PublisherInfo		
PublisherName	:	BioMed Central
PublisherLocation	:	London
PublisherImprintName	:	BioMed Central

Mammalian RNAi

ArticleInfo		
ArticleID	:	4336
ArticleDOI	:	10.1186/gb-2002-3-5-reports0024
ArticleCitationID	:	reports0024
ArticleSequenceNumber	:	18
ArticleCategory	:	Paper report
ArticleFirstPage	:	1
ArticleLastPage	:	4
ArticleHistory	:	RegistrationDate : 2002–2–25 Received : 2002–2–25 OnlineDate : 2002–4–25
ArticleCopyright	:	BioMed Central Ltd2002
ArticleGrants	:	

ArticleContext	:	130593355

Chris Berrie

Abstract

Long hairpin-forming double-stranded RNAs provoke specific suppression of gene expression in murine embryonic cells that is strongly suggestive of conventional RNA interference

Significance and context

Since the original observations of endogenous gene silencing when transgenes were introduced into plants, it has become evident that double-stranded (ds) RNAs can provoke gene silencing in numerous biological contexts. The identical size of the putative specificity determinants (dsRNA-derived guide or small interfering RNAs, siRNAs) in plants and animals predicted conservation of both the mechanisms and components of this dsRNA-induced, sequence-specific, post-transcriptional gene silencing, which is now commonly known as RNA interference (RNAi).

The use of RNAi as a genetic tool in mammals has taken two different directions. The first has relied upon the relative ease of use of siRNAs. Introduction of selected siRNAs into cells bypasses the initial dsRNA-processing enzyme (an RNase III, encoded by the *dicer* gene in *Drosophila*) while still allowing the formation of the RNA-induced silencing complex (RISC) responsible for the specificity of RNAi. This approach has led to the transfection of selected siRNAs into mammalian tissue culture cells, resulting in the transient silencing of a range of major cellular proteins.

The second approach directly introduces large dsRNAs (around 500 nucleotides), and has already significantly advanced understanding of gene function in *Caenorhabditis elegans*. Furthermore, there have been recent demonstrations of heritable gene silencing by transgene expression of hairpin dsRNA in plants, *C. elegans* and *Drosophila*. In mammals, however, this approach has been greatly limited by the accompanying activation of the dsRNA-activated protein kinase PKR, which causes a global inhibition of gene expression, and activation of the nonspecific ribonuclease RNase L.

Key results

Paddison and colleagues initially report the non-specificity of dsRNAs on the expression of reporter genes in human embryonic kidney (293) cells and mouse embryo fibroblasts, presumably as a result of the activation of PKR. They also screened a number of murine cell lines of embryonic origin in search

of dsRNA-induced, sequence-specific gene silencing. Although some showed a reduced, but still evident, activation of PKR, in both mouse embryonic stem (ES) cells and P19 embryonal carcinoma cells transfection of non-homologous dsRNAs had no effect on the reporter genes. They thus selected P19 cells for enforced endogenous expression of long-hairpin-forming dsRNAs and were able to induce stable, sequence-specific gene silencing.

Conclusions

This study extends the potential of RNAi as a tool for probing gene function in mammalian cells. As outlined by Paddison *et al*., the creation of stable cell lines with a loss-of-function phenotype of choice will greatly enhance our knowledge of gene function in mammals.

Reporter's comments

In this study, Paddison *et al*. have combined a series of standard experimental techniques. They have applied these to mammalian cells in culture, working on the basis that previous reports have shown that the nonspecific inhibition of gene expression is seen to be reduced in early development. Hence, they initially quantified the extent of dsRNA-induced, sequence-specific gene silencing in a number of cell lines of embryonic origin. The cotransfection of a mixture of plasmids for firefly and *Renilla* luciferases with selected dsRNAs allows for an enhanced sensitivity of marker-gene response detection through the firefly/*Renilla* luciferase activity ratios.

Paddison *et al.* do not examine a large selection of cell lines, however, and only report on continuous expression of hairpin dsRNAs to provide the stable, sequence-specific silencing of their target gene in P19 cells. Should it be possible to extend this to the ES cells, and to combine it with attenuation of the more generalized gene silencing by PKR in other cell lines, the potential use of RNAi for investigating the biological effects of specific gene silencing becomes evident. There also remains the need to show that the mechanistic aspects of this dsRNA-induced, sequence-specific gene silencing do indeed represent the RNAi pathways seen in lower organisms.

Over the few years since its initial discovery, RNAi has been proposed to be a generalized defense mechanism against unwanted nucleic acids, and many plant viruses have indeed developed proteins that counteract RNAi. Nevertheless, there remains the question of whether RNAi has a role in regulating the normal expression of cellular genes. This has already been suggested by leaf and flower developmental abnormalities seen with RNAi-resistant mutants in *Arabidopsis thaliana*.

Therefore, although RNAi has been used extensively for systematic investigation of the function of the entire genome of *C. elegans* and has proved of great use in probing *Drosophila* embryology, maybe we can only now begin to explore the potential role for RNAi in mammalian cells.

Table of links

Proceedings of the National Academy of Sciences of the United States of America

References

1. Paddison PJ, Caudy AA, Hannon GJ: Stable suppression of gene expression by RNAi in mammalian cells. Proc Natl Acad Sci USA. 2002, 99: 1443-1448. 0027-8424