RNA Interference in Fungi

Subjects: Microbiology | Biochemistry & Molecular Biology | Genetics & Heredity

Contributor: Silvia Calo

RNA interference (RNAi) was discovered at the end of last millennium, changing the way scientists understood regulation of gene expression. Within the following two decades, a variety of different RNAi mechanisms were found in eukaryotes, reflecting the evolutive diversity that RNAi entails. The essential silencing mechanism consists of an RNase III enzyme called Dicer that cleaves double-stranded RNA (dsRNA) generating small interfering RNAs (siRNAs), a hallmark of RNAi. These siRNAs are loaded into the RNA-induced silencing complex (RISC) triggering the cleavage of complementary messenger RNAs by the Argonaute protein, the main component of the complex. Consequently, the expression of target genes is silenced. This mechanism has been thoroughly studied in fungi due to their proximity to the animal phylum and the conservation of the RNAi mechanism from lower to higher eukaryotes. However, the role and even the presence of RNAi differ across the fungal kingdom, as it has evolved adapting to the particularities and needs of each species. Fungi have exploited RNAi to regulate a variety of cell activities as different as defense against exogenous and potentially harmful DNA, genome integrity, development, drug tolerance, or virulence. This pathway has offered versatility to fungi through evolution, favoring the enormous diversity this kingdom comprises.

RNA Interference RNAi fungi

1. Introduction

RNA interference (RNAi) or RNA silencing has been deeply studied in the last two decades, as its discovery entailed a revolution in the understanding of the regulation of gene expression. This RNAi pathway, broadly conserved in eukaryotes, uses small interfering RNAs (siRNAs) to suppress gene expression of homologous sequences. These siRNAs, of 20–30 nucleotides (nt) long, are produced from double-stranded RNA (dsRNA) by an RNase III called Dicer (Dcr) and loaded into an RNA-induced silencing complex (RISC), which contains an Argonaute protein (Ago) that drives the selective degradation of homologous messenger RNAs (mRNA), as well as translational or transcriptional repression of target sequences. Moreover, in fungi and other organisms, an RNA-dependent RNA polymerase (Rdp or RdRP) generates dsRNA from certain single-stranded RNA (ssRNA) or from the target messenger RNA, activating or amplifying the silencing response, respectively[1][2].

Fungi have proven to be excellent model organisms for the study of the RNAi pathway, since many of the discoveries accomplished in these organisms were later extended to higher eukaryotes. In fact, one of the first RNA silencing phenomena reported was found in *Neurospora crassa*, which is an essential model organism to study modern genetics. *N. crassa* has developed different RNAi mechanisms, but the two that were originally found are the best described. The first is called quelling, a post-transcriptional gene silencing (PTGS) guided by

siRNAs[3], which suppresses transposons and virus infections[4]. Quelling is triggered by the introduction of transgenes homologous to an endogenous gene. After the transgene is transcribed, it follows a canonical pathway to activate silencing, carried out by an Rdp protein (QDE-1), two Dicer-like proteins (Dcl1 or Dcl2), and an Argonaute (QDE-2). The second mechanism is called meiotic silencing of unpaired DNA (MSUD) and is involved in silencing genes that are not paired with their partner on the homologous chromosome during meiosis[5]. This mechanism is present not only in N. crassa but also in other ascomycetes, such as Gibberella zeae, and operates during prophase I[6][7]. Some of the elements involved in the canonical RNAi pathway, such as Dcl1, are necessary for this other mechanism, as well as a MSUD-specific Rdp (SAD-1), a second Argonaute (SMS-2), and the helicase SAD-3. These proteins form a multiprotein complex located at the perinuclear region that acts generating MSUD-associated siRNAs (masiRNAs)[8]. After these first discoveries in *N. crassa*, the RNAi mechanism was found in several other fungi, such as *Schizosaccharomyces pombe*[9], *Cryptococcus*[10], or *Mucor*[11].

When RNAi was discovered, it was thought to be a defense system against exogenous and potentially harmful DNA, including transposons, virus, and transgenes. However, very soon, its involvement in other cellular functions, such as genome integrity or gene regulation, was found. Recent studies in fungal pathogens stated that the RNAi pathway is also implicated in development, drug tolerance, and virulence. Thus, fungi have exploited RNAi to tune their cellular processes, reaching unsuspected limits.

2. Defense against Viruses

The first predicted function of RNAi as a defense against viral infections would explain the conservation of the pathway through the evolution of eukaryotes, since viral infections affect every phylum on the tree of life. This mechanism has been widely explored in the ascomycete filamentous fungus *Cryphonectria parasitica*[12][13]. In *Aspergillus nidulans* RNAi also acts as a defense mechanism against virus[14].

3. Control of Transposable Elements

Transposable elements (TEs) are described as DNA sequences that have the ability to change their position within a genome. Although TEs were initially considered junk DNA, they have been associated with several important activities since their discovery, including centromere function, genome reorganization, and gene expression regulation[15][16][17]. TEs are also considered “selfish” DNA because they try to be perpetuated whilst the host tries to curtail their spread and, thus, their consequences on genome integrity. As a result, many organisms have developed mechanisms to ensure the control of TE activity[18][19]. Some of those mechanisms are well described in the literature and include DNA methylation[20], histone methylation[21], and heterochromatin-inducing protein[22]. Another mechanism that controls TE spread, probably the most ancient of all mentioned, is RNAi. Small RNAs associated with proteins can act at the transcriptional or post-transcriptional level against TE activity. The role of RNAi in TE repression has been well characterized in the plant kingdom[23] and other organisms, such as *Drosophila melanogaster* and *Caenorhabditis elegans*[24][25]. Studies on the RNAi role in TE

control in fungi, such as *Neurospora crassa*[26], *Schizosaccharomyces pombe*[27], *Magnaporthe oryzae*[28], *Mucor lusitanicus*[29], or *Cryptococcus neoformans*[30][31] have served to further understand these mechanisms.

4. Regulation of Endogenous Genes

As described above, the development of RNAi mechanisms represents an evolutive advantage regarding the defense against exogenous nucleic acids. However, specialization of those RNAi mechanisms has led to the establishment of novel post-transcriptional regulation networks of endogenous genes according to the use of Rdp, Dicer, and Argonaute proteins. The human pathogenic fungus *M. lusitanicus,* for instance, shows an intricate RNAi mechanism as a function the interplay of the silencing proteins in three different pathways, named the canonical, epimutational, and noncanonical RNAi pathways. The crosstalk of the RNAi pathways creates a complex network that regulates both basic cellular activities, such as metabolism or vegetative growth, and elaborated mechanisms, including sexual reproduction and pathogenesis[11][32][33]. *N. crassa*[34], *Coprinopsis cinerea*[35], *Fusarium graminearum*[35] and *Magnaporthe oryzae*[28][36], also produce sRNAs that regulate endogenous genes.

5. Heterochromatin Formation

Heterochromatin constitutes a highly condensed state of DNA. It is considered to have no transcriptional activity due to the limited access of the regulatory proteins to the promoter regions[37]. Generally, heterochromatin is concentrated in the telomeric, centromeric, ribosomal, and mating type regions of the eukaryotic chromosome[38]. Heterochromatin assembly is strictly regulated for accurate chromosome segregation, maintenance of telomere integrity, transcriptional silencing, and transposon control[37][39]. Heterochromatin formation in *S. pombe* is triggered by the production of siRNAs derived from centromeric regions with numerous repeats[40][41]. Remarkably, heterochromatin formation and the RNAi pathway can also regulate the epigenetic inheritance of gene silencing in *S. pombe*[42].

6. Adaptation to Stressful Conditions

The epimutational pathway in *M. lusitanicus* was discovered after the emergence of isolates resistant to the antifungal drug FK506 with no apparent mutations in the target genes[43]. The isolates, called epimutants, produced siRNAs from the mature mRNA of *fkbA* gene, which encodes FKBP12, the FK506-interacting protein[43][44]. Epimutants developing resistance to other antifungal agents, such as 5-fluoroorotic acid (5-FOA), have also been isolated[44]. Therefore, the epimutation process does not appear to occur at a specific gene locus, suggesting it might constitute a general mechanism that generates phenotypic plasticity in Mucor by silencing key genes and allowing rapid and reversible adaptation to environmental stresses[45][46].

7. Pathogenesis

RNAi has also been found to play an important role in pathogenesis, more thoroughly studied in plant pathogens. Many crops with worldwide importance are susceptible to being infected by pathogenic fungi, which translates into economic losses. Thus, alternative methods of infection control have been investigated, allowing a deeper understanding of fungal pathogenesis and the involvement of RNAi. Some of those pathogenic fungi, such as *Colletotrichum gloeosporioides*[47], *M. oryzae*[48], *Sclerotinia sclerotiorum*[49] have active RNAi pathways which influences their pathogenicity. RNAi is also involved in virulence of animal pathogens, such as *M. lusitanicus*[50].

An important feature of fungal pathogenesis is the mechanism called cross-kingdom RNAi, which has evolved to regulate the host–pathogen interaction. The existence of sRNA trafficking between the host and the pathogen and silencing target genes of the counterparty in trans was first discovered in plants, but afterward extended to mammal systems. Some fungal pathogens, such as *Phytophthora sojae*[51] or *Botrytis cinerea*[52], have been found to produce sRNAs that function as RNA effectors to suppress host immunity.

8. Loss of RNAi

In essence, RNAi has crucial regulatory and defense roles in eukaryotes, suggesting that this key mechanism has been positively selected through evolution in plants, nematodes, animals, and fungi. Yet, some members of the fungal kingdom have lost key components of the RNAi pathway[53], resulting in its inactivation. A hypothesis to explain this contradiction could be that those species may have other defensive mechanisms more advantageous than RNAi. Alternatively, perhaps, this RNA-based mechanism constitutes a disadvantage for them, forcing the survival of the RNAi-deficient species.

References

1. Shwu-Shin Chang; Zhenyu Zhang; Yi Liu; RNA Interference Pathways in Fungi: Mechanisms and Functions. *Annual Review of Microbiology* **2012**, *66*, 305-323, 10.1146/annurev-micro-092611-150138.
2. Megha Ghildiyal; Phillip D. Zamore; Small silencing RNAs: an expanding universe. *Nature Reviews Microbiology* **2009**, *10*, 94-108, 10.1038/nrg2504.
3. Nicoletta Romano; Giuseppe Macino; Quelling: transient inactivation of gene expression in Neurospora crassa by transformation with homologous sequences. *Molecular Microbiology* **1992**, *6*, 3343-3353, 10.1111/j.1365-2958.1992.tb02202.x.
4. Carlo Cogoni; Giuseppe Macino; Gene silencing in Neurospora crassa requires a protein homologous to RNA-dependent RNA polymerase. *Nature* **1999**, *399*, 166-169, 10.1038/20215.

5. Hitoshi Nakayashiki; RNA silencing in fungi: Mechanisms and applications. *FEBS Letters* **2005**, *579*, 5950-5957, 10.1016/j.febslet.2005.08.016.
6. Hokyoung Son; Kyunghun Min; Jungkwan Lee; Namboori B. Raju; Yin-Won Lee; Meiotic silencing in the homothallic fungus Gibberella zeae. *Fungal Biology* **2011**, *115*, 1290-1302, 10.1016/j.funbio.2011.09.006.
7. Yizhou Wang; Kristina M. Smith; John W. Taylor; Michael Freitag; Jason E. Stajich; Endogenous Small RNA Mediates Meiotic Silencing of a Novel DNA Transposon. *G3: Genes|Genomes|Genetics* **2015**, *5*, 1949-1960, 10.1534/g3.115.017921.
8. Logan M. Decker; Erin C. Boone; Hua Xiao; Benjamin S. Shanker; Shannon F. Boone; Shanika L. Kingston; Seung A. Lee; Thomas M. Hammond; Patrick K. T. Shiu; Complex Formation of RNA Silencing Proteins in the Perinuclear Region ofNeurospora crassa. *Genetics* **2015**, *199*, 1017-1021, 10.1534/genetics.115.174623.
9. Yasuhiro Hirano; Haruhiko Asakawa; Takeshi Sakuno; Tokuko Haraguchi; Yasushi Hiraoka; Nuclear Envelope Proteins Modulating the Heterochromatin Formation and Functions in Fission Yeast. *Cells* **2020**, *9*, 1908, 10.3390/cells9081908.
10. R. Blake Billmyre; Silvia Calo; Marianna Feretzaki; Xuying Wang; Joseph Heitman; RNAi function, diversity, and loss in the fungal kingdom.. *Chromosome Research* **2013**, *21*, 561-72, 10.1007/s10577-013-9388-2.
11. Santiago Torres-Martínez; Rosa M. Ruiz-Vázquez; The RNAi Universe in Fungi: A Varied Landscape of Small RNAs and Biological Functions. *Annual Review of Microbiology* **2017**, *71*, 371-391, 10.1146/annurev-micro-090816-093352.
12. Qihong Sun; Gil H. Choi; Donald L. Nuss; A single Argonaute gene is required for induction of RNA silencing antiviral defense and promotes viral RNA recombination. *Proceedings of the National Academy of Sciences* **2009**, *106*, 17927-17932, 10.1073/pnas.0907552106.
13. Xuemin Zhang; Donald L. Nuss; A host dicer is required for defective viral RNA production and recombinant virus vector RNA instability for a positive sense RNA virus. *Proceedings of the National Academy of Sciences* **2008**, *105*, 16749-16754, 10.1073/pnas.0807225105.
14. T. M. Hammond; M. D. Andrewski; M. J. Roossinck; N. P. Keller; Aspergillus Mycoviruses Are Targets and Suppressors of RNA Silencing. *Eukaryotic Cell* **2007**, *7*, 350-357, 10.1128/ec.00356-07.
15. Savannah J. Klein; Rachel J. O'neill; Transposable elements: genome innovation, chromosome diversity, and centromere conflict. *Chromosome Research* **2018**, *26*, 5-23, 10.1007/s10577-017-9569-5.
16. Cory D. Hirsch; Nathan M. Springer; Transposable element influences on gene expression in plants. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer* **2017**, *1860*, 157-165, 10.1016/

j.bbagrm.2016.05.010.

17. Adam G Diehl; Ningxin Ouyang; Alan P. Boyle; Transposable elements contribute to cell and species-specific chromatin looping and gene regulation in mammalian genomes. *Nature Communications* **2020**, *11*, 1796-18, 10.1038/s41467-020-15520-5.
18. Marc Friedli; Didier Trono; The Developmental Control of Transposable Elements and the Evolution of Higher Species. *Annual Review of Cell and Developmental Biology* **2015**, *31*, 429-451, 10.1146/annurev-cellbio-100814-125514.
19. Raúl Castanera; Leticia López-Varas; Alessandra Borgognone; Kurt LaButti; Alla Lapidus; Jeremy Schmutz; Jane Grimwood; Gúmer Pérez; Antonio G. Pisabarro; Igor V. Grigoriev; et al.Jason E. StajichLucía Ramírez Transposable Elements versus the Fungal Genome: Impact on Whole-Genome Architecture and Transcriptional Profiles. *PLOS Genetics* **2016**, *12*, e1006108, 10.1371/journal.pgen.1006108.
20. Déborah Bourc'his; Timothy H. Bestor; Meiotic catastrophe and retrotransposon reactivation in male germ cells lacking Dnmt3L. *Nature* **2004**, *431*, 96-99, 10.1038/nature02886.
21. Aydan Bulut-Karslioglu; Inti A. De La Rosa-Velázquez; Fidel Ramirez; Maxim Barenboim; Megumi Onishi-Seebacher; Julia Arand; Carmen Galán; Georg E. Winter; Bettina Engist; Borbala Gerle; et al.Roderick J. O'SullivanJoost H.A. MartensJörn WalterThomas MankeMonika LachnerThomas Jenuwein Suv39h-Dependent H3K9me3 Marks Intact Retrotransposons and Silences LINE Elements in Mouse Embryonic Stem Cells. *Molecular Cell* **2014**, *55*, 277-290, 10.1016/j.molcel.2014.05.029.
22. Simon Quenneville; Priscilla Turelli; Karolina Bojkowska; Charlène Raclot; Sandra Offner; Adamandia Kapopoulou; Didier Trono; The KRAB-ZFP/KAP1 System Contributes to the Early Embryonic Establishment of Site-Specific DNA Methylation Patterns Maintained during Development. *Cell Reports* **2012**, *2*, 766-773, 10.1016/j.celrep.2012.08.043.
23. Deborah Bourc'his; Olivier Voinnet; A Small-RNA Perspective on Gametogenesis, Fertilization, and Early Zygotic Development. *Science* **2010**, *330*, 617-622, 10.1126/science.1194776.
24. Alexei A. Aravin; Natalia M. Naumova; Alexei V. Tulin; Vasilii V. Vagin; Yakov M. Rozovsky; Vladimir A. Gvozdev; Double-stranded RNA-mediated silencing of genomic tandem repeats and transposable elements in the D. melanogaster germline. *Current Biology* **2001**, *11*, 1017-1027, 10.1016/s0960-9822(01)00299-8.
25. Titia Sijen; Ronald H. A. Plasterk; Transposon silencing in the Caenorhabditis elegans germ line by natural RNAi. *Nature* **2003**, *426*, 310-314, 10.1038/nature02107.
26. Tony Nolan; Laura Braccini; Gianluca Azzalin; Arianna De Toni; Giuseppe Macino; Carlo Cogoni; The post-transcriptional gene silencing machinery functions independently of DNA methylation to

repress a LINE1-like retrotransposon in Neurospora crassa. *Nucleic Acids Research* **2005**, *33*, 1564-1573, 10.1093/nar/gki300.

27. Soichiro Yamanaka; Sameet Mehta; Francisca E. Reyes-Turcu; Fanglei Zhuang; Ryan T. Fuchs; Yikang Rong; Gregory B. Robb; Shiv I. S. Grewal; RNAi triggered by specialized machinery silences developmental genes and retrotransposons. *Nature* **2012**, *493*, 557-560, 10.1038/nature 11716.
28. Cristiano C Nunes; Malali Gowda; Joshua K Sailsbery; Minfeng Xue; Feng Chen; Douglas E Brown; YeonYee Oh; Thomas K. Mitchell; Ralph A. Dean; Diverse and tissue-enriched small RNAs in the plant pathogenic fungus, Magnaporthe oryzae. *BMC Genomics* **2011**, *12*, 288-288, 1 0.1186/1471-2164-12-288.
29. Lysett Wagner; Sybren De Hoog; Ana Alastruey-Izquierdo; Kerstin Voigt; Oliver Kurzai; Grit Walther; A Revised Species Concept for OpportunisticMucorSpecies Reveals Species-Specific Antifungal Susceptibility Profiles. *Antimicrobial Agents and Chemotherapy* **2019**, *63*, e00653-19, 1 0.1128/aac.00653-19.
30. Guilhem Janbon; Shinae Maeng; Dong-Hoon Yang; Young-Joon Ko; Kwang-Woo Jung; Frédérique Moyrand; Anna Floyd; Joseph Heitman; Yong-Sun Bahn; Characterizing the role of RNA silencing components in Cryptococcus neoformans. *Fungal Genetics and Biology* **2010**, *47*, 1070-1080, 10.1016/j.fgb.2010.10.005.
31. Xuying Wang; Yen-Ping Hsueh; Wenjun Li; Anna Floyd; Rebecca Skalsky; Joseph Heitman; Sex-induced silencing defends the genome of Cryptococcus neoformans via RNAi. *Genes & Development* **2010**, *24*, 2566-2582, 10.1101/gad.1970910.
32. Carlos Lax; Carlos Pérez-Arques; María Isabel Navarro-Mendoza; José T. Cánovas-Márquez; Ghizlane Tahiri; José A. Pérez-Ruiz; Macario Osorio-Concepción; Eusebio Navarro; Eusebio Navarro; Victoriano Garre; et al.Francisco E. Nicolás Genes, Pathways, and Mechanisms Involved in the Virulence of Mucorales. *Genes* **2020**, *11*, 317, 10.3390/genes11030317.
33. Francisco E. Nicolás; Laura Murcia; Eusebio Navarro; María Isabel Navarro-Mendoza; Carlos Pérez-Arques; Victoriano Garre; Mucorales Species and Macrophages. *Journal of Fungi* **2020**, *6*, 94, 10.3390/jof6020094.
34. Heng-Chi Lee; Liande Li; Weifeng Gu; Zhihong Xue; Susan K. Crosthwaite; Alexander Pertsemlidis; Zachary A. Lewis; Michael Freitag; Eric U. Selker; Craig C. Mello; et al.Yi Liu Diverse Pathways Generate MicroRNA-like RNAs and Dicer-Independent Small Interfering RNAs in Fungi. *Molecular Cell* **2010**, *38*, 803-814, 10.1016/j.molcel.2010.04.005.
35. Amy Yuet Ting Lau; Xuanjin Cheng; Chi Keung Cheng; Wenyan Nong; Man Kit Cheung; Raymond H Chan; Jerome Ho Lam Hui; Hoi Shan Kwan; Discovery of microRNA-like RNAs during early fruiting body development in the model mushroom Coprinopsis cinerea. *PLOS ONE* **2018**, *13*, 325217, 10.1101/325217.

36. Jialan Yuan; Zhao Wang; Junjie Xing; Qing-Yong Yang; Xiao-Lin Chen; Genome-wide Identification and characterization of circular RNAs in the rice blast fungus Magnaporthe oryzae.. *Scientific Reports* **2018**, *8*, 6757, 10.1038/s41598-018-25242-w.

37. Jing Liu; Mujahid Ali; Qi Zhou; Establishment and evolution of heterochromatin. *Annals of the New York Academy of Sciences* **2020**, *1476*, 59-77, 10.1111/nyas.14303.

38. Robin C. Allshire; Hiten D. Madhani; Ten principles of heterochromatin formation and function. *Nature Reviews Molecular Cell Biology* **2017**, *19*, 229-244, 10.1038/nrm.2017.119.

39. Francesca Taglini; Elliott Chapman; Rob Van Nues; Emmanuelle Theron; Elizabeth H. Bayne; Mkt1 is required for RNAi-mediated silencing and establishment of heterochromatin in fission yeast. *Nucleic Acids Research* **2019**, *48*, 1239-1253, 10.1093/nar/gkz1157.

40. Robert A Martienssen; Danesh Moazed; RNAi and Heterochromatin Assembly. *Cold Spring Harbor Perspectives in Biology* **2015**, *7*, a019323, 10.1101/cshperspect.a019323.

41. Thomas A. Volpe; Catherine Kidner; Ira M. Hall; Grace Teng; Shiv I. S. Grewal; Robert A. Martienssen; Regulation of Heterochromatic Silencing and Histone H3 Lysine-9 Methylation by RNAi. *Science* **2002**, *297*, 1833-1837, 10.1126/science.1074973.

42. Ruby Yu; Xiaoyi Wang; Danesh Moazed; Epigenetic inheritance mediated by coupling of RNAi and histone H3K9 methylation. *Nature* **2018**, *558*, 615-619, 10.1038/s41586-018-0239-3.

43. Silvia Calo; Cecelia Shertz-Wall; Soo Chan Lee; Robert J. Bastidas; Francisco E. Nicolás; Joshua A. Granek; Piotr Mieczkowski; Santiago Torres-Martínez; Rosa M. Ruiz-Vázquez; Maria E. Cardenas; et al.Joseph Heitman Antifungal drug resistance evoked via RNAi-dependent epimutations. *Nature* **2014**, *513*, 555-558, 10.1038/nature13575.

44. Zanetta Chang; R. Blake Billmyre; Soo Chan Lee; Joseph Heitman; Broad antifungal resistance mediated by RNAi-dependent epimutation in the basal human fungal pathogen Mucor circinelloides. *PLOS Genetics* **2019**, *15*, e1007957, 10.1371/journal.pgen.1007957.

45. Silvia Calo; Francisco E. Nicolás; Soo Chan Lee; Ana Vila; Maria Cervantes; Santiago Torres-Martinez; Rosa M. Ruiz-Vazquez; Maria E. Cardenas; Joseph Heitman; A non-canonical RNA degradation pathway suppresses RNAi-dependent epimutations in the human fungal pathogen Mucor circinelloides. *PLOS Genetics* **2017**, *13*, e1006686, 10.1371/journal.pgen.1006686.

46. Zanetta Chang; Vikas Yadav; Soo Chan Lee; Joseph Heitman; Epigenetic mechanisms of drug resistance in fungi. *Fungal Genetics and Biology* **2019**, *132*, 103253-103253, 10.1016/j.fgb.2019.103253.

47. Qiannan Wang; Bang An; Xingrong Hou; Yunfeng Guo; Hongli Luo; Chaozu He; Dicer-like Proteins Regulate the Growth, Conidiation, and Pathogenicity of Colletotrichum gloeosporioides from Hevea brasiliensis. *Frontiers in Microbiology* **2018**, *8*, 2621, 10.3389/fmicb.2017.02621.

48. Vidhyavathi Raman; Stacey A. Simon; Feray Demirci; Mayumi Nakano; Blake C. Meyers; Nicole M. Donofrio; Small RNA Functions Are Required for Growth and Development of Magnaporthe oryzae. *Molecular Plant-Microbe Interactions®* **2017**, *30*, 517-530, 10.1094/mpmi-11-16-0236-r.
49. Achal Neupane; Chenchen Feng; Pauline K. Mochama; Huma Saleem; Shin-Yi Lee Marzano; Roles of Argonautes and Dicers on Sclerotinia sclerotiorum Antiviral RNA Silencing. *Frontiers in Plant Science* **2019**, *10*, 976, 10.3389/fpls.2019.00976.
50. Carlos Pérez-Arques; María Isabel Navarro-Mendoza; Laura Murcia; Eusebio Navarro; Victoriano Garre; Francisco E. Nicolás; A non-canonical RNAi pathway controls virulence and genome stability in Mucorales. *PLOS Genetics* **2020**, *16*, e1008611, 10.1371/journal.pgen.1008611.
51. Yongli Qiao; Lin Liu; Qin Xiong; Cristina Flores; James Wong; Jinxia Shi; Xianbing Wang; Xigang Liu; Qijun Xiang; Shushu Jiang; et al.Fuchun ZhangYuanchao WangHoward S JudelsonXuemei ChenWenbo Ma Oomycete pathogens encode RNA silencing suppressors. *Nature Genetics* **2013**, *45*, 330-333, 10.1038/ng.2525.
52. Arne Weiberg; Ming Wang; Feng-Mao Lin; Hongwei Zhao; Zhihong Zhang; Isgouhi Kaloshian; Hsien-Da Huang; Hailing Jin; Fungal Small RNAs Suppress Plant Immunity by Hijacking Host RNA Interference Pathways. *Science* **2013**, *342*, 118-123, 10.1126/science.1239705.
53. Francisco E. Nicolás; Victoriano Garre; RNA Interference in Fungi: Retention and Loss. *The Fungal Kingdom* **2017**, -, 657-671, 10.1128/9781555819583.ch31.