COMMENTARY

Dampening the silencing effect of RNA interference in mammals

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RNAi (RNA interference) refers to the suppression of expression of a target gene (mainly at the post-transcriptional or translational level) induced by small (21–23 nucleotides) RNA molecules, including siRNA (small interfering RNA). Suppression of gene expression by RNAi represents an important part of the regulation of gene expression. Interestingly, recent advancements in RNAi research support the notion that RNAi can be regulated just as an ordinary gene. In this issue of the *Biochemical Journal*, Hong et al. report their finding that suppression of RNAi is triggered by a high dose of siRNA in mice, and the suppression of RNAi in mice is related to *eri-1* (enhanced RNA interference). *Eri-1*

is an RNaseT enzyme initially found in *Caenorhabditis elegans* that can degrade double-stranded RNA with 3' overhangs. The results presented by Hong et al. have the potential to be extended and contribute to our knowledge about the regulation of RNAi in mammals.

Key words: enhanced RNA interference (*eri*), *Escherichia coli*-expressed and enzyme-digested RNA (*esiRNA*), hydrodynamic injection, RNA interference (RNAi), RNA interference regulation, small interfering RNA (siRNA).

RNAi (RNA interference) refers to the suppression of expression of target gene (mainly at the post-transcriptional or translational level) induced by small (21–23 nucleotides) homologous RNA molecules, including double-stranded siRNA (small interfering RNA) and single-stranded miRNAs (microRNAs). RNAi has been observed in a wide spectrum of organisms, from plants to worms to mammals. RNAi is involved in important and fundamental biological functions, such as the immune response in plants, the control of developmental timing in *Caenorhabditis elegans* and early development in mice [1,2]. Rapid progress has been made in RNAi research both as a research tool and as a potential class of new drugs. As a research tool, RNAi has the advantage of knocking down gene expression without altering genomic DNA structure and function. *In vitro* and *in vivo* models of conditional knocking down of gene expression using RNAi have been established. In therapeutic applications, RNAi could be used to treat viral diseases, such as AIDS, or non-viral diseases, such as the metabolic syndrome/type II diabetes, as siRNA can reduce plasma cholesterol levels, as shown in animal studies [3].

Advances have also been achieved in understanding how RNAi is regulated. Emerging evidence supports the notion that suppression of gene expression by RNAi can be regulated just as the regulation of an ordinary gene, and a cell or organism may become more or less sensitive to the gene-silencing effect of RNAi (positive/negative regulation of RNAi). In *C. elegans* and plants, the RNAi effect can be 'amplified' in a mechanism similar to PCR. The 'amplification' process is catalysed by RDRP (RNA-directed RNA polymerase) [4], and a few molecules of dsRNA (double-stranded RNA) per cell or embryo can completely silence the corresponding gene. On the other hand, a 19 kDa protein (p19) encoded by an invading virus can suppress RNAi in the host plants (negative regulation). In *C. elegans*, the negative regulation of RNAi is found to be related to the *eri-1* gene (named after enhanced RNAi) [5]. Mutation in the *eri-1* gene increases the sensitivity of *C. elegans* to the gene-silencing effect of RNAi [5]. *C. elegans* *eri-1* belongs to the family of RNaseT enzymes, and can partially degrade siRNAs with two-nucleotide 3' overhangs [5]. As the 3' overhangs of siRNAs are essential for efficient

siRNA-mediated mRNA degradation [6], degraded siRNAs may be non-functional, and may be relevant to reduced RNAi efficiency [5].

Apart from *eri-1*, ADARs (adenosine deaminases) also negatively regulate RNAi in *C. elegans* and mice [7,8]. ADARs catalyse the conversion of adenosine (A) residues into inosine (I) in RNA substrates containing a short dsRNA. Conversion of A to I generates an I–U mismatch, which is a poor substrate for Dicer (an RNase III enzyme that degrades dsRNAs), and thus suppresses RNAi. Gene silencing by siRNA is more effective in homozygous ADAR-1-deficient murine fibroblasts than in wild-type cells [8], whereas overexpressing ADAR-1 decreases the RNAi effect [8].

In this issue of the *Biochemical Journal*, Hong et al. [9] show that gene silencing by RNAi may be negatively regulated by *eri-1* and ADAR in mice. HBsAg (hepatitis B virus surface antigen) was used as a marker, and HBsAg was detectable in cells or mice transfected with plasmids expressing HBsAg. Levels of HBsAg were examined at different time points following treatment with specific siRNA *in vitro* and *in vivo*. They showed that the reduced level of HBsAg (due to synthetic siRNA treatment) had recovered by approx. 3–4 days post-siRNA treatment, a process described as 'rebound'. The rebound phenomenon represents the disappearance of the siRNA effect, which has been widely observed as the transient effect using synthetic siRNA. The contribution of these authors was that, when a high dose of synthetic siRNA was administered either *in vitro* or *in vivo*, the rebound happened more quickly and to a greater extent. Interestingly, even non-specific siRNA at similar high doses to specific siRNA induced a comparable rebound effect. Their work suggests that a high siRNA dose might have induced a suppressive effect on RNAi (negative regulation of RNAi).

Hong et al. [9] studied further whether the rebound phenomenon was linked to *eri-1* and ADAR-1, given that *eri-1* and ADAR-1 negatively regulate RNAi in *C. elegans* and murine fibroblasts. They found that the rebound effect induced by a high dose of siRNA was eliminated by the co-transfection of another siRNA against *eri-1*. Using semi-quantitative RT (reverse transcription)–PCR, they found that a high dose of siRNA increased hepatic

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mRNA levels of both *eri-1* and *ADAR-1*, in parallel to the rebound effect, whereas co-transfection of *eri-1* siRNA eliminated the rebound phenomenon, with a parallel reduction in hepatic mRNA levels of *eri-1* and *ADAR-1*. Their data suggest that both *eri-1* and *ADAR-1* may be involved in the negative regulation of RNAi in mice.

The work of Hong et al. [9] suggests that the function of *eri-1* may be conserved in a wide range of organisms. The notion that *eri-1* may suppress RNAi in mice as it does in *C. elegans* may be biologically significant, as the 'amplification' phenomenon observed in *C. elegans* has not been reported in mice so far. Their data may be relevant to the widely observed transient effect of knocking down gene expression using siRNA. Their work has raised a series of interesting questions yet to be addressed. For example, in *C. elegans*, *eri-1* is expressed abundantly in the gonad and a subset of neurons, suggesting tissue-specific expression [5]. It is yet to be seen if *eri-1* expression varies in a tissue-specific way in mice. If it does, will the dose of siRNA required to 'trigger' *eri-1* or *ADAR-1* expression also differ in different tissues? Furthermore, what regulates tissue-specific expression of *eri-1* or *ADAR-1*? Answers to these questions will further our understanding about the negative regulation of RNAi.

Data from a very recent publication [10] show that expression of miRNA (miR-1s) can be regulated by transcription factors. The two related miRNA genes, *miR-1-1* and *miR-1-2* (collectively known as miR-1s), are enriched in the heart and muscle. miR-1s regulate cardiac differentiation (specialization) through inhibiting expression of *Hand2*, a critical transcription factor in cardiac differentiation [10]. Interestingly, *miR-1s*, like ordinary genes, are also regulated by transcription factors such as serum-response element and myoD [10]. Serum-response element and myoD regulate muscle differentiation through acting on their response genes. These advancements suggest that expression of specific gene(s) (such as *Hand2*) can be negatively regulated via an interaction between transcription factors and small RNA molecules (miR-1s). This prompts us to speculate whether expression of *eri-1* may be regulated by specific transcription factors, or even

whether the negative regulation of RNAi may be gene-specific (i.e. suppression of RNAi depends on the sequence of siRNA administered). Answering these questions not only will facilitate application of RNAi in characterizing gene function and in therapeutic application, but also may shed light on understanding development and cell differentiation and the aetiology of common diseases, such as the developmental origins of cardiovascular disease and type II diabetes.

REFERENCES

- Banerjee, D. and Slack, F. (2002) Control of developmental timing by small temporal RNAs: a paradigm for RNA-mediated regulation of gene expression. *BioEssays* **24**, 119–129
- Bernstein, E., Kim, S. Y., Carmell, M. A., Murchison, E. P., Alcorn, H., Li, M. Z., Mills, A. A., Elledge, S. J., Anderson, K. V. and Hannon, G. J. (2003) Dicer is essential for mouse development. *Nat. Genet.* **35**, 215–217
- Soutschek, J., Akinc, A., Bramlage, B., Charisse, K., Constien, R., Donoghue, M., Elbashir, S., Geick, A., Hadwiger, P., Harborth, J. et al. (2004) Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. *Nature (London)* **432**, 173–178
- Nishikura, K. (2001) A short primer on RNAi: RNA-directed RNA polymerase acts as a key catalyst. *Cell* **107**, 415–418
- Kennedy, S., Wang, D. and Ruvkun, G. (2004) A conserved siRNA-degrading RNase negatively regulates RNA interference in *C. elegans*. *Nature (London)* **427**, 645–649
- Elbashir, S. M., Martinez, J., Patkaniowska, A., Lendeckel, W. and Tuschl, T. (2001) Functional anatomy of siRNAs for mediating efficient RNAi in *Drosophila melanogaster* embryo lysate. *EMBO J.* **20**, 6877–6888
- Tonkin, L. A. and Bass, B. L. (2003) Mutations in RNAi rescue aberrant chemotaxis of ADAR mutants. *Science* **302**, 1725
- Yang, W., Wang, Q., Howell, K. L., Lee, J. T., Cho, D. S., Murray, J. M. and Nishikura, K. (2005) ADAR1 RNA deaminase limits short interfering RNA efficacy in mammalian cells. *J. Biol. Chem.* **280**, 3946–3953
- Hong, J., Qian, Z., Shen, S., Min, T., Tan, C., Xu, J., Zhao, Y. and Huang, W. (2005) High doses of siRNAs induce *eri-1* and *adar-1* gene expression and reduce the efficiency of RNA interference in mouse. *Biochem. J.* **390**, 627–631
- Zhao, Y., Samal, E. and Srivastava, D. (2005) Serum response factor regulates a muscle-specific microRNA that targets Hand2 during cardiogenesis. *Nature (London)* **436**, 214–220

Received 25 July 2005; accepted 28 July 2005

Published on the Internet 5 September 2005, doi:10.1042/BJ20051206