery. This spreading effect has been observed in plants, planarians, Coleoptera and Cnidaria. In addition, human homolog of Sid1 transmembrane protein responsible for systemic RNAi in *C. elegans* has also been identified. However, RNAi spreading has not yet been reported in mammals. In this study we investigated cell- to-cell spreading of RNAi in cultured human cells. We constructed a reporter plasmid that transcribed a bicistronic RNA coding for *Renilla* and firefly luciferase. The expression of the two homologous reporters could be specifically inhibited by different small hairpin RNAs (shRNAs). Next, we established two stable cell lines derived from HEK293 cells. One cell line expressed the bicistronic reporter and the other expressed shRNA targeting the *Renilla* luciferase. Co-culture of both cell lines resulted in the reduction of *Renilla* luciferase expression in the reporter cell line, but did not affect the expression of firefly luciferase. RNA and protein analysis confirmed that the suppression of luciferase expression in the reporter cell line was mediated by small interfering RNA. Taken together, our findings suggest that RNAi spreads from one mammalian cell to another in culture.