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HIGHLIGHT

RNA silencing mechanisms are highly differentiated in eukaryotes

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Abstract Posterior to the discovery of the double-stranded RNA mediated gene silencing two decades ago, RNA interference or RNA-mediated gene silencing has received unusual intensity of study in the biology-related research fields. RNA silencing represents a large spectrum of gene regulation mechanisms in all kingdoms of eukaryotes. The power and necessity of RNA silencing has been unambiguously appreciated in both animals and plants, although the mechanisms engaged are divergent in some aspects. Interestingly, as comprehensively reviewed by Schumann et al. in this issue, RNA silencing in the simple eukaryotic fungi strikingly differs from those of animals and plants, and among fungal species as well.

The classification of living things into different kingdoms has extensively evolved in the last three centuries. Fungi are simple eukaryotes, with most of them being unicellular microorganisms. It starts to be recognized as a separate kingdom by Robert Whittaker in his five-kingdom system proposed in 1969 based mainly on differences in nutrition styles (Whittaker, 1969). Fungi are now separated from plants, animals and bacteria with the major difference lying in its cell-wall component of chitin. The formally documented fungi species has reached about 100000 with a wide range of growth habitats (http://en.wikipedia.org/wiki/Fungus), playing very important roles in most ecosystems. Many fungi are pathogens of plants and animals (including humans), which significantly increases the public interest in studying the gene regulation mechanisms of this kingdom of tiny living beings.

The core molecular mechanisms of central dogma including DNA replication, transcription (RNA synthesis) and translation (protein synthesis) are very similar in all eukaryotes. Most gene regulation mechanisms mediated by proteins are well conserved across different kingdoms of eukaryotes.

Nevertheless, RNA silencing mechanisms seem highly differentiated among eukaryotes. RNA silencing mechanisms are characterized by two separable processes, production of small RNAs and the small RNA-mediated gene silencing either at the transcriptional level (TGS) or post-transcriptional level (PTGS). The protein machineries engaged in these two processes, the small RNA species generated and the silencing mechanisms followed are distinct among different kingdoms of eukaryotes. For example, human encodes over 1000 different microRNAs, *Arabidopsis* encodes over 200. About 50 microRNAs have been identified in the unicellular alga *Chlamydomonas reinhardtii* and 2 in the single-celled amoeba *Dictyostelium discoideum* (http://www.mirbase.org, release 16, Sept. 2010), but no microRNA has yet been identified in any fungi examined thus far. On the contrary, the number of genes encoding Dicer protein for microRNA processing and AGO protein for effecting small RNA-mediated gene silencing are not correlated with the complexities of different eukaryotes. For example, human encodes one Dicer while *Arabidopsis* encodes four distinct Dicer-like enzymes. Human contains four *AGO* genes, while *Arabidopsis* contains ten (Ghildiyal and Zamore, 2009). Most fungal species studied contain one or more *DCL* (dicer-encoded gene) and *AGO* genes (Schumann et al., 2010).

The genome sizes of eukaryotes are generally increased with the organism complexity, while the numbers of the protein-coding genes do not vary greatly because of the decreased gene density. It is now becoming clear that the genomes of all studied eukaryotes are almost entirely transcribed, underlining that the different kingdoms of eukaryotes mainly differs in their encoded non-coding RNAs but not proteins (Amaral et al., 2008). Fungal genomes are small but dense in protein-coding genes, and therefore has a low encoding capacity for non-coding RNA species. It is very interesting that the genome fitness of

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simple eukaryotes favors the complete set of protein machineries for all kinds of molecular process, regardless of their application efficiency. In addition to the presence of the *DCL* and *AGO* genes even in the absence of miRNAs in most studied fungi, the budding yeast genome contains the full set of spliceosome genes for splicing of only a few hundreds of introns (Davis et al., 2000).

RNA silencing is a combined effort of non-coding RNAs and effector proteins, and thus the large diversity in non-coding RNAs of different eukaryotes may significantly contributes to the highly differentiated RNA silencing mechanisms in eukaryotes. The lack of miRNAs in fungi suggests that miRNA-mediated gene silencing may not be important for fungal growth and reproduction. Although RNA silencing mechanisms have been demonstrated in several fungi and a number of budding yeasts using transfected reporter plasmids (Drinnenberg et al., 2009), the physiologic role of the known RNA silencing pathways has not been clearly assigned yet (Schuman et al., 2010). It could be possible that RNA silencing mechanisms in fungi are dispensable, which explains their striking differences in the model fungi *Neurospora crassa* and *Schizosaccharomyces pombe* (Schumann et al., 2010). Consistently, fungi might serve as a unique reservoir in nature for a large variety of RNA silencing mechanisms due to the lack of selection pressures.

Another divergent point of RNA silencing in eukaryotes lies in the mechanisms of recruiting the silencing proteins alone or the small RNA-protein complexes into different target sites for action. For example, the AGO-miRNA complex in metazoans is generally recruited to the partially complementary miRNA target sites in the 3′ untranslated regions (3′UTR) of the mRNA, resulting in the translational repression and mRNA degradation associated with 3′ deadenylation and 5′ decapping (Eulalio et al., 2008). The availability of some target sites is also regulated by the absence or presence of specific RNA binding proteins in the 3′ UTR of the mRNA (Kedde et al., 2007). In plants, the AGO-miRNA complex is commonly recruited to the fully complementary target sites both in the 3′ UTR and the more often coding region, which results in the cleavage of mRNA at the target sites (Ghildiyal and Zamore, 2009). The lack of miRNA in fungi suggests that this class of post-transcriptional gene silencing mechanism is not biologically relevant in this kingdom of simple eukaryotes. Instead, the RNA silencing proteins play more direct roles in mediating heterochromatin formation and maintaining genome stability (Schumann et al., 2010), which are recruited to the sites of action by small RNAs other than miRNAs.

Systematic identification of the small RNAs associated with different AGOs and their target sites shall expedite the comprehensive understanding of the diverse RNA silencing mechanisms in different kingdoms of eukaryotes. The advantages in their relative small genomes, fast growth and easy genetic manipulation predict a large contribution from fungi. Meanwhile, the therapeutic potential of small RNA-mediated gene silencing for human and plant diseases is attractive (Nakayashiki, 2005; Schumann et al., 2010). Although efficient delivery of small RNAs into fungal cells remain quite challenging, successful inhibition of the growth of a pathogenic budding yeast by antisense oligonucleotides targeting ribozyme RNA folding and function encourages further exploration of more efficient delivery approaches (Disney et al., 2003; Zhang et al., 2009).

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