Role of Non-coding RNAs in Fungal Pathogenesis and Antifungal Drug Responses

Sourabh Dhingra[1]

Abstract

Purpose of Review Non-coding RNAs (ncRNAs), including regulatory small RNAs (sRNAs) and long non-coding RNAs (lncRNAs), constitute a significant part of eukaryotic genomes; however, their roles in fungi are just starting to emerge. ncRNAs have been shown to regulate gene expression in response to varying environmental conditions (like stress) and response to chemicals, including antifungal drugs. In this review, I highlighted recent studies focusing on the functional roles of ncRNAs in pathogenic fungi.

Recent Findings Emerging evidence suggests sRNAs (small RNAs) and lncRNAs (long non-coding RNAs) play an important role in fungal pathogenesis and antifungal drug response. Their roles include posttranscriptional gene silencing, histone modification, and chromatin remodeling. Fungal pathogens utilize RNA interference (RNAi) mechanisms to regulate pathogenesis-related genes and can also transfer sRNAs inside the host to suppress host immunity genes to increase virulence. Hosts can also transfer sRNAs to induce RNAi in fungal pathogens to reduce virulence. Additionally, sRNAs and lncRNAs also regulate gene expression in response to antifungal drugs increasing resistance (and possibly tolerance) to drugs.

Summary Herein, I discuss what is known about ncRNAs in fungal pathogenesis and antifungal drug responses. Advancements in genomic technologies will help identify the ncRNA repertoire in fungal pathogens, and functional studies will elucidate their mechanisms. This will advance our understanding of host-fungal interactions and potentially help develop better treatment strategies.

Introduction

Fungi are a major contributor to animal and plant pathogenesis and crop loss [1]. It is estimated that fungi cause 1.6 million deaths, and over one billion people are affected by fungal infections [2]. Global warming and climate change have resulted in increased incidences of fungal infections, with some animal species on the verge of extinction [3]. Reverse and forward genetic approaches have focused on protein-coding genes to identify molecular mechanisms associated with fungal pathogenesis; however, mortality rates are still high, and

crop losses are increasing [1, 2]. The central dogma, DNA → mRNA → protein, explains protein as a functional unit [4]; however, it exhibits incomplete information regarding gene regulation, timing, and rates of protein production and fails to explain organismal complexity, referred to as the G-value paradox [5]. Recent genomic advances have identified that only 2–3% of the transcriptome is translated into proteins [6, 7], thus indicating potential widespread roles of untranslated transcripts or non-coding RNAs (ncRNAs) [8]. Interestingly, organismal complexity highly correlates with the number of ncRNAs but not protein-coding genes [9].

ncRNAs have been identified in most eukaryotic genomes [10, 11], prokaryotic genomes [12, 13], and in archaea [14, 15]. They can be classified based on their functional roles as either structural ncRNAs including transfer RNAs, ribosomal RNAs, small nuclear and nucleolar RNAs, or regulatory RNAs including small RNAs (sRNAs) and long ncRNAs (lncRNAs) (> 200 nucleotides) [16] including natural antisense transcripts (NATs) [17]. In eukaryotes, sRNAs can be

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✉ Sourabh Dhingra
    sdhingr@clemson.edu

1   Department of Biological Sciences; Eukaryotic Pathogen Innovation Center, Clemson University, 190 Collings St, Life Sciences Building, Clemson, SC, USA

further classified as short-interfering RNAs (siRNAs), microRNAs (miRNAs), and Piwi-interacting RNAs [18].

siRNAs and miRNAs are both small ncRNAs (20–30 nt long); however, siRNAs stem from exogenous dsRNAs (e.g., viruses, transposons, or transgene) and are excised from completely complementary target sequences, whereas miRNAs are endogenously expressed, processed from precursors that form stem loops, and are not fully complementary to the target. Once processed, both siRNA and miRNAs form active complexes called RNA-induced silencing complexes (RISC) to silence genes in a process called RNA interference (RNAi) [19].

The components of RNAi machinery consist of RNA-dependent RNA polymerase (RdRP) [20], ribonuclease enzyme, Dicer [21], and the small RNA-binding partner, Argonaute proteins [22]. In the model fungus, *Neurospora crassa*, where RNAi was first discovered in fungi [23], RdRP has both RNA-dependent and DNA-dependent (DdRP) RNA polymerase activity with DdRP activity much stronger than RdRP [24, 25]. Once aberrant DNA (aDNA) is detected in *N. crassa*, a helicase unwinds the DNA and RdRP with its DdRP activity synthesizes ssRNA and then using RdRP activity to form dsRNA [24]. Thus, using DdRP activity, the fungus can differentiate endogenous RNAs and is specific to targets originating from aDNA production [20]. *N. crassa* has two Dicer-like enzymes with overlapping roles, which bind to dsRNA and cleave them into 20–25 nt long sRNAs [26]. These sRNAs bind to Argonaute proteins and form inactive RISC complex [27]. The Argonaute protein nicks the non-targeting strand and recruits additional exonuclease, QIP, to degrade it, leading to activation of the RISC complex [28]. Based on sequence complementarity of sRNA, the RISC complex is targeted, leading to posttranscriptional gene silencing. These core RNAi components (RdRP, Dicer, and Argonaute proteins) have been identified in the majority of eukaryotes, including pathogenic fungi. Notably, *Saccharomyces cerevisiae* lacks Dicer and Argonaute proteins; however, expression of genes encoding these proteins from *S. castelli* can activate RISC-mediated gene silencing in *S. cerevisiae* [29]. This cross-species complementation study indicates the presence of some RNAi machinery components that are functional in *S. cerevisiae*. Interestingly, in pathogenic fungi *Cryptococcus deuterogattii* and *Ustilago maydis*, loss of RNAi has been shown to correlate with the loss of active full-length retroelements and shorter centromeres [30]. Given that RNAi is present in most eukaryotes, it is possible that this defense mechanism was present in a common ancestor and, in the absence of selection pressure, including loss of full-length transposable elements, subsequently became dispensable in some fungi [29–32].

Piwi-interacting RNAs are mostly identified in metazoan genomes due to their association with Argonaute proteins belonging to the Piwi clade [33] and are therefore not discussed in this review. On the other hand, lncRNAs are > 200 nt long and have been shown to play a critical role in diseases like cancer, Alzheimer's disease, diabetes, and cardiovascular and neurological disorders [34, 35]; however, functional studies on the role of lncRNAs are lacking in fungi [36]. Additionally, unlike protein-coding genes, there is a lack of sequence conservation across species that makes identification and functional prediction hard (if not impossible) for these ncRNAs [37]. It is important to note that the structure of lncRNAs determines its function and lack of sequence conservation does not mean lack of function [37]. Interestingly, the promoter regions of these lncRNAs show high degrees of sequence similarity across species [38]. However, both the identification and regulatory roles of lncRNAs in pathogenic fungi are lacking. lncRNAs are generally transcribed from RNA pol II, can be polyadenylated or not, and may or may not include a 5′ cap and function *in cis* or *trans*. lncRNAs have a wide variety of functions, including transcriptional interference, chromatin remodeling, and histone modifications [39–42].

Another form of regulatory lncRNAs—natural antisense transcripts (NATs)—is commonly found in eukaryotic genomes and can range from hundreds to thousands of nucleotides. They can be transcribed in sense or antisense direction and form complementary base pairs with 5′ or 3′ end of the coding transcript [43, 44]. NATs have widespread mechanisms of controlling gene expression by forming a sense-antisense pair and blocking other factors or by remodeling chromatin [44, 45]. This review will focus on recent advances on the roles of ncRNAs, including sRNAs and their role in RNAi, lncRNAs, and NATs in fungal pathogenesis and antifungal drug responses.

Role of ncRNAs in Fungal Pathogenesis

Role of sRNAs and RNAi in Fungal Pathogenesis

Fungi imperfecti *Verticillium dahliae*, the causative agent of verticillium wilt, expresses core RNAi machinery [46]. sRNAs detected in *V. dahliae* are processed from 70 to 400 bp long precursors into 18–20 nt in length with G preferred at position 1; however, the exact mechanism of this processing is unknown [47•]. Blotting analysis to identify highly expressed sRNAs identified only one RNA pol II expressed sRNA—Vd mi-like RNA 1, VdmILR1 (processed from precursor VdMILR1), which regulates fungal pathogenesis by chromatin remodeling. Interestingly, the deletion of canonical Dicer proteins, which are involved in cleaving precursors, increased the expression of VdmILR1, indicating that canonical dicers do not play a role in the *V. dahliae* RNAi mechanism. Further analysis revealed the presence of a third RNAse III domain-containing protein, named Vdr3, whose

expression directly correlated with VdmiRNA1 expression [47•]. Canonical Dicer proteins contain two RNAse III domains and a PAZ domain (Piwi/Argonaute/Zwille domain); however, VdR3 consists only of a single RNAse III domain [21, 47•]. Interestingly, budding yeasts *S. castellii* and *Kluyveromyces* also contain a non-canonical single RNAse III containing a functional dicer protein, *dcr-1* [29, 48]. It is fair to assume that fungal systems have evolved to express non-canonical RNAi components, possibly due to selective pressures that may include host-pathogen interactions and warrants future investigation.

Comparative mRNA analysis identified an uncharacterized protein-coding gene, VdHy1, as a target for milR1 with predicted sRNA-binding site in 3' UTR. VdmiR1 repressed the transcription but did not cleave VdhY1, which is a positive regulator of virulence. Chromatin regulation by histone modification is known to cause changes in transcription levels [49, 50], and methylation of histones (H3K9me3) is a classical hallmark of heterochromatin formation [51, 52]. H3K9me3 antibody-mediated chromatin immunoprecipitation (ChIP) followed by quantitative PCR identified heterochromatin enrichment in overexpression (OE) VdMILR1 (precursor) and less enrichment in ΔvdmilR1 strains [47•]. Surprisingly, OE of VdR3, which increased milR1 levels, showed increased virulence. As VdR3 is indispensable for *V. dahliae* and functions as a non-canonical RNAse III domain protein, it is likely that VdR3 has additional, yet unknown functions in fungal virulence [47•]. More studies are needed to understand the role of non-canonical Dicer-like proteins in regulating gene expression and RNA processing in fungal pathogenesis.

Additionally, host miRNA-mediated gene silencing has also been shown to regulate *V. dahliae* virulence. Sequencing analysis in fungi recovered from cotton *in planta* infection but not in vitro cultures revealed higher accumulation of host miRNAs, miR166 and miR159, indicating host control of fungal RNAi machinery [53••]. Target analysis revealed binding sites of miR166 and miR159 in the Ca^{2+}-dependent calcium protease calpain, clp-1, and isotrichodermin C-15 hydroxylase, HiC-15, respectively; null mutants of both genes show significant virulence defects. Moreover, sequences of Clp-1 and HiC-15 are conserved, especially in miRNA-binding regions in various strains of *V. dahliae*, and mir166 Clp-1 silencing also plays a role in tomato infections by VdLs.17 strain [53••]. Even though it was evident that miRNAs can lead to cross-species gene silencing, it was unclear how sRNAs were transferred from host to pathogen. Recently, Jin et al. identified *Arabidopsis thaliana* sRNAs in a careful examination of isolated *Botrytis cinerea* cells from infected plants, many of which can target the *B. cinerea* genome. *A. thaliana* secretes exosome-like selective sRNA containing extracellular vesicles (EVs), which are taken up by fungi [54••]. In support of EVs-mediated cross-kingdom transfer of sRNAs, plant EVs-specific marker tetraspanins TET8 and TET9 are accumulated at the site of infection, and *tet8* and *tet9* mutants showed increase susceptibility to *B. cinerea* infection [54••, 55].

Interestingly, the opposite phenomenon of sRNA transfer from pathogen to host has also been shown to regulate virulence. Recently, a similar EVs-dependent cross-kingdom transfer of sRNA was observed in a mosquito fungal pathogen *Beauveria bassiana*. Bba-milR1, a dicer-dependent sRNA, plays a dual role in pathogenesis by silencing host immune genes to initiate infection and preventing immune recognition for fungal proliferation. To initiate infection, *B. bassiana* upregulates bba-milR1 expression, which gets transported to the host via EVs and silences toll immune pathways by binding to toll ligand Spätzle, Spz4. This effect is mediated by host Argonaute protein, Ago1. This initial response helps the fungus invade the cell hemolymph [56•]. Melanization is a key immune pathway in invertebrates resulting from the detection of microbes in the hemocoel. Once a pathogen is detected, melanin can encapsulate pathogens by forming crosslinks with microbial surface pathogen-associated molecular patterns (PAMPs) [57]. In mosquitos, melanization is regulated by the serine protease CLIPB9 that cleaves prophenoloxidase (PPO) into active phenoloxidase (PO), a key enzyme in the melanization process [58]. *B. bassiana* bba-milR1 interacts with and induces CLIPB9 expression. However, once in hemolymph, *B. bassiana* downregulates expression of bba-milR1 preventing CLIPB9-mediated cleavage of PPO to PO to inhibit melanization allowing fungal proliferation [56•]. It is interesting to note that host hijacking of the fungal virulence machinery is preserved during evolution. One possible reason can be a long-term survival strategy by fungi to keep the host alive by downregulating its virulence during infection.

Similarly, *B. cinerea in planta* infection of *A. thaliana* and *Solanum lycospericum* (tomato) revealed enrichment of 73 Bc sRNAs compared with in vitro cultures, the majority of which can target the plant genome [59••]. Specifically, mitogen-activated protein kinase 2 (MPK2) and MPK1, oxidative stress-related gene peroxiredoxin (PRXIIF), and cell wall-associated kinase (WAK)—all of which play a role in plant immunity against fungal infection—were targeted by Bc sRNAs, siR3.2, siR3.1, and siR-5, respectively, and are mediated by host Argonaute protein, Ago1. Additionally, Bc sRNAs expressing transgenic *A. thaliana* lines were hypervirulent when infected with *B. cinerea*, a phenotype that can be reversed by destroying sRNA target sites [59••] confirming that fungal sRNAs regulate virulence by suppressing host immunity genes.

Levels of ncRNAs are differentially regulated in both host and fungi upon infection of the host in many pathogenic fungi [60]. Further research is needed to identify the regulatory roles of these ncRNAs in response to fungal infections. What is unclear is if these pathways can regulate cross-kingdom RNAi or rather the presence of additional components to mediate host-pathogen interactions.

It is important to note that functional RNAi systems have been identified in major human fungal pathogens *Candida albicans* [61], *Cryptococcus neoformans* [62], and *Aspergillus fumigatus* [63] and major crop pathogen *Magnaporthe oryzae* [64] and *Fusarium* spp. [65]; however, their role in virulence of these fungal pathogens remains to be fully explored. Interestingly, in fungi lacking core RNAi components, active transposition of shorter retroelements may provide a pathogenic advantage; however, a direct correlation between loss of RNAi and virulence is not known at this time [30, 31]. Further research will elucidate the full potential of RNAi-associated mechanisms or evolutionary lack thereof in important fungal pathogens.

Roles of Long ncRNAs (lncRNAs) in Fungal Pathogenesis

Functional analyses of lncRNAs in pathogenic fungi are limited. In the human pathogenic fungus, *C. neoformans*, a random mutagenesis screen identified the long ncRNA RZE1 as a regulator of Znf2—a yeast to hyphae morpho switch regulator [66, 67]. Deletion of *znf2* locks cells in the pathogenic yeast form and increases its virulence [67]. Mechanistically, RZE1 is a nuclear localized lncRNA that reduces the cytoplasmic accumulation of *znf2* mRNA [66]. It is unclear if decreased cytoplasmic localization or increased nuclear localization is due to increased cytoplasmic degradation or reduced transport; however, this mislocalization possibly affects Znf2 protein levels. Although direct role(s) of RZE1 in fungal pathogenesis are not described to date, it can be speculated that OE RZE1 will lead to hypervirulence (correlate with ΔZnf2 strain) and ΔRZE1 to be hypovirulent (correlate with OE Znf2 strain) [67]. Further research is needed to test these hypotheses.

In the plant pathogen, *Ustilago maydis*, a developmentally regulated lncRNA, ncRNA1, has been identified with a regulatory role in the expression of natural antisense transcripts (see the next section for details) and pathogenesis [68•, 69]. Interestingly, ectopically complementing ncRNA1 under the influence of a constitutive promoter in ΔncRNA1 strain did not complement the pathogenesis phenotype suggesting *in cis* roles in pathogenesis [68•, 69]. The location-specific function might be due to regulatory elements present at that genomic locus, or the constitutive promoter uncouples development and stress-specific regulation leading to loss of function. Further research is needed to identify the regulation of ncRNA1 in plant fungal pathogenesis. A gene, *in trans*, *UMAG_12316*, shares sequence similarity with ncRNA1, and a Δ*UMAG_12316* strain showed reduced virulence, however, not as severe reduction as ΔncRNA1 [68•, 70]. Given that ncRNA1 is

a negative regulator of *UMAG_12316* transcript levels, it is possible that stochiometric levels of *UMAG_12316* are important for pathogenesis or ncRNA1 has other yet unidentified targets with a potential role in pathogenesis.

Non-sense mediated decay (NMD) pathway plays an important role in clearing aberrant mRNAs to protect cells from errors of RNA biogenesis and preventing protein mutations [71]. However, it is unclear if the NMD pathway recognizes ncRNAs and plays an important role in maintaining their levels [71, 72]; however, its role in fungal pathogenesis is limited. In *S. cerevisiae,* the NMD pathway is controlled by Xrn1 exonuclease that destabilizes Xrn1-sensitive lncRNAs [73]. In entomopathogenic fungi, *Cordyceps militaris*, deletion of the NMD pathway regulator *xrn1* results in a significant virulence defect [74]. Further analysis revealed upregulation of 10 lncRNAs and downregulation of 8 of their target genes controlling virulence and sexual development. Even though not direct, this study shows the potential role of lncRNAs in pathogenesis and their regulation by the NMD pathway in *C. militaris* [72].

As we are in the genomic revolution and the quality of fungal genomes is improving, future research will identify additional lncRNAs in fungi, and functional analyses are expected to reveal diverse roles for lncRNAs in gene regulation, chromatin remodeling, and fungal pathogenesis. Given that chromatin remodeling and gene regulation play a major role in fungal development process and secondary metabolite production, it is easy to speculate on their potential roles in pathogenesis and host-pathogen interactions.

Role of Natural Antisense Transcripts in Fungal Pathogenesis

NATs are widely present in eukaryotic genomes and can be expressed in sense or antisense direction to the ORF. They usually form a sense-antisense pair by complementary base pairing; however, their roles in gene regulation are more complex [45]. In the fungal plant pathogen *U. maydis*, ncRNAs and NATs are expressed in similar numbers as mRNAs, indicating very widespread roles of ncRNAs in this pathogen [75]. Interestingly, like *S. cerevisiae* canonical components of RNAi, viz., RdRP, Dicer, and Argonaute proteins are absent in this fungus [76]. Functional analysis of one genomic locus identified two NATs *as-UMAG_02150* and *as-UMAG_02151* (expressed at the same locus as ncRNA1 (discussed above)) with functional roles in gene regulation, teliospore formation, and pathogenesis [68•, 69]. As NATs *as-UMAG_02150* and *as-UMAG_02151* are both synthesized in the antisense direction overlapping ORF *UMAG_02150* and *UMAG_02151*, respectively, it is not possible to study the effects of one deletion without impacting other. Regulatory study identified a region upstream of *as-UMAG_02151* termed as $P_{as\text{-}UMAG\_02151}$, as a

negative regulator of *as-UMAG_02150* and *as-UMAG_02151* expression and a positive regulator of virulence [68•, 69]. Ectopic complementation of P~as-UMAG_02151~ did not complement the pathogenesis defect of the deletion mutant strongly indicating the role(s) of regulatory control at this genomic locus. Interestingly, deletion of the protein-coding gene *UMAG_02151* (3′ end of gene overlaps *as-UMAG-02151*) did not affect virulence, whereas *UMAG_02150* deletion (which overlaps *as-UMAG_0215*) reduced virulence; however, functional characterization failed to establish a direct role of *as-UMAG_02150* in pathogenesis. UMAG_02150 is a xylitol hydrogenase and is necessary to use xylitol as the sole carbon source. Ectopic expression of UMAG_02150 complemented the xylose growth phenotype of the deletion mutant but did not complement virulence defect, indicating a locus-specific regulatory role of P~as-UMAG_02151~ in NAT expression but not ORF function [68•]. Importantly, *UMAG_12316* (a gene regulated by ncRNA1 as mentioned in the previous section) expression was altered in Δ*UMAG_02150* strain. Further research is needed to evaluate direct or indirect roles of *as-UMAG_02150* in regulating *UMAG_12316* expression and if these are mediated by lncRNA, ncRNA1.

In another plant, pathogen *A. flavus*, genome-wide analysis identified temperature-regulated expression of NATs, with a NAT antisense to *aflD* gene upregulated at 37 °C [77]. *aflD* gene encodes a protein in the biosynthetic gene cluster of virulence factor, aflatoxin, that is highly produced at 28 °C but is repressed at 37 °C [78]. It is possible but unclear if *aflD* NAT regulates temperature-dependent expression of aflatoxin [77, 79]. Further research is needed to understand the complex roles of NATs in fungal pathogenesis and toxin production.

Role of ncRNAs in the Antifungal Drug Response

Role of RNAi in Fungal Drug Response

ncRNAs have been implicated in drug response in many different forms of cancers and function by regulating protein-coding genes, signaling pathways, cell death pathways, and drug efflux [80], with less known about their roles in fungi. In the human fungal pathogen, *Mucor circinelloides*, a spontaneous unstable and reversible RNAi-based drug resistance mechanism has been identified and termed as epimutation [81••, 82•]. *M. circinelloides* expresses both canonical and non-canonical RNAi machinery to induce endogenous RNA and exogenous transgene-dependent gene silencing [83]. The antifungal drug FK506 interacts with and forms a complex with the FKBP12 gene product, *fkbA* (peptidylprolyl isomerase), inhibiting the highly conserved protein phosphatase calcineurin [84]. Mutations in either *fkbA* or calcineurin genes

confer resistance to FK506. However, transient mutant strains or epimutants, with no mutations in *fkbA* or calcineurin genes, were identified. In these epimutants, endogenous sRNA derived from an mRNA precursor silences the *fkbA* gene [81••]. Interestingly, strains lacking the dicer enzyme *dcl2*, argonaute protein *ago1,* or RNA-dependent RNA polymerase *rdrp2*—all components of RNAi core machinery—did not produce any epimutants [81••, 85], indicating an important role of RNAi in acquiring transient fungal drug resistance and epimutations. A similar mechanism for 5-fluoroorotic acid (5-FOA) resistance, a different class of molecule, has been reported in *M. circinelloides* suggesting a conserved RNAi mechanism to regulate xenobiotic stress-mediated gene expression [82•]. This phenotypic plasticity allows an organism to rapidly adapt to a given stress; however, given its reversible nature allows the organism to revert to "wild-type" phenotype in a stress-free environment without introducing genetic mutations in the presence of the stress, which may or may not be beneficial long term. It will be interesting to see if in the presence of drugs, endogenous sRNAs are generated from mRNAs of drug target genes in other fungal pathogens and if this mechanism is conserved to mitigate additional stresses observed during host-pathogen interactions and infections.

Role of lncRNAs in Fungal Drug Response

A major study in the fission yeast *S. pombe* identified a role for a lncRNA, ncRNAtgp1, in drug sensitivity against a wide variety of drugs [86••]. Deletion of ncRNAtgp1 resulted in > 50-fold increase in mRNA levels of the neighboring gene, tgp1, while other neighboring genes expression was unaltered, suggesting a role *in cis*. Functionally, ncRNAtgp1 alters the nucleosome density upstream of *tgp1*, hindering binding of transcription factor Pho7 in the presence of phosphate, thus altering its expression and regulating drug responses [86••]. This shows the complex role of lncRNAs in gene regulation to mediate stress-specific functions. Given conserved synteny of this lncRNA in other *Schizosaccharomyces* species [86••, 87], further research will elucidate if lncRNA-mediated gene regulation is a conserved mechanism.

Chromatin modification involving histone acetylation and deacetylation has also been shown to play a role in the fungal drug response in many fungal species [85]; however, it is unclear at this time if this is mediated by ncRNAs. This represents an interesting area of future research. Interestingly, upon fungal infection, differential ncRNAs are detected in host cells [60]. Recent reports indicate roles of ncRNAs in host cell regulation of drug-metabolizing enzymes CYP450, UDP-glucuronosyltransferase, and drug transporters [88]. CYP450 enzymes represent an important class of enzymes responsible for metabolizing the azole class of antifungal drugs [89]. As evident from the examples above that the host and pathogen can regulate a cross-kingdom sRNA response,

future research will seek to uncover if fungal interactions with the host cells cause differential ncRNA-mediated drug metabolism and responses to alter disease outcomes.

Conclusions and Perspectives

In this review, the known roles of ncRNAs in fungi and fungal pathogenesis and drug response are described. ncRNAs have widespread functions, including indirect transcriptional regulation by recruiting regulatory protein complex or methylation of specific genes and by directly regulating histone modifications or other chromatin regulatory complexes to modify chromatin structure [20, 90, 91]. Even though a lot is known about ncRNAs in mammalian cells, more work is needed to understand their functional roles in pathogenic fungi. We have gained many insights from deep sequencing analyses of fungal genomes and transcriptomes; however, identification of ncRNAs in fungal genomes is still challenging. For example, in *A. fumigatus*, ncRNAs have only been detected to date by a complementary cDNA library approach and not by advanced sequencing technologies [92]. Furthermore, a majority of fungal transcriptome studies has utilized polyA+ based mRNA sequencing; however, it is clear that fungal lncRNAs are not necessarily polyadenylated; thus, they are not detected/identified. As the sequence similarity for ncRNAs is low, advancements in genomics technologies will help uncover the ncRNA repertoire in fungal pathogens. This will still require functional studies to understand the complex roles of ncRNAs (lncRNA and sRNAs) in fungal pathogenicity and drug response. Recently, drug tolerance, which is distinct from drug resistance, has been described in *C. albicans* [93]. Given that ncRNAs play an important role in stress responses, including antifungal drugs [81••, 82•], it is tempting to speculate that tolerance phenotypes are mediated in part by ncRNAs; however, functional studies are needed to test this hypothesis. As drug tolerance can lead to drug resistance [94], understanding the roles of ncRNAs in fungal drug response is crucial in designing new treatment strategies. These studies in pathogenic fungi will not only tell us the specific roles of ncRNAs in pathogenesis and drug response but will also add to our understanding of molecular mechanisms involved in host-pathogen interactions.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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Papers of particular interest, published recently, have been highlighted as:
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•• Of major importance

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