RNA interference: a new and powerful tool for functional genomic analysis

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Abstract

In many species, the introduction of double-stranded RNA induces potent and specific gene silencing, referred to as RNA interference. This phenomenon, which is based on targeted degradation of mRNAs and occurs in almost any eukaryote, from trypanosomes to mice including plants and fungi, has sparked general interest from both applied and fundamental standpoints. RNA interference, which is currently used to investigate gene function in a variety of systems, is linked to natural resistance to viruses and transposon silencing, as if it were a primitive immune system involved in genome surveillance. Here, we review the mechanism of RNA interference in post-transcriptional gene silencing, its function in nature, its value for functional genomic analysis, and the modifications and improvements that may make it more efficient and inheritable. We also discuss the future directions of this versatile technique in both fundamental and applied science.

Resum

En moltes espècies, la introducció de RNAs de doble cadena indueix un silenciament gènic molt específic i potent, anomenat interferència induïda per RNA. Aquest fenomen, basat en la degradació dirigida de mRNAs, es produeix en gairebé tots els eucariotes, des de tripanosomes a ratolins, incloent-hi fongs i plantes. Les seves possibles aplicacions en ciència bàsica i aplicada han despertat un interès general. La interferència induïda per RNA, que actualment s'empra per investigar la funció gènica en sistemes molt diversos, està relacionada amb la resistència natural a virus i al silenciament de transposons, com si fos un sistema immunitari primitiu implicat en la vigilància i control del genoma. En aquest article, fem una revisió dels mecanismes d'actuació del silenciament gènic postranscripcional induït per la interferència de RNA, de la seva funció a la natura, de la importància que té la seva aplicació en estudis de genòmica funcional i de les modificacions i millores que el fan més efectiu i heretable, i discutim les perspectives de futur d'aquesta tècnica tan versàtil en ciència bàsica i aplicada.

Keywords: RNA interference, double-stranded RNA, developmental biology, vertebrates, invertebrates

When double-stranded RNA (dsRNA) from sense and antisense sequences of an endogenous mRNA is introduced into a cell of almost all eukaryotes, the sequence-corresponding mRNA is degraded and the gene is silenced [1-3]. This post-transcriptional gene silencing (PTGS) was first discovered by Guo and Kempues in the nematode *Caenorhabditis elegans* [4]. To describe this phenomenon, Fire and co-workers coined the term RNA interference (RNAi) [5]. Their discovery was based on the puzzlinaz g observation that sense and antisense RNA were equally effective in suppressing specific gene expression [4]. Further research revealed that the active agent was small amounts of dsRNA that usually contaminate DNA transcription of both sense and antisense RNAs *in vitro* [5]. RNAi has since been found to be effective in a wide variety of animals, including trypanosomes [6-8], hydra [9], platyhelminths [10-12, work in progress], nematodes [5, 13-16], insects [8, 12, 17-19], zebrafish [20, 21], *Xenopus* [22], chicken [work in progress] and mice [23, 24], and it may be associated with gene silencing in plants («co-suppression») [25-28] and fungi («quelling») [29-31].

RNAi is a powerful new tool that provides geneticists and molecular biologists of very diverse fields another approach to the study of gene function as an alternative to loss-of-function transgenics. However, RNAi function, if it is to be stably

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inherited, needs the creation of a transgenic organism carrying a transgene with both sense and antisense information.

Mechanism of RNAi post-transcriptional gene silencing

Experimental aspects

Before discussing the proposed mechanism for RNAi gene silencing, several aspects of RNAi experimental design should be considered. To produce an RNAi effect by means of PTGS, the dsRNA introduced into the organism should contain exonic sequences of the gene whose expression is to be disrupted, as the rapid maturation of heterogeneous nuclear RNAs (hnRNAs) does not allow dsRNA intronic sequences to silence the corresponding gene [5]. However, in the plant *Arabidopsis*, the introduction of dsRNA from the promoter causes DNA methylation in the promoter of the corresponding gene [32], which leads to the inhibition of its expression by transcriptional gene silencing (TGS) [1]). The RNAi process is homology-dependent and so the sequences to be included in the dsRNA should be carefully selected to avoid interference between homologous sequences of related genes [10].

The delivery system of the dsRNA may vary among organisms. Whereas microinjection is suitable for introducing dsRNA into the germ line or into early embryos of multicellular eukaryotes (zebrafish, *Xenopus*, chicken and mice) [21, 23, 24, work in progress], and in whole or regenerating organisms (nematodes and platyhelminths) [5, 33, work in progress], electroporation is more effective in simpler organisms (trypanosomes and hydra) [6, 22]. Moreover, dsRNA can be introduced into the nematode *C. elegans* by feeding the organisms with food mixed with dsRNA or producing the desired dsRNA (i.e. expressed by the feeding bacteria), or by simply soaking them in a medium containing dsRNA [34, 35].

Another aspect to be considered is the dilution of the dsRNA when it is introduced into the organism, as it spreads to most tissues. Moreover, when introduced into early embryos, dsRNA is diluted as cells steadily divide during development. This may not be a problem for small organisms, but it can be a limiting factor in more complex organisms. Indeed, dilution of dsRNA may explain the variable penetrance of phenotypes observed after RNAi with some genes in *Drosophila* [10], zebrafish [21] and chicken [work in progress] (Figure 2). However, RNAi may also involve amplification of the dsRNA signal by an RNA-dependent RNA
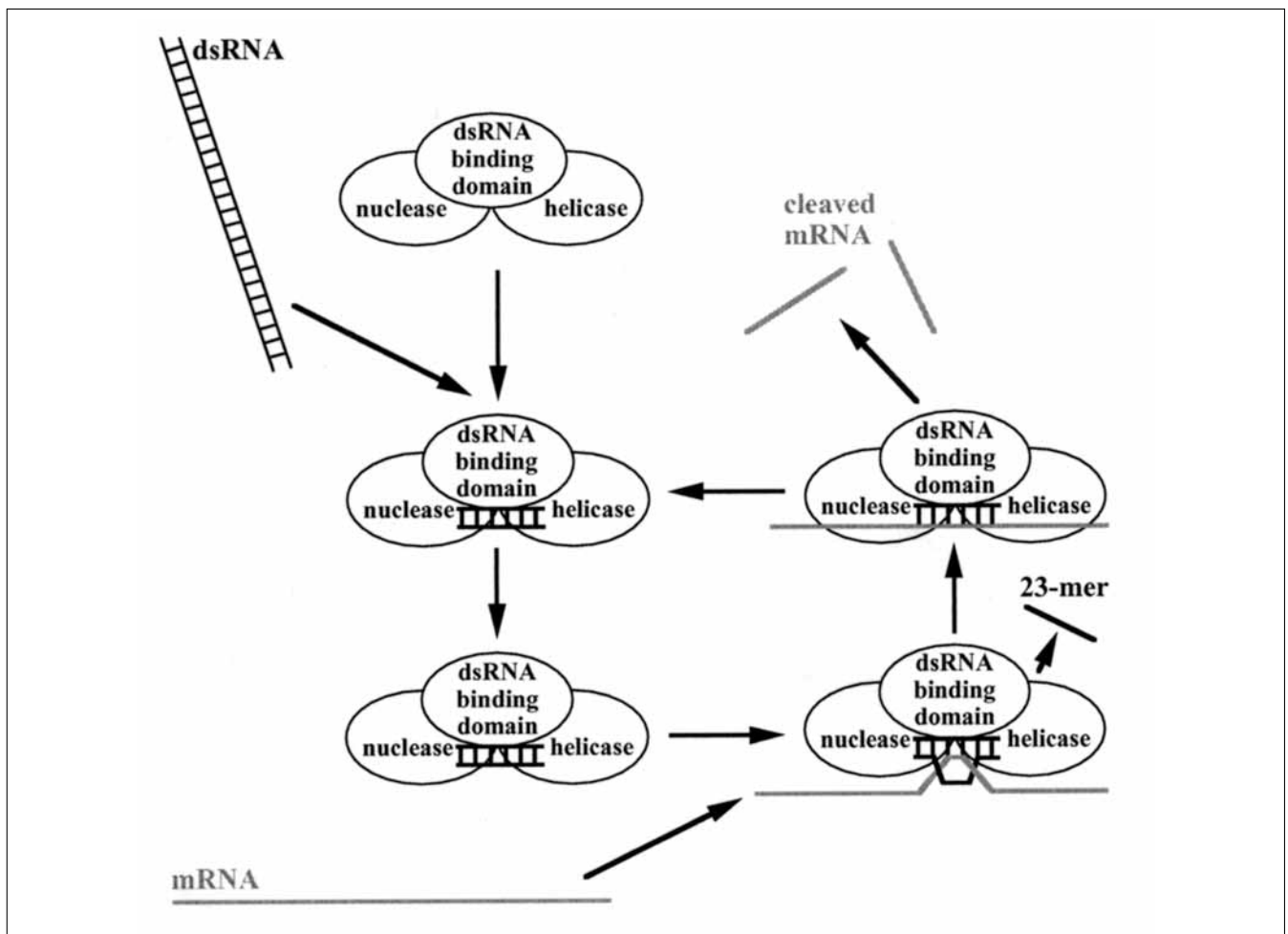

Figure 1. Proposed model in which the degradation of the mRNA by RNAi is catalysed by a hypothetical enzyme that contains a dsRNA binding domain, a ribonuclease domain and a helicase domain. Modified from [3].
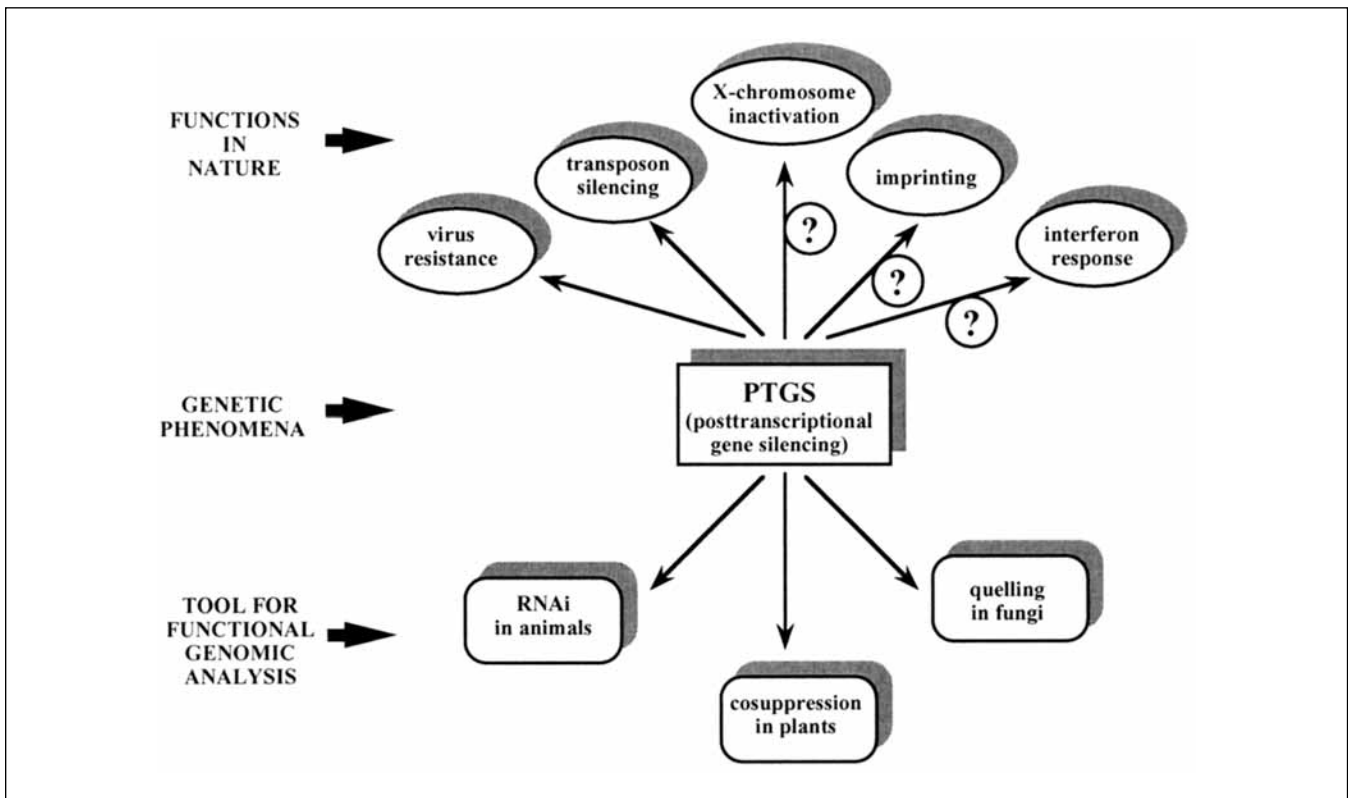
Figure 2. Diagram showing the link between natural virus resistance, transposon silencing, RNAi in animals, PTGS co-suppression in plants and quelling in fungi.

polymerase (RdRP), whose activity may delay the effect of the dilution [29, 36]. Generally, the higher the amount of dsRNA introduced, the more effective is the PTGS [2, 37]. However, relatively small quantities of dsRNA can degrade vast amounts of the corresponding mRNA [5].

dsRNA sequence determines the cleavage sites of the mRNA

RNAi is active mainly in cytoplasm [6, 10], but it can also affect nuclear transcripts [38]. Although the precise mechanism of RNAi is unknown, the involvement of permanent gene modification and the disruption of transcription have been ruled out experimentally [15]. It is generally accepted that RNAi in animals, PTGS co-suppression in plants and quelling in fungi are post-transcriptional, targeting RNA transcripts for degradation [15, 39]. By using three different dsRNAs from a single gene, it has been shown that the cleavage sites of the RNA transcripts are precisely determined by the sequence of the dsRNA [40]: each of the three dsRNAs produced a ladder of bands corresponding to a set of mRNA cleavage products characteristic of that particular dsRNA.

A specific nuclease cleaves both sense and antisense strands of the dsRNA into small pieces of 21-25 nucleotides (21– 25-mers) both *in vivo* and *in vitro* (cell-free system) [3, 37, 39-44]. Moreover, the mRNA is degraded at specific sites, spaced 21-23 nucleotides apart, suggesting that the cleavage is templated by the small pieces of dsRNA. However, each individual mRNA is cleaved only once or twice along its entire sequence [40]. The cleavage of the mRNA is

unaffected by several translation inhibitors but it is ATP-dependent, suggesting the involvement of specific enzymes.

The above data have led to a model in which the degradation of the mRNA by RNAi is catalysed by a hypothetical enzyme that contains a dsRNA binding domain, a ribonuclease domain and a helicase domain [3, 40] (Figure 1). In this model, the dsRNA is bonded to the hypothetical enzyme (dsRNA binding domain) and degraded to ~23-mers (nuclease activity), and the small dsRNA pieces remain bonded to the enzyme. Thereafter, these small dsRNA pieces template sequence-specific cleavage of the mRNA (nuclease activity), replacing the sense strand of the ~23-mers with the mRNA strand (helicase activity). The dsRNA is regenerated and so after targeting mRNA for degradation, it is ready to template the next round of mRNA degradation. The regeneration of the small dsRNA pieces after mRNA degradation explains why relatively small amounts of dsRNA degrade vast amounts of the corresponding mRNA.

Genes involved in RNAi

Genetic screens in both the nematode *C. elegans* and the fungus *Neurospora* have identified genes required for RNAi, but the scenario is rather incomplete and the genetic mechanism of RNAi in animals, PTGS co-suppression in plants and quelling in fungi is unclear. RNAi-resistant mutants and the genes involved can be classified in several categories. In the nematode *C. elegans*, some of these genes, including *rde-2*, *rde-3*, *mut-2* and *mut-7*, when mutated, result in the mobilisation of transposons [45-47], suggesting that the machinery involved in the two processes is linked (see below, *RNAi in*

nature; Figure 2). Another subset of genes, including *rde-1* and *rde-4*, is required for the initiation of inheritable RNAi [47] (see below, *Heredity of RNAi*). It has been shown that *rde-2* and *mut-7* are required downstream in the tissue where inhibition occurs. *rde-1* and *rde-4* may respond to dsRNA by producing a secondary extragenic agent that is used by the downstream genes *rde-2* and *mut-7* to target specific mRNAs for PTGS. That is, *rde-1* and *rde-4* may be the initiators of RNAi and *rde-2* and *mut-7* may be the effectors.

As for the hypothetical enzyme mentioned above, the characteristics of the small nucleotide pieces (~23 mers) suggest they were generated by RNase III or a closely related enzyme, as this is the only identified nuclease known to cleave dsRNA at specific sites, spaced <22 nucleotides [48, 49]. However, RNase III does not bind dsRNA fragments. Interestingly, open reading frames (ORF) in metazoa with RNase III and helicase domains, and dsRNA binding motifs have been found [48]. These ORF may correspond to that hypothetical enzyme. In the same way, *mut-7* from the nematode *C. elegans* is homologous to RNase D [45].

In the fungus *Neurospora*, the *qde-3* gene, a member of the RecQ DNA helicase family, may be an example of an initiation gene for fungi quelling [30]. It is relevant that most genes whose products are involved in RNAi have their homologous genes in other metazoa in which RNAi has been successfully tested, like the fly *Drosophila* and the plant *Arabidopsis* [2]. For example, the gene *rde-1* from the nematode *C. elegans* is homologous to gene *qde-2* from the fungus *Neurospora* and to the gene *ago-1* from the plant *Arabidopsis*, all of which are involved in their respective PTGS [50]. This points to common mechanisms for RNAi in animals, co-suppression in plants and quelling in fungi, which must have appeared before the evolutionary split of these groups.

Heredity of RNAi

One of the limitations to the use of injected, fed or electroporated dsRNA for RNAi is that descendants do not inherit it. The only reported partial exception is *C. elegans*, in which injected dsRNA leads to heritable gene silencing in the F2 generation and beyond [47]. This requires the above-mentioned genes *rde-1* and *rde-4* [47]. This limitation can be overcome by expressing dsRNA as an extended hairpin-loop RNA. The hairpin-RNA is expressed from a transgene exhibiting dyad symmetry in a controlled temporal and spatial pattern, under the control of a strong, heat-shock-inducible promoter. This approach has recently generated RNAi in the nematode *C. elegans*, in the fly *Drosophila melanogaster* and in the plant *Arabidopsis thaliana* [51-53]. It should be applicable to any organism in which RNAi is effective and the generation of transgenic organisms technically possible.

RNAi is linked to chromosome methylation

In several plant systems, RNA-triggered gene silencing is accompanied by DNA methylation. This process may be associated with PTGS. Three models of DNA methylation-PTGS have been postulated [1]: (1) DNA methylation is completely distinct from PTGS and it results from an independent interaction between the interfering RNA and the template DNA; (2) there is some kind of causal relationshipbetween RNA-triggered methylation of DNA causing aberrant transcription and the resulting transcripts inducing PTGS; and (3) conversely, the methylation of target DNA sequences may result from PTGS. As mentioned above, the introduction of dsRNA from gene promoters in *Arabidopsis* causes DNA methylation in the promoter of the corresponding genes [32], suggesting that methylation and PTGS are related gene-silencing phenomena, perhaps sharing part of the machinery.

RNAi self-propagation

RNAi is characterised by crossing cell boundaries and spreading throughout the organism [5] and, in some cases, to subsequent generations *via* a dominant extragenic agent, possibly the ~23-mers dsRNA molecules, as mentioned above [47]. However, the RNAi from introduced dsRNA is not maintained forever, probably because cell division and degradation dilute dsRNA. Yet small amounts of dsRNA can target the degradation of many corresponding mRNAs, as revealed by calculations of the dilution of introduced dsRNA [5]. As mentioned above, in the mechanism proposed for RNAi [3, 40] (Figure 1), the ~23-mers dsRNA fragments are regenerated after each round of mRNA cleavage, which may explain why RNAi behaves as a catalyst.

Two genes involved in PTGS, *qde-1* in the fungus *Neurospora* [30] and *ego*-1 in the nematode *C. elegans* [36], are homologous to a tomato gene that displays a RNA-dependant RNA-polymerase (RdRP), which suggests that RNAi may also involve amplification of the dsRNA signal by an RdRP. With the available data, both dsRNA regeneration and RdRP amplification may account for RNAi self-propagation.

RNAi in nature

RNAi has been poetically described as «a genetic wand and a genetic watchdog» [2]. Genetic wand refers to its potential for functional genomic analysis, and genetic watchdog refers to its function in nature. dsRNA is not a requisite product of normal gene expression but it is produced, at least transiently, by many viruses and transposons. This dsRNA is not associated with normal gene expression, and it is used by the organism to recognise potentially dangerous situations, to which the organism responds by blocking the expression of the potentially harmful RNAs.

Studies in plants strongly implicate PTGS as an antiviral mechanism [reviewed in 1] (Figure 2). Viral RNAs can be targets for PTGS [26], whose effects are spread among cells by direct dissemination of interfering RNA, as described in the nematode *C. elegans* during RNAi [5, 54]. Moreover, some viruses produce specific proteins that interfere with PTGS to overcome this defensive mechanism [55].

In addition, some of the genes involved in RNAi in the nematode *C. elegans* (*rde-2*, *rde-3*, *mut-2* and *mut-7*), when

mutated, allow the mobilisation of transposons. Thus, their wild-type function may be linked to transposon silencing in this organism [45-47]. This suggests that dsRNA-based PTGS is a general mechanism that controls the mobilisation of these mobile elements and their subsequent spreading throughout the genome (Figure 2).

This is also the basis for plant co-suppression and fungi quelling. These two PTGS phenomena are observed in transgenic plants and fungi, as transgenes can be regarded as foreign genetic objects, like viruses and transposons. In these cases, transgenes can induce their own silencing and simultaneously silence the homologous endogenous gene [56-58]. Recently, two *Arabidopsis* genes, *sgs2* and *sgs3*, necessary for both plant PTGS co-suppression and natural virus resistance, have been isolated. *Sgs2* is similar to an RdRP from the fungus *Neurospora crassa* (*qde-1*), which controls quelling, and to *ego-1* from the nematode *C. elegans*, which controls RNAi [59].

In summary, RNAi in nature may be a primitive immune system involved in genome surveillance, a general mechanism that limits aberrant or unwanted gene expression, developed to allow the genome of an organism to survive in a hostile environment with foreign genetic objects (like transposons and viruses).

RNAi as a tool for functional genomic analysis

The ability of dsRNA to silence gene expression through RNA-mediated genetic interference mechanisms is used by geneticists and molecular biologists for functional genomic analysis in a great and growing variety of organisms. RNAi has been successfully applied in plants, *Trypanosome*, and several invertebrates and vertebrates.

RNAi in plants

RNAi has been used in various plant species, like *Nicotiana tabacum*, *Oryza sativa* and *Arabidopsis thaliana* [27, 52]. In the first two, it was used to demonstrate the link between RNAi gene silencing and virus resistance [27], whereas in the latter, it was used to analyse the function of four genes involved in floral formation (*agamous*, *clavata3*, *apetala1* and *perianthia*) [52]. A genetically engineered DNA coding for RNA capable of duplex formation (dsRNA) was introduced into the genome of *Arabidopsis* by *Agrobacterium*-mediated transformation, which caused specific and heritable genetic interference.

RNAi in trypanosomes

In *Trypanosome*, transfection of cells with α-tubulin 5' untranslated region (5' UTR) dsRNA, resulted in the formation of multinucleated cells as a result of the specific block of cytokinesis [6]. Analysis of cytoskeletal structures from these trypanosomes revealed defects in the microtubules of the flagellar axoneme and attachment zone, a complex cortical structure that may be essential for establishing the path of the cleavage furrow at cytokinesis. More recently, it has

been shown that genetic interference in *Trypanosome* by dsRNA can be achieved in a heritable and inducible fashion by transgenesis, introducing into the genome a construct expressing the dsRNA in the form of stem-loop structure under the control of a tetracycline-inducible promoter [7, 60].

RNAi in invertebrates

The metazoa in which RNAi has been most used are, by far, invertebrates: hydra, platyhelminths, nematodes and insects. In *Hydra* [9], dsRNA-mediated interference demonstrated the role of *ks1* in head development, introducing the dsRNA into polyps by electroporation.

In platyhelminths, RNAi has been used in leech [12] and planaria. In the latter, the effectiveness of RNAi was first revealed by analysis of the specific effects of myosin, a-tubulin and opsin dsRNAs on regenerating and adult organisms [33]. As there is no available technique for transgenesis in these organisms [61], the use of RNAi is the only way to generate loss-of-function mutants for the study of gene function [33]. dsRNA-dependent gene silencing has recently been used to analyse the function of the *sine oculis* planarian gene (*Gtsix-1*), involved in the genetic cascade of eye formation in flies and vertebrates, and of planarian photoreceptors during head regeneration [11]. This study has corroborated the evolutionary conservation of the initial eye genetic pathway in invertebrates and vertebrates.

RNAi has also been used in planarians to analyse the function of newly discovered genes, not homologous with any other known gene [62 and work in progress] (Figure 3). It has been shown that *tcen49*, a gene involved in anterior-posterior regionalisation of the planarian body [62-65], contributes to the maintenance of gross planarian body regions and, specifically, of the organs from the central body region, i.e. the pharynx.

Among invertebrates, the group of metazoa in which RNAi has been most used is, by far, the nematodes. As mentioned above, RNAi was first discovered in the nematode *Caenorhabditis elegans* [4]. Apart from the studies aimed at elucidating the mechanism of RNAi action, RNAi has been extensively used in *C. elegans* to create loss-of-function mutants of many genes involved in various cellular and developmental processes.

Of special interest is the huge work of functional genomic analysis of *C. elegans* chromosomes I and III, whose genomic sequences have been completed, by systematic RNAi [66, 67]. RNAi was used to target nearly 90% of the predicted genes of chromosome I [66], and function has been assigned to 13.9% of the genes analysed, thus increasing the number of sequenced genes with known phenotypes from 70 to 378. RNAi has also been used to target 96% of the predicted ORF of chromosome III [67], in search for genes involved in cell division. As ORF sequences do not directly assign function to genes, RNAi could be used to elucidate the function of predicted ORF in the distinct Genome Projects.

Finally, RNAi has been used to create loss-of-function mutants of several genes involved in various cellular and developmental processes in several species of insects, like the
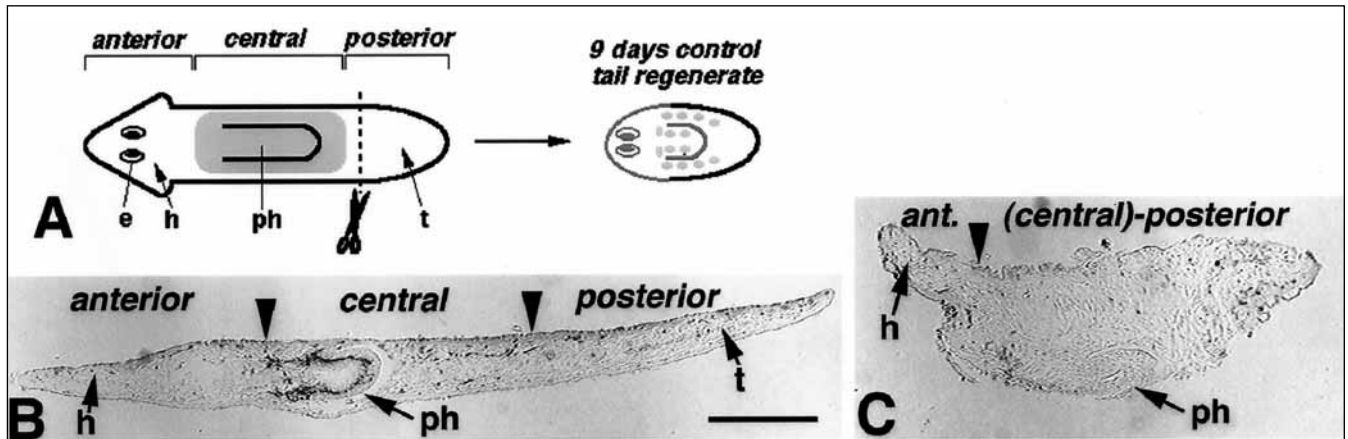
Figure 3. RNAi in regenerating planarians. RNAi has been used to analyse the function of *tcen49* [62-64], a new gene coding for a secreted protein involved in anterior-posterior regionalisation of the planarian body that is not homologous to any other known gene. Regenerating tails of 9 days of regeneration. Anterior is to the left, and dorsal to the top. Organisms were micro-injected with *tcen49* dsRNA or water (negative control) just after amputation following standard protocols [33], sacrificed after 9 days of regeneration, and analysed by immunohistochemistry on paraffin sagittal sections using a monoclonal antibody specific to TCEN49 protein [63, 76]. A) Diagram showing the area of amputation (dashed line) and the distribution of secreted TCEN49 protein (in dark yellow). A regenerate of 9 days is also shown. Note the structures and regions newly regenerated (in grey) and the localisation of cells expressing *tcen49* (in dark yellow), in a regeneration stage just starting TCEN49 secretion from *tcen49* expressing cells (cianophylic secretory cells[63]). B) Negative control regenerate microinjected with water. The regenerate has built up a new pharynx and a new head, and TCEN49 is restricted to expressing cells of the central body region, including the pharynx (dark blue staining). At this stage, TCEN49 starts to be secreted [63]. C) Regenerating tail micro-injected with *tcen49* dsRNA. Note that the gene is completely silenced, as no traces of the corresponding protein are detected after immunohistochemistry, and that the pharynx is expelled from the central region through the ventral side. Anterior region is mainly unaffected, but central and posterior regions seem to be fused. The malformations in the central and posterior region started at day 9 of regeneration, when TCEN49 should start to be secreted, and the organism died shortly afterwards. These results suggest that *tcen49* is critical for central body regional identity from day 9 on, and that in these loss-of-function mutants, the central body region identity is replaced by posterior body regional identity [62, 63]. Abbreviation: e, eye; h, head; ph, pharynx; t, tail. Scale bar: 0.5 mm.

fruit fly *Drosophila* [8, 10, 17], the fly *Megarelia* [19] and the milkweed bug *Oncopeltus fasciatus* [18]. As *Drosophila* is one of the genetically best-known organisms and the sequence of its genome has been completed, RNAi may be a powerful tool for analysing the function of predicted ORF, as in nematodes.
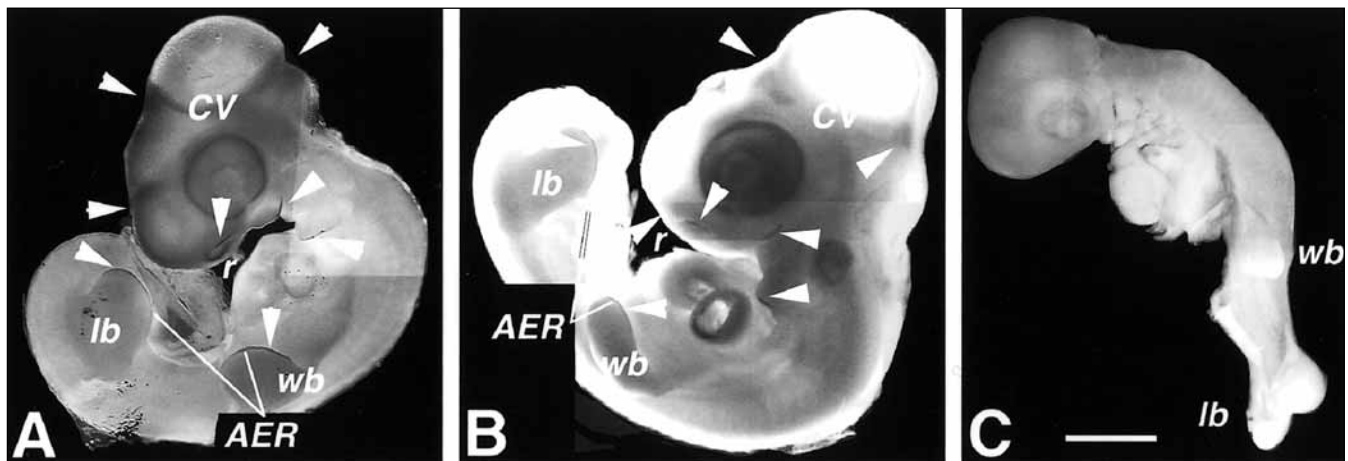

Figure 4. RNAi in chicken embryos. The specific effect of dsRNA-mediated gene silencing in chicken was tested by injection of several dsRNAs *in ovo*. Embryos of 1.5-2 days of development (Hamburger and Hamilton stages 12-14) were micro-injected at various sites along the anterior-posterior axis with chicken *fgf8* (Fibroblast Growth Factor – 8) dsRNA [77], planarian *tcen49* dsRNA (see Figure 3) or Dulbecco's Modified Eagle Medium (dsRNA solvent; DMEM). Microinjected embryos were analysed at several stages after microinjection (4-5 days of development) by *in situ* hybridisation with *fgf8* antisense riboprobe [77]. The head is to the top. A) Negative control embryo micro-injected with DMEM. Development proceeded normally. Note the areas of *fgf8* expression (arrowheads) [77]. B) Negative control embryo micro-injected with planarian *tcen49* dsRNA. Development proceeded normally. The presence of a dsRNA not homologous with any chicken gene did not produce any morphological effect and did not silence *fgf8* gene expression (arrowheads). C) Embryo micro-injected with chicken *fgf8* dsRNA. Regardless of the microinjection site, *fgf8* gene expression was completely silenced in most embryos, although some variability was also observed (not shown). At this stage, *fgf8* should be expressed at discrete sites in the cephalic vesicles and rostrum, and in the apical ectodermal ridge of wing and limb buds (see Figures 2A and 2B). Note the malformations produced by *fgf8* loss-of-function in the cephalic vesicles and the rostrum, as described elsewhere [78-80]. Also note that wing and limb buds remain largely unaffected, probably owing to a redundant function between *fgf4* and *fgf8*, two distinct members of the *fgf* gene family that are coexpressed in these areas, promoting growth and patterning, and that have been described as partially redundant [81-83]. Abbreviations: AER, apical ectodermal ridge; cv, cephalic vesicles; lb, limb bud; r, rostrum; wb, wing bud. Scale bar: 0.25 mm.

RNAi in vertebrates

The specific ability of dsRNA to silence genes in vertebrates has given rise to an intense debate, with some contradictory reports [21, 68]. It is generally accepted that RNAi in vertebrates degrades the corresponding mRNAs, with specific loss-of-function effects. The presence of both multiple sites for single-gene expression at various developmental stages and of gene families with redundant functions in vertebrates, which hinders the analysis of loss-of-function phenotypes, may account for these contradictory reports [work in progress]. dsRNA has been used to silence genes in zebrafish [20, 21, 68], *Xenopus* [22], chicken [69 and work in progress] and mice [23, 24].

Generally, dsRNA is introduced by microinjection into oocytes, 1-cell stage embryos or one cell of 2-cell stage embryos, to target mRNAs involved in early developmental stages [20-23, 68] and dormant maternal mRNAs [24]. However, dsRNA has been micro-injected *in ovo* of chicken at several developmental stages, producing the phenotypic defects associated with the corresponding loss-of-function mutant (Figure 4) [69 and work in progress]. The adventage of introducing dsRNA at later developmental stages is that the interference does not affect former stages. The function of essential genes for the embryos can thus be defined, while avoiding the progressive accumulation of mutant phenotypes that hinder the analysis of function at one specific point. However, it is not yet possible to directly silence gene expression at a single site of the embryo by directed dsRNA microinjection, as RNAi effects spread all over the embryo, given its remarkable ability to cross cell boundaries. RNAi is a powerful tool for interfering with gene expression in a wide range of organisms, thus facilitating functional genomic analysis.

Future perspectives

Research on RNAi is growing rapidly and generating exciting results in many fields, from screens to identify essential genes for completion of the first cell cycle or early embryonic or germline development [70] to elucidate the function of predicted ORF in the distinct Genome Projects by systematic RNAi [66, 67]. RNAi is used as a general technique for determining gene function [71]. This approach is very valuable for several reasons. First, it provides the fastest link between sequence and function. Second, homology-based cross-interference may be useful to silence highly homologous and redundant genes simultaneously. Finally, it allows comparison of the function of homologous genes of distinct species that have not yet been cloned, such as orthologous homeotic genes during the development of distantly related species [72].

In addition, the link between RNAi, antiviral mechanisms and transposon mobilisation may be extended to X-chromosome inactivation, imprinting and interferon response (Figure 2) [73-75]. Moreover, RNAi offers great economic and therapeutic potential, both for agriculture, where it can improve the response to viral infections and help develop transgene/host association overriding gene silencing to allow the expression of proteins of interest, and for mammals, in the fight against certain diseases like cancer and virus/parasite infection. It will probably be regarded as one of the major scientific breakthroughs of the end of the 2nd and the beginning of the 3rd millennium.

It has been recently reported the targeted degradation of mRNAs in cultured mammalian cells by RNAi [84, 85], and that the 21-25 nt long dsRNAs (currently named small interfering RNAs; siRNAs) serve as primers to transform the target mRNA into dsRNA by the action of a RNA-dependent RNA polymerase (RdRP) [86, 87, 88], which is degraded to eliminate the targeted mRNA while generating new siRNA in a cycle of dsRNA synthesis and degradation that leads to RNAi.

Acknowledgements

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Dr. David Bueno i Torrens was born in Barcelona in 1965. He graduated from the University of Barcelona in 1989 with a degree in Biology, and received a PhD in Genetics (Developmental Genetics) at the beginning of 1994 for work on planarian regeneration at cellular and molecular level. His PhD Thesis was awarded cum laude; he received the PhD Extraordinary Award (Premi Extraordinari de Doctorat) of the Faculty of Biology for the most significant Thesis finished in the Faculty during 1994; and also received Special Mention in the 1st Prize of the Doctors' Assembly (1r Premi Claustre de Doctors) of Barcelona University as the second most significant Thesis finished in the University during the biennium 1994-1995. He did postdoctoral work at the University of Oxford (England) in a group funded by the Cancer Research Campaign. During this period, his research focused on the analysis of Fibroblast Growth Factor functions during vertebrate development, using transgenic mice as a model system. Since the beginning of 1996, he has worked at the Department of Genetics of the University of Barcelona, spending short periods of time at the European Molecular Biology Laboratory (Heidelberg, Germany) and at the University of Innsbruck (Austria). In 2000, he became Associate Lecturer at the University of Barcelona. He is a founder member of the Spanish Society of Developmental Biology, and member of the European Life Scientists Organisation, the Spanish Society of Genetics and the Catalan Society of Biology. His current research in Developmental Biology and Genetics focuses on both planarian regeneration, and cephalic and rostrum formation during vertebrate development. He is leading a young team of scientists, which is part of the team headed by Dr. Rafael Romero, which in turn is part of the Developmental Biology and Genetics group of the University of Barcelona led by Prof. Jaume Baguñà. He became interested in RNAi as a tool to induce loss-of-function mutants and thus override transgenesis in planarians to analyse gene function during planarian regeneration and simplify the development of knock-out transgenics in vertebrates to analyse gene function during cephalic and rostrum development. He is author or co-author of over 25 articles in international peer-review journals, 35 papers to inter-

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