MILESTONE 8

Small interfering RNAs silence genes in mammals

The discovery that exogenous double-stranded RNA (dsRNA) could silence specific genes in *Caenorhabditis elegans* (MILESTONE 4) was closely followed by the observation that, in *Drosophila melanogaster*, this phenomenon was dependent on the processing of the dsRNAs into fragments of ~21–25 nucleotides (MILESTONE 7). This observation was pivotal to the development, in 2001, of dsRNAs that could silence specific genes in mammalian cells.

Indeed, despite the efficiency of dsRNA-mediated gene silencing in insect cells, dsRNAs of 38–1,662 base pairs (bp) in mammalian cells did not seem to silence specific genes, perhaps because dsRNAs of >30 bp activate a cellular interferon response that triggers non-specific degradation of mRNA. In an attempt to circumvent this problem, Elbashir et al. worked downstream of the dsRNA-processing step by directly generating 21-bp small interfering RNA (siRNA) duplexes with symmetric, two-nucleotide 3′ overhangs. Transfecting siRNAs against a reporter gene into mouse, monkey and human cells repressed reporter gene expression by 2–25-fold, indicating that siRNAs function in mammalian cells. Substituting uridine with thymidine in the 3′ overhang, to protect the siRNA from nuclease degradation and to reduce the cost of siRNA synthesis, did not compromise knockdown (repression) efficiency or specificity. Furthermore, an siRNA concentration of just 1.5 nM, which was several orders of magnitude below the concentration of conventional antisense molecules required for gene silencing, could silence target genes. Together, these findings suggested that siRNAs were powerful tools for gene silencing in mammalian cells.

Comparing siRNAs with dsRNAs of 50 bp and 500 bp in gene targeting assays confirmed that the short length of siRNAs was key to gene-specific silencing in mammalian cells. Longer dsRNAs triggered the degradation of non-cognate reporter genes through the interferon response, rendering their effect on the target gene difficult to detect.

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Credit: Lara Crow / Springer Nature Limited

The final confirmation that siRNAs could silence genes in mammalian cells came from the observation that, 40–45 hours after transfection, siRNA specifically reduced the endogenous expression of the gene encoding lamin A/C (by >90%) and of the genes encoding lamin B1 and nuclear mitotic apparatus protein (to low levels). Therefore, although the underlying mechanism was not yet clear, this 2001 study uncovered the promise of siRNAs for the study of gene function and highlighted their potential utility for gene-specific therapies.

The first proof-of-principle that siRNAs could be harnessed to treat disease came from a study published in 2003, showing that siRNAs could protect mice against fulminant hepatitis, which can result from excessive apoptosis in the liver. As hepatocytes express high levels of the FAS receptor, and are thus susceptible to FAS-mediated apoptosis, inhibiting *Fas* expression in hepatocytes was of interest for treating liver diseases.

Song et al. showed that tail vein injection of siRNA targeting *Fas* into mice could markedly reduce the expression of FAS at the gene and protein level compared with mice injected with *GFP* siRNA; these reductions were stable for 10 days after siRNA injection and were achieved at low doses. Importantly, whereas mice treated with *GFP* siRNA before or after injection with concanavalin A (to induce fulminant hepatitis) showed signs of liver damage, mice injected with *Fas* siRNA before or after concanavalin A treatment did not. Therefore, this study highlighted the potential of siRNAs in both preventing and treating liver injury.

The discovery that siRNAs could silence genes in mammalian cells, and indeed in whole mammalian organisms, revolutionized the study of gene function. Several advances in siRNA technology have been made in the years since their discovery, including tangible improvements in siRNA delivery (MILESTONES 11, 13), with the result that siRNAs are still widely used in the laboratory today.

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MILESTONE STUDIES Elbashir, S. M. et al. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature* **411**, 494–498 (2001) | Song, E. et al. RNA interference targeting Fas protects mice from fulminant hepatitis. *Nat. Med.* **9**, 347–351 (2003).