ELSEVIER

British Mycological Society promoting fungal science

journal homepage: www.elsevier.com/locate/fbr

Review

RNAi silencing: A tool for functional genomics research on fungi

Anahí ARMAS-TIZAPANTZI[a,b], *Alba Mónica MONTIEL-GONZÁLEZ*[a,*]

[a]*Laboratorio de Biología Molecular, Centro de Investigaciones en Ciencias Biológicas, Universidad Autónoma de Tlaxcala, 90120 Tlaxcala, Mexico*

[b]*Doctorado en Ciencias Biológicas, Universidad Autónoma de Tlaxcala, 90120 Tlaxcala, Mexico*

ARTICLE INFO

Article history:
Received 2 March 2016
Received in revised form
27 May 2016
Accepted 31 May 2016

Keywords:
Functional genomics
Quelling
RNAi
Silencing in fungi

ABSTRACT

RNA interference (RNAi) is a mechanism of post-transcriptional gene silencing which is triggered by the presence of double stranded RNA molecules homologous to a gene, resulting in degradation of the messenger RNA produced attenuating thereby the target gene expression, as a mechanism of regulation of gene expression. The potential use of this mechanism in several areas of life sciences is tangible. In the case of biotechnology, the use of fungi as production models of human interest compounds, invites studies on the mechanisms of regulation of gene expression, for the better use of its qualities. This review tries to concentrate most of the existing information about the mechanism and components that direct the silencing, using RNAi to fungus as a model organism. Also, it was included available information about the role of RNAi in organisms that contain it. Strategies that have been developed as tools for the study of gene function using the RNAi mechanism are mentioned, too. Finally, some of the research in fungi where RNAi silencing has been used to assess gene function is included.

1. Introduction

RNA silencing, also known as RNA interference (RNAi) or gene silencing in eukaryotes is a conserved mechanism where small double stranded RNA (siRNA) molecules are involved in regulating the gene expression of a target gene. This mechanism was first observed in 1990 in petunia plants, when trying to over-express the enzyme chalcone synthase (CHS), a key enzyme in the anthocyanin pathway that produces a purple pigment in these plants. In this study, an additional copy of the CHS gene was introduced into the genome of petunias with the expectation that over-expression of CHS would produce a more intense purple color. However, only white petunias were observed, with white patches or pale flowers (Napoli *et al.*, 1990; Van der Krol *et al.*, 1990). This finding suggested to the authors that the overexpression of the transferred gene (transgene) caused both the introduced gene, and their endogenous counterparts to be suppressed. This process was therefore called "co-suppression" or post-transcriptional gene silencing (PTGS) of the endogenous CHS gene.

The first report of RNA silencing in fungi was released by Romano and Macino in 1992, where they worked with two genes; albino -1 and albino -3 (*al-1*, and *al-3*), encoding

* *Corresponding author*. Tel./fax: +52 248 48 154 82.
E-mail address: amonicamg@yahoo.com (A. M. Montiel-González).
http://dx.doi.org/10.1016/j.fbr.2016.05.003
1749-4613/

sequences of enzymes involved in carotenoid biosynthesis from *Neusospora crassa* wild-strain with an orange phenotype. The alterations of these genes can result in an albino phenotype. In this study, they induced silencing of these genes, integrating coding sequences of the *al-1* and *al-3* with different genes portions into somatic cells. In this investigation, the most representative transformants showed albino phenotypes that ranged from white, pale yellow, dark yellow and dark/orange mycelia and conidia, indicating that the endogenous genes, *al-1* and *al-3*, were suppressed to different degrees. The albino transformants showed levels of mRNA from the *al-1* gene to be between 10 and 20 fold lower than the wild-type, indicating that albino phenotypes were characterized by transcriptional inactivation. They suggested that one possible mechanism to explain this phenomenon was DNA methylation, which plays an essential role in the regulation of gene expression in other eukaryotes. This phenomenon was termed "quelling".

Subsequently, in 1998 Fire *et al.*, (1998) analyzed RNA-mediated genetic interference in *Caenorhabditis elegans*. They found that injection of double stranded RNA (dsRNA) complementary to a target sequence was substantially more efficient in producing interference than the use of any of their RNA strains injected individually. Such interference resulted in silencing of the target gene expression, demonstrating that genetic interference is mediated by dsRNA. These results could explain why inverted-repeat structures or features of the dsRNA viruses are involved in transgene dependent co-suppression in plants and why quelling occurs during the introduction of duplicated sequences in *N. crassa*. This phenomenon could also explain both the co-suppression and the quelling and was named RNA interference (RNAi). For this work, the authors received the Nobel Prize for Physiology and Medicine in 2006.

Currently, the use of this mechanism has been exploited in numerous studies to extend the knowledge about the function of gene products. RNAi enables researchers the ability to attenuate the expression of specific genes at different levels while keeping the organism viable even when applied to genes vital for cell survival (Nakayashiki *et al.*, 2005). In addition, RNAi enables the silencing of a complete gene family, simultaneously (Salame *et al.*, 2010).

2. Components and mechanism of action in fungi

In the case of fungi, most studies of RNA silencing have been undertaken in the model multicellular filamentous fungus *N. crassa* and in the model unicellular yeast *Schizosaccharomyces pombe*. The gene silencing mechanism in fungi is commonly referred to as quelling and occurs at the post-transcriptional level during the vegetative stage of the life cycle of *N. crassa* (Fulci and Macino, 2007; Romano and Macino, 1992). These studies have provided important information for the understanding of the mechanism of silencing by RNAi in other eukaryotes. Gene quelling in fungi is equivalent to RNAi in animals or post-transcriptional gene silencing in plants, since the basic components of gene silencing such as Dicer, Argonaute and RNA-dependent RNA polymerase (RdRP) are also used in these pathways (Fagard *et al.*, 2000).

In *N. crassa*, both the components and the mechanism involved in the quelling pathway have been identified have been shown to be necessary to control transposons, but apparently do not play a role in the assembly and maintenance of heterochromatin (Fulci and Macino, 2007). One component is QDE-1, is a dual-function enzyme with RNA-dependent RNA polymerase (RdRp) and DNA-dependent RNA polymerase (DdRP) activities. QDE-1 can act as a DdRP to produce aberrant RNA (aRNA) from aberrant repetitive DNA sequences and then act as an RdRP to convert aRNA to dsRNA. Therefore, QDE-1 both produces and amplifies the dsRNA (Chang *et al.*, 2012; Cogoni and Macino, 1999a; Lee *et al.*, 2009; Lee *et al.*, 2010a). The QDE-3 protein belongs to the family of RecQ helicases that are involved in homologous recombination, replication, DNA repair (Cogoni and Macino, 1999b) and in the production of aRNA which is converted to dsRNA by QDE-1 (Cogoni and Macino, 1999a; Kato *et al.*, 2005; Lee *et al.*, 2010a). Cogoni and Macino (1999b) suggested a role of QDE-3 in the unwinding of dsDNA which may be required for the DNA–DNA interactions between transgenic repeats, with ssDNA serving as a target for the production of aRNA by QDE-1. The presence of two homologous Dicer-like genes (*dcl-1* and *dcl-2*) was subsequently revealed from the sequencing of the genome of *N. crassa*. Dicer-like encoding endonucleases belonging to the RNase family III and are also involved in the quelling pathway with *dcl-2* making a greater contribution (Catalanotto *et al.*, 2004). These enzymes cut dsRNA to generate small interfering mature double stranded RNA (siRNA) with a length between 21 and 25 nucleotides (nt), where 19 nt form double-stranded RNA and other two nt are overhanging. The siRNA is incorporated into a multiprotein complex with ribonuclease activity called the RNA-induced silencing complex (RISC) (Hammond *et al.*, 2001). This complex contains the QDE-2 gene encoding the protein Argonaute. Normally this protein has the PAZ and PIWI domains, which work as siRNA binding site and the catalytic core and cutting machinery, respectively. Separation into individual strands of siRNA is essential for activation of RISC (Chang *et al.*, 2012; Song *et al.*, 2004). In the biochemical derivative purification of QDE-2 in *N. crassa*, the QIP protein was identified which has an exonuclease domain. It is suggested that QIP cleaved and removed the passenger strand of the siRNA duplex in a QDE-2 dependent pathway (Maiti *et al.*, 2007). Once the RISC complex is activated, it recognizes complementary mRNA from the incorporated siRNA, resulting in gene silencing, either as mRNA cleavage or translational repression.

micro RNA (miRNA) is another type of small RNA duplex with an approximated length of 21 nt and are endogenous molecules processed by Dicer from precursors of small sRNA (ssRNA) with a hairpin structure. The miRNAs have been found in various eukaryotic organisms and may play important regulatory roles in animals and plants in mRNA cleavage or translational repression (Bartel, 2004). In plants for example, they have been implicated in stress tolerance and the development of bloom (Achard *et al.*, 2004; Aukerman and Sakai, 2003; Khraiwesh *et al.*, 2012; Sunkar *et al.*, 2012). In insects, they have been implicated in the development of their