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The 5′ → 3′ Exoribonuclease 2 as a Potential Target for Developing Fungicides to Control the Panama Disease

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Abstract

An outbreak of *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 is currently threatening the global production of bananas. Due to the clonal nature of commercial banana plants, selecting resistant cultivars does not seem feasible; therefore, alternative approaches to crop protection must be developed. The 5′ → 3′ exoribonuclease XRN2/RAT1 is involved in 5′ → 3′ RNA decay. Fungal studies with *XRN2* and conditional mutants have illustrated the crucial role of this enzyme, suggesting *XRN2* should be considered as target to searching for novel inhibitors that might be used as fungicides to control Panama disease. Our *in silico* analysis of Tropical Race 4 *XRN2* (*FocTR4*XRN2) revealed characteristic features of 5′ → 3′ exoribonuclease such as the catalytic domain that recognizes 5′-monophosphorylated RNA and catalyses the processed cleavage of mononucleotides. A delimited cavity showing the potential for substrate uptake appears prone to interacting with small molecules that might inhibit its activity. The catalytic domain in *FocTR4XRN2* harbors a CCHC motif, which is conserved in orthologous proteins from filamentous fungi but lacking in yeasts. The residues involved in the interaction with the pyrophosphohydrolase RAI1 are also conserved. Molecular docking reveals the potential interaction of *FocTR4XRN2* with the natural inhibitor adenosine 3′, 5′ bisphosphate, and suggests this approach is reliable to screen for novel enzyme inhibitors that could be help in suppressing the progression of causal agents of Panama disease.

Keywords: *Fusarium oxysporum* f. *sp*; *Cubense* Tropical race 4; 5′ → 3′ exoribonuclease; RNA processing; Enzyme inhibitor; Molecular docking

Introduction

The *Fusarium oxysporum* species complex (FOSC) comprises soil-borne saprophytic fungi and plant pathogenic isolates that cause vascular wilt and root rot in plants, including some agronomically important species [1]. The members of the FOSC complex are asexual, but like other pathogenic fungi, they are prone to acquiring DNA via horizontal gene transfer which acts as an alternative to provide the genetic information necessary for plant colonization [2]. Although the FOSC complex causes disease in dozens of plant species, the host range of each virulent strain is limited to one or a few related species. The term *forma specialis* (f. sp.) is attributed to an individual strain to denote the host specificity [3]. *Fusarium oxysporum* f. sp. *cubense* (*Foc*) is the causal agent of the fusarium wilt in banana plants (*Musa* spp.), also known as Panama disease [4]. *Foc* is classified into four races based on the banana cultivars affected. *Foc* Race 1 is a pathogen of Gros Michel bananas and related cultivars such as Pome, Silk, and Pisang Awak. It caused Gros Michel epidemics during the 19th Century that destroyed banana production, but it is avirulent in the resistant Cavendish cultivar on which current banana production is based. *Foc* Races 2 and 3 affect the banana cultivar Bluggoe and plants in the genus *Heliconia*, respectively. *Foc* Race 4 can cause disease in both *Foc* Race 1 hosts and the Cavendish cultivar. Depending on the climate conditions under which the disease develops, *Foc* Race 4 is further classified into Subtropical Race 4 (*FocSTR4*) or Tropical Race 4 (*FocTR4*) when it occurs in the subtropics or tropics, respectively [5]. Because they are parthenocarpic and propagated by vegetative methods, commercial banana cultivars have reduced genetic variability, which impedes selection for resistant cultivars and favors the establishment of novel enhanced virulent races such as *FocTR4*. Due to the wide host range of *FocTR4* and the lack of resistant commercial cultivars, wilting caused by this race is considered the most deleterious factor affecting banana production worldwide, compromising the welfare of producers in developing countries [4]. Thus far, there are no effective strategies to control or abolish *FocTR4*. Therefore, research focused on this pathogen is required to give rise to novel alternatives for protecting this important crop. Resistance genes have been identified in wild *Musa* plants, and although their expression in transgenic Cavendish banana plants conferred resistance to *FocTR4*, implementation is technically limited because there are roughly 5000 accessions of *Musa* spp. [6]. The molecular docking approach is a tool used for investigating protein-ligand interactions that has been helpful for discovering and screening for new drugs and antibiotics [7,8]. Virtual screening of novel inhibitors is an early step in designing novel compounds with antifungal activity that could be used to protect banana plants, regardless of the cultivar or environmental growth conditions. This approach was used to identify compounds that could function as G-protein coupled receptor (GPCR) inhibitors in *Fusarium graminearum*, the causal agent of head blight in wheat [9]. Enzymes involved in RNA degradation, an essential molecular process in all organisms, including fungi, are possible targets to control Panama disease and other *F. oxysporum*-caused diseases [10]. Endo- and exo-nucleases are required for eliminating unnecessary mRNAs as a part of general turnover, specific transcript regulation, and aberrant mRNA degradation to control RNA quality. Nucleolytic activity also participates in the processing, maturation, and decay of functional noncoding RNAs [10,11]. Using divalent cations as cofactors, 5′ → 3′ exoribonucleases catalyze the removal of nucleoside monophosphates from 5′-monophosporylated RNA [12]. These enzymes were initially identified in the yeast *Saccharomyces cerevisiae*, which has two 5′ → 3′ exoribonucleases referred to as XRN1 [13] and

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XRN2, originally known as Rat1 (ribonucleic acid trafficking protein 1) [14]. XRN1 and the orthologous proteins in eukaryotes generally participate in the 5′ → 3′ decay of mRNAs in the cytoplasm [15]. *XRN2* localizes to the nucleus, and is involved in noncoding RNA processing such as the exoribonucleolytic trimming of rRNA precursors [16], snoRNAs biogenesis [17] and degradation of hypomodified tRNAs that lack complete functionality to protein synthesis [18]. These activities suggest *XRN2* influences protein biosynthesis by processing the noncoding RNAs involved in translation. In yeast, Rat1 is involved in the degradation of the 3′-end products of transcription termination, and it might collide with RNA polymerase II to induce its dissociation from DNA [19]. The telomeric repeat-containing RNA (TERRA) is produced from telomere sequences located at the end of the chromosomes [20]. The accumulation of TERRA inhibits telomere elongation in S. *cerevisiae*, possibly by repressing telomerase activity. Telomere elongation, and thus, chromosome stability is maintained by the Rat1- mediated degradation of TERRA [21]. The lethality of the *XRN2* (*rat1, hke1, tap1*) mutant in the fission yeast *Schizosaccharomyces pombe* suggests that the reactions catalysed by this enzyme are essential for fungal development. Expressing the *XRN2* gene (driven by the inducible *GAL7* promoter) in the human pathogen *Cryptococcus neoformans* suggests this exoribonuclease is essential for cell viability, as growth is established upon *XRN2* induction and abolished under restrictive conditions [22]. Thus far, there have been no reports concerning the role of this enzyme in phytopathogens. Although this is an unexplored topic, the evidence suggests that understanding XRN2's structure and its mode of action in phytopathogens could be helpful in proposing approaches to suppress invasions by economically important phytopathogens such as the causal agents of Panama disease. The availability of fungal genome sequences, including *FocTR4*, and the crystal structures of 5′ → 3′ exoribonucleases facilitates this endeavor. In this study, we performed an *in silico* analysis of *FocTR4XRN2* as an initial step to elucidate its structural features, which could lead to the screening and identification of novel inhibitors.

Materials and Methods

Protein sequences and phylogenetic analysis

The sequences of the 5′ → 3′ exoribonucleases XRN1 and *XRN2* from representative fungal species were analyzed. The protein sequences were retrieved from the Universal Protein Resource (UniProt) database [23] by using the XRN1(P40383) and XRN2/Rat1 (P40480) sequences from *S. pombe* as queries. Human XRN1 (Q8IZH2) and *XRN2* (Q9H0D6) were used as controls. The protein annotation in UniProt automatically predicts the catalytic domain from the InterPro 5′ → 3′ exoribonuclease IPR027073. The *Fusarium oxysporum* f. sp. *cubense* TR4 strain 54006 sequence was subjected to further analysis as presented below. The software cNLS Mapper (http://nls-mapper.iab.keio.ac.jp/cgi-bin/NLS_Mapper_form.cgi) was used to identify putative nuclear localization signals (NLS). The list of species and ID entry names for each protein are included in the Supplementary Table 1. Protein sequences were aligned using the MUSCLE algorithm [24]. PhyML was used to estimate a maximum likelihood phylogenetic tree with 100 bootstrap replicates [25]. The MUSCLE algorithm was also used to align *S. pombe Sp* Rai1 (O13836) and the homolog *FOC4TR4*RAI1 (X0K375).

Modelling of *FocTR4*XRN2

Once *FocTR4XRN2* was identified (X0JIN1), the Robetta server (http://robetta.bakerlab.org/) was utilized to predict the tridimensional structure based on the full sequence surrounding the catalytic domain (IPR027073) with homology to 5′ → 3′ exoribonucleases [26]. The previously reported structure *Sp*Rat1 (3FQD) was selected as the template to generate the *FocTR4XRN2* models (pdb files), which were visualized in PyMOL (Schrödinger). The models were structurally similar, and one was selected for further analysis and detailed comparisons with *Sp*Rat1. The structure of the *FocTR4XRN2* fragment with a potential for interacting with *FocTR4*RAI1 was generated in Swiss-Model by using the corresponding *Sp*Rat1 fragment as template [27]. The sequence and structure of the activating partner *Sp*Rai1has also been previously reported [28].

Molecular docking

The PatchDock algorithm was used to test adenosine 3′, 5′ bisphosphate (PAP) as a ligand of the *FocTR4XRN2* protein predicted by the Robetta server [29]. The PAP structure was retrieved from the ZINC database (http://zinc.docking.org/), and the 2D structure was drawn using ChemDraw^{R} 17 (PerkinElmer). An energy minimization of the structures was performed with VEGA ZZ suite (http://nova.disfarm.unimi.it) prior to the molecular docking. The two solutions with the highest geometric score were selected for further analysis and visualization in UCSF Chimera [30].

Results

Identification of XRN1 and *XRN2*

Differences in the XRN1 and *XRN2* sequences and structures must be distinguishable, as each enzyme catalyzes the removal of mononucleotides from specific RNA substrates. The 5′ → 3′ exoribonuclease sequences from 26 representative fungal species were aligned, and a phylogenetic tree was constructed by maximum likelihood (Figure 1). XRN1 and *XRN2* were clustered into two independent clades that were supported by the bootstrap value. While the analyzed protein sequences harbor the characteristic 5′ → 3′ exoribonuclease domain, the context surrounding the catalytic domain could influence substrate recognition and be the determining factor responsible for XRN2's crucial role in fungal development.

Structural features of the predicted *FocTR4*XRN2

A more detailed analysis of the *FocTR4XRN2* identified a CCHC motif and the potential NLS in the catalytic domain, which was absent in the *FocTR4*XRN1 (Figure 2). These additional motifs are lacking in the 5′ → 3′ exoribonucleases form yeasts [28,31]. A glycine-rich in the C-terminal portion of *FocTR4XRN2* is evident, but thus far, the function of this region in 5′ → 3′ exoribonucleases is unknown. A MUSCLE alignment was performed to determine whether the catalytic domain is functional. Selected *XRN2* sequences were aligned, including the already reported *Sp*Rat1 (Figure S1, supplementary material). The N-terminal of *Sp*Rat1 includes residues involved in the cleavage of mononucleotides and acidic residues involved in binding of cations such as Mn^{2+} or Mg^{2+}, which is necessary for catalysis [28]. All those residues are conserved. The alignment also reveals that the additional CCHC knuckle motif (C-X2-CX3GH-X4-C) that is unique to *XRN2* from filamentous fungi, and present in plant 5′ → 3′ exoribonucleases [32]. In *S. pombe*, the pyrophosphohydrolase Rai1 removes pyrophosphate form the first triphosphorylated nucleotide of a single stranded RNA [28]. *Sp*Rai1interacts with *Sp*Rat1, and most of the residues of *FocTR4XRN2* involved in the potential interaction are conserved (Figure S1, supplementary material), suggesting this interaction might also occur in *FocTR4*. Despite the slight differences and the presence of the CCHC motif, the highly conserved catalytic

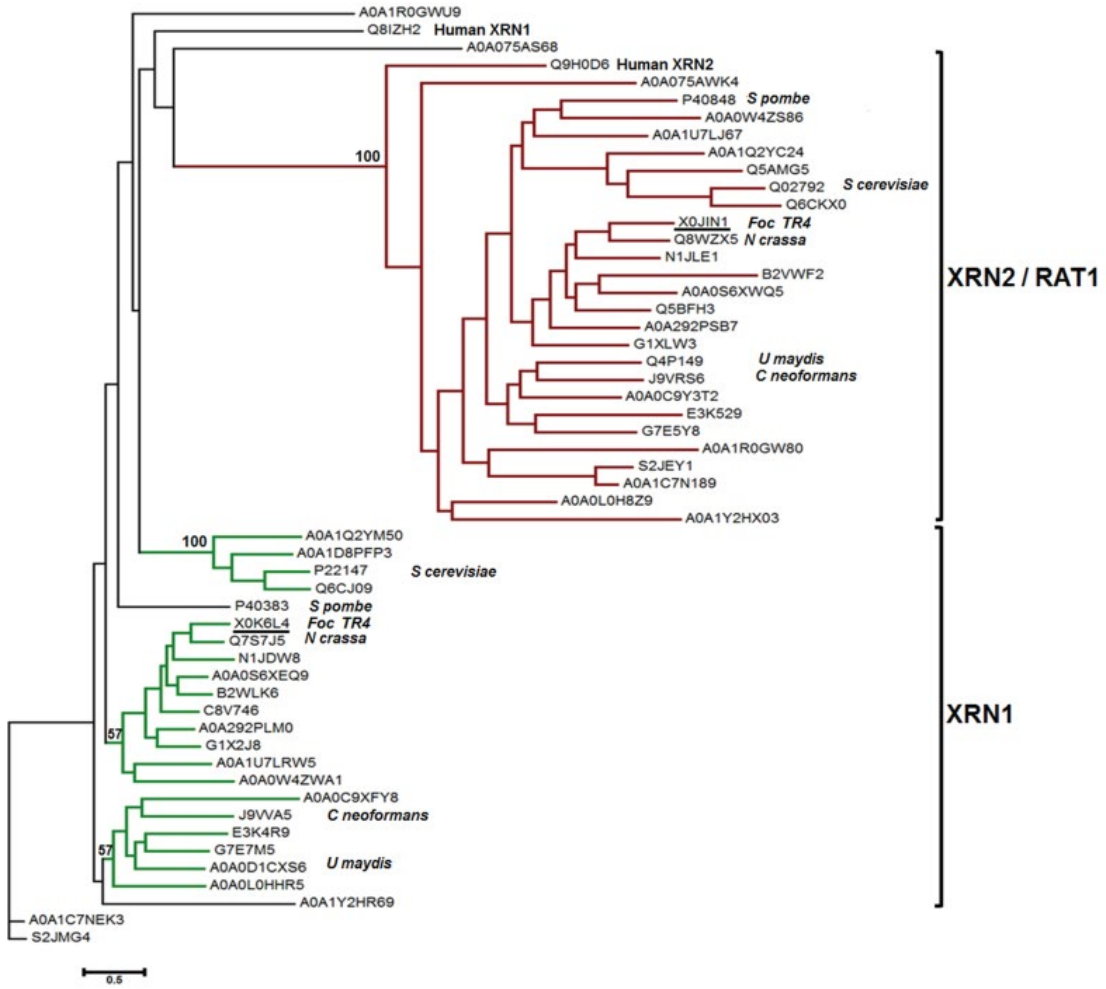

Figure 1: The 5′→3′ exoribonucleases in fungi. A maximum likelihood phylogenetic tree shows the homologs of XRN1 and XRN2/RAT1. The UniProt entry ID and name of the selected species are shown at the right of the branches. Bootstrap support values > 50 are listed on the branches. A complete list of the species is included in the Supplemental Material.

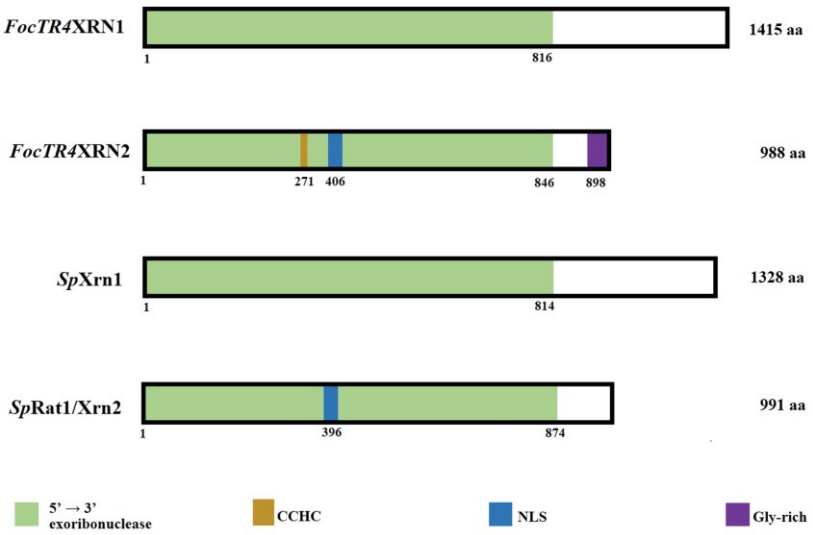

Figure 2: Structural comparisons of the 5′→3′ exoribonucleases in *FocTR4* and *Schizosaccharomyces pombe* showing the catalytic domain (green), CCHC motif (yellow), glycine-rich region (purple), and putative nuclear localization signal (NLS; blue). The putative NLS in *S. pombe* was reported in [41].

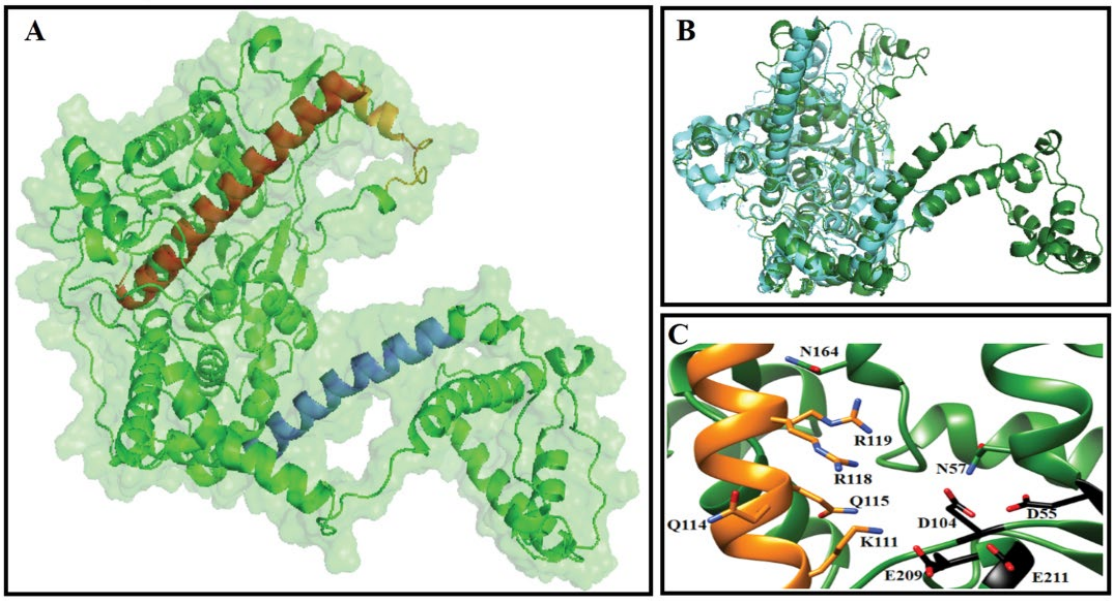

Figure 3: Predicted 3D model and ribbon diagram of *FocTR4*XRN2. (A) The surface is presented in transparent green, the tower domain involved in catalysis is indicated in red, the CCHC motif is in orange, and the putative nuclear localization signal is in blue. (B) Merging of the predicted *FocTR4*XRN2 and *Sp*Rat1 structures in green and cyan, respectively. (C) Lateral residue chains involved in catalysis in the predicted *FocTR4*XRN2 structure are highlighted. The tower domain is in orange, and the acidic residues for metal binding are in black. Hydrogen atoms were omitted to simplify the diagram; the oxygen and nitrogen atoms of the residues are shown in red and blue, respectively.

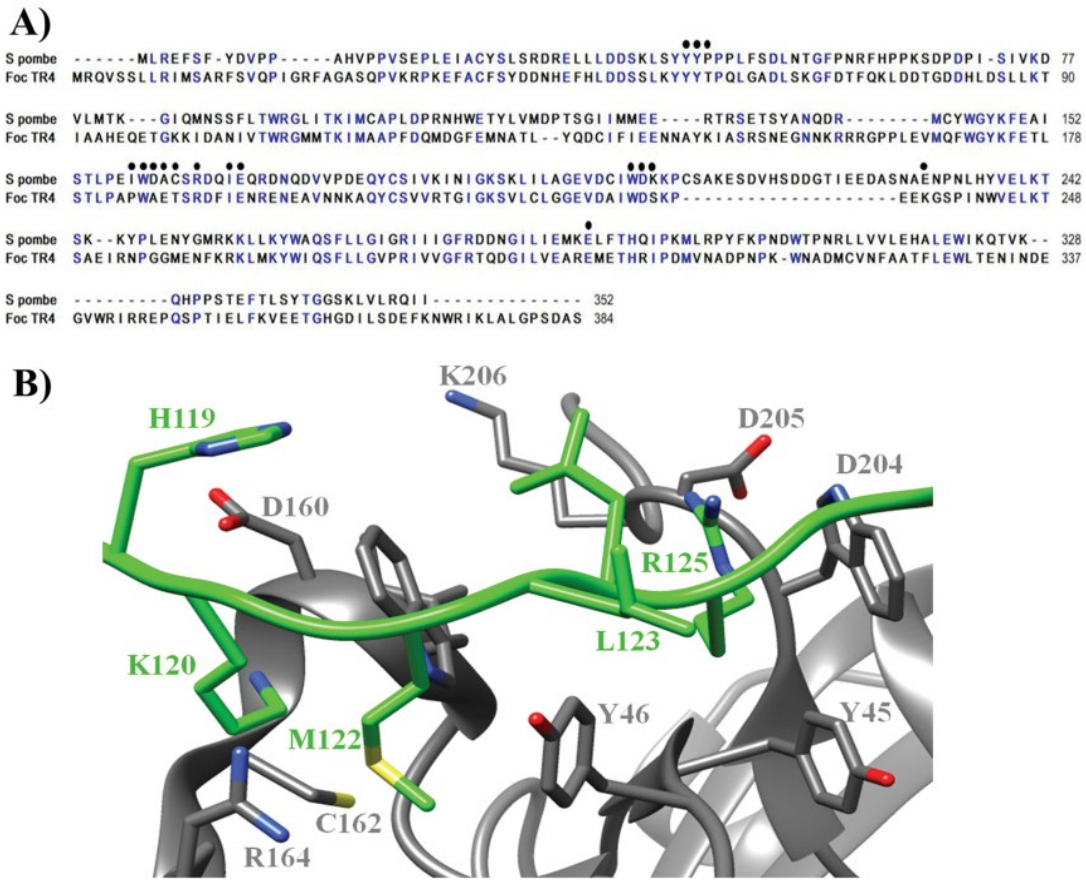

Figure 4: Residues on the surface of *FocTR4*XRN2 involved in the interaction with RAI1 are conserved. MUSCLE alignment of *Sp*Rai1 and the homolog *FocTR4*RAI1, black spots mark residues involved interacting with *Sp*Rat1, which are conserved in *FocTR4*RAI1 (A). Predicted model of the surface of *FocTR4*XRN2 interacting with *Sp*RAI1 (B).

domain in 5′ → 3′ exoribonuclease homologs, including *FocTR4*XRN2, implies the formation of a tridimensional structure capable of removing mononucleotides from 5′-phosphorylated RNA, using divalent cations as cofactors. The structure of *FocTR4XRN2* as predicted by the Robetta server is illustrated in Figure 3A. The overall structure highly resembles that of *Sp*Rat1 (Figure 3B). The 5′ → 3′ exoribonucleases possess 17 α-helices. The fourth α-helix, known as the tower domain, contains the basic residues critical for catalysis and are hypothetically oriented towards the pocket where the 5′-phosphate of the substrate is located, which agrees with the structure of the 5′ → 3′ exoribonuclease XRN1/PCM in the fruit fly [12] (Figure 3C). As expected, the residues involved in metal binding are located near the tower domain (Figure 3C). The CCHC motif is a putative α-helix located at the surface of the predicted protein and does not seem to be directly involved in catalysis, but its conservation in *XRN2* homologs suggests it might confer a specific biochemical function such as interacting with proteins involved in RNA processing (Figure S2, supplementary material).

RAI1 as a potential interactor of *FocTR4XRN2*

The sequence alignment revealed conserved residues involved in the interaction with RAI1. To obtain some insight into the relevance of these residues, the *Sp*Rai1 and *FocTR4*RAI1 sequences were aligned. As fission yeast, the conservation of residues involved in interacting with Rat1/*XRN2* suggests that both enzymes co-evolved to associate in *FocTR4* (Figure 4A). The Figure 4B shows the structure of the tract of residues H819 to G826 located at the *FocTR4XRN2* C-terminal that hypothetically interact with RAI1 is represented in the Figure 4B.

Virtual interaction between *FocTR4XRN2* and adenosine 3′, 5′ bisphosphate

Visualizing the cavities that converge in the *FocTR4XRN2* catalytic site reveals potential sites for inhibitors (Figure 5A). The *in vitro* relative activities of the S. *cerevisiae* 5′ → 3′ exoribonucleases XRN1p and Rat1p decreases by addition of adenosine 3', 5' bisphosphate (PAP), a bypass

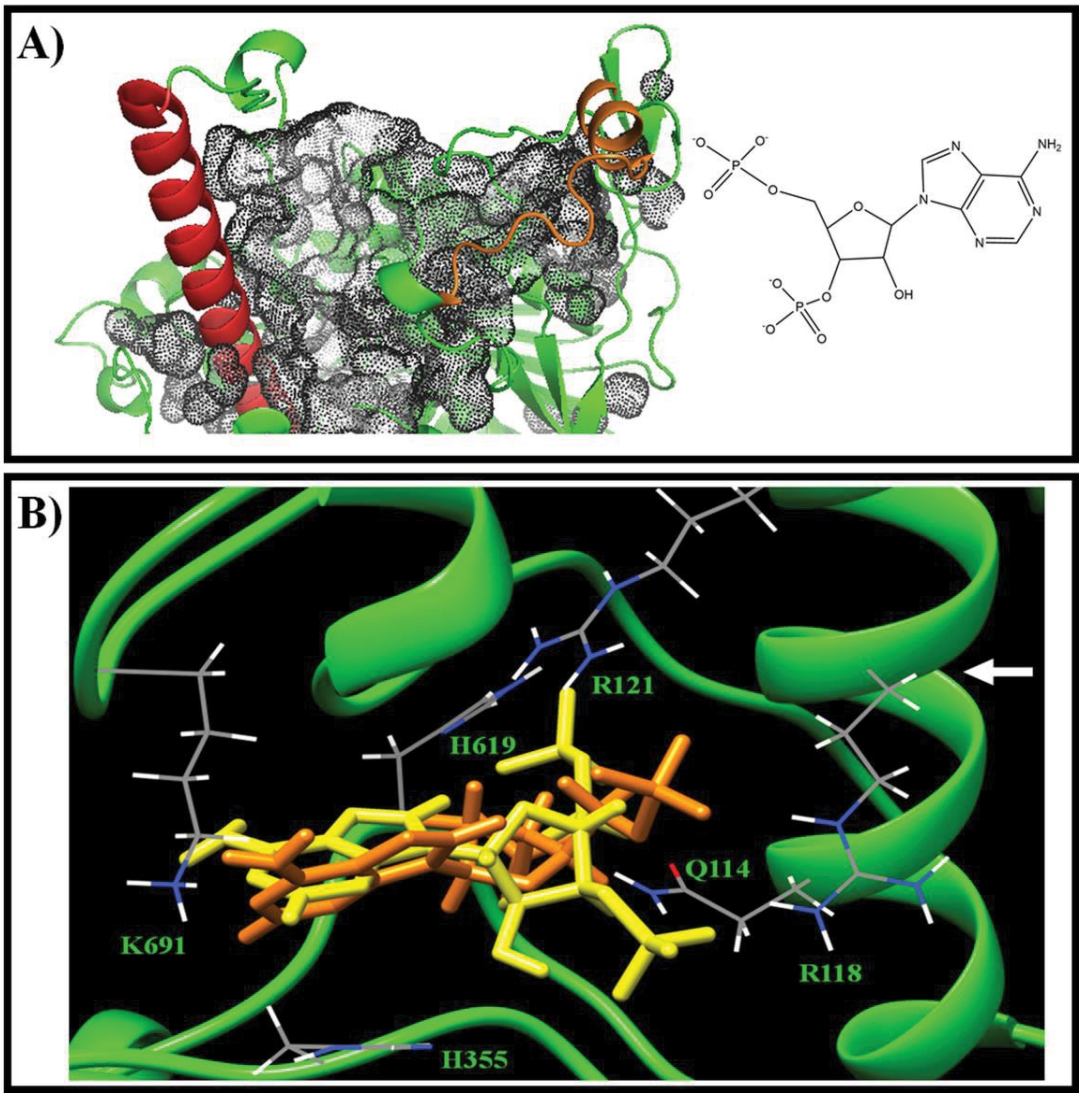

Figure 5: Docking of adenosine 3′, 5′ bisphosphate (PAP) into the catalytic site of *FocTR4*XRN2. (A) The cavity in the *FocTR4*XRN2 structure in the proximity of the catalytic site is represented by dotted black mesh; the tower domain is in red, and the CCHC motif is in orange; PAP is illustrated at the right. (B) The two orientations of PAP that displayed the highest scores are represented in orange and yellow. The residues in *FocTR4*XRN2 involved in docking are represented by colored lines (grey, carbon; white, hydrogen; red, oxygen; blue, nitrogen); the tower domain is indicated by an arrow.

product of sulfate assimilation (Figure 5A) [33]. As a proof of concept, molecular docking was performed with PatchDock to assess PAP as potential inhibitor of the 5′ → 3′ exoribonucleases. The two solutions produced by this virtual procedure with the highest geometric scores (4766 and 4702) demonstrated that PAP can be placed at the catalytic site as a competitive inhibitor, close to the tower helix and neighboring residues (Figure 5B).

Discussion

Currently, the most outstanding factor that hampers the world production of bananas is the emergence of *FocTR4*. Both natural and synthetic products have been tested as *FocTR4* inhibitors. Treatment with demethylation-inhibiting fungicides decreases symptom development in banana plants inoculated with *FocSTR4*, however, these compounds can be toxic to mammals [34]. Bacterial volatiles have been demonstrated to act as suppressors of spore germination and mycelial growth in *FocTR4* [35,36]. Crude extracts obtained from *Streptomyces violaceusniger* are also inhibitors of *FocTR4* spore germination of [37]. Synthetic Cu(II) complexes with benzophenone derivatives have also shown antifungal activity against *FocTR4* [38]. Although these results are promising, more compounds need to be screened to increase the repertoire of innocuous-to-humans and environmentally friendly fungicides. Molecular docking is an emergent approach to screening for novel antibiotics with high affinity, including compounds to control the agents of Panama disease. Research aimed at determining which enzymes are indispensable for fungal growth and viability is necessary to accomplish this task. In this study, the 5′ → 3′ exoribonuclease *XRN2* was selected to perform an *in silico* structural analysis. In fungi, nuclear-localized *XRN2* is the predominant 5′ → 3′ exoribonuclease, performing the RNA trimming required for the maturation of rRNAs and snoRNAs [18]. Thus, its activity is an early event that secures protein synthesis. The degradation of TERRA by Rat1p in *S. cerevisiae* is indicative of a requirement for 5′ → 3′ exoribonuclease activity for maintaining chromosome integrity [39]. Furthermore, the failed attempts to generate *XRN2* knockout mutants in filamentous fungi suggest that the disruption of this gene is lethal [22,40]. These reports strengthen the proposition that *XRN2* might have unique features that confer its prominent role in RNA processing. The differences in the fungal XRN1 and *XRN2* primary structures were notable and allowed the phylogenetic reconstruction of 5′ → 3′ exoribonucleases into two clades. Thus, detailed about the structure of *XRN2* can be utilized to design novel fungicides. Furthermore, the catalytic residues of *XRN2* are under selective pressure that would limit the probability to select a functional enzyme resistant to competitive inhibitors. As a pre-requisite to accomplishing this task, we predicted the *in vitro* structure of *FocTR4*XRN2. The protein has a quite similar structure to that previously reported for *Sp*Rat1p, including the tower domain that harbors the residues required for catalysis and the acidic residues involved in binding divalent cations. When XRN1p was targeted to the nucleus in *S. cerevisiae*, it fulfilled the functions of Rat1p [41]. This indicates that the nuclear localization of *XRN2* is critical for it to play its role. The identification of a putative NLS in *FocTR4XRN2* is *in silico* evidence that it localizes to the nucleus to rapidly and efficiently recognize substrates such pre-rRNAs. The CCHC motif is specific to *XRN2* in filamentous fungi, including *FocTR4*, and according to the predicted tridimensional model, it is an α-helix exposed at the surface of the protein. Thus far, the function of the CCHC motif is unknown, but in *S. cerevisiae,* the CCHC-containing protein Mpe1is a subunit of the cleavage and polyadenylation factor (CPF) involved in terminating transcription [42]. Specific subunits of the holo-CPF immunoprecipitates with snoRNA genes [42], which leads us to suggest that the CCHC motif might be involved in binding of the RNA substrate or interaction with other proteins that mediate its activity or recruitment into nuclear complexes. It is feasible to search for small molecules that could interact with this exposed motif, and thus interrupt protein-protein interactions and cause deleterious effects in the fungus. Since the catalytic site of *FocTR4XRN2* was resolved *in silico*, it might be possible to test the docking of small molecules.

Conclusion

Knocking out the *hal2* gene in yeast caused the accumulation of its substrate PAP, consequently inhibiting XRN1p and Rat1p activity and provoking the accumulation of pre-rRNAs and pre-snoRNAs. There is no evidence that 5′ → 3′ exoribonucleases recognize PAP, but as it is a nucleotide, it is plausible that PAP's inhibitory effect might be due to its insertion into the XRN1p and Rat1p catalytic sites, a process that might be conserved in fungi. The virtual result of the molecular docking suggests that the catalytic sites of 5′ → 3′ exoribonucleases, especially in XRN2, are prone to being docked by small molecules that disrupt their function, which could provide a way to screen for novel natural products that could be utilized as fungicides.

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References

1. Fravel D, Alabouvette C (2003) *Fusarium oxysporum* and its biocontrol. New Phytol 157: 493-502.
2. Mehrabi R, Bahkali AH, Abd-Elsalam KA, Moslem M, Ben M'Barek S, et al. (2011) Horizontal gene and chromosome transfer in plant pathogenic fungi affecting host range. FEMS Microbiol Rev 35: 542-554.
3. Lievens B, Rep M, Thomma BPHJ (2008) Recent developments in the molecular discrimination of *formae speciales* of *Fusarium oxysporum*. Pest Manag Sci 64: 781-788.
4. Ploetz RC (2015) Fusarium wilt of banana. Phytopathology 105: 1512-1521.
5. Fourie G, Steenkamp ET, Ploetz RC, Gordon TR, Viljoen A (2011) Current status of the taxonomic position of *Fusarium oxysporum* formae forma *cubense* within the *Fusarium oxysporum* complex. Infect Genet Evol 11: 533-542.
6. Ruas M, Guignon V, Sempere G, Sardos J, Hueber Y (2017) Database tool MGIS : Managing banana (Musa spp .) genetic resources information and high-throughput genotyping data. Database.; 1-12.
7. Meng XY, Zhang HX, Mezei M, Cui M (2011) Molecular docking: A powerful approach for structure-based drug discovery. Curr Comput Aided Drug Des 7: 146-157.
8. Tech JAB, Dar AM, Mir S (2017) Analytical & bioanalytical techniques molecular docking : approaches, types, applications and basic challenges. J Anal Bioanal Tech 8: 8-10.
9. Bresso E, Togawa R, Hammond-Kosack K, Urban M, Maigret B (2016) GPCRs from fusarium graminearum detection, modeling and virtual screening - the search for new routes to control head blight disease. BMC Bioinformatics 17: 463.
10. Houseley J, Tollervey D (2009) The many pathways of RNA degradation. Cell 136: 763-776.
11. Parker R (2012) RNA Degradation in Saccharomyces cerevisae. Genetics 191: 671-702.
12. Jinek M, Coyle SM, Doudna JA (2011) Coupled 5' nucleotide recognition and processivity in Xrn1-Mediated mRNA Decay. Mol Cell. 41: 600-608.
13. Kenna M, Stevens A, McCammon M, Douglas MG (1993) An essential yeast gene with homology to the exonuclease-encoding XRN1/KEM1 gene also encodes a protein with exoribonuclease activity. Mol Cell Biol 13: 341-50.
14. Hampshire N, The A, Words K, March R (1992) Isolation and characterization of RATI : an essential gene of Saccharomyces cerevisiae required for the efficient nucleocytoplasmic trafficking of mRNA. Genes Dev 6: 1173-1189.

15. Nagarajan VK, Jones CI, Newbury SF, Green PJ (2013) XRN 5' → 3' exoribonucleases: Structure, mechanisms and functions. Biochim Biophys Acta 1829: 590-603.

16. Geerlings TH, Vos JANC, Raué HA (2000) The final step in the formation of 25S rRNA in Saccharomyces in Saccharomyces cerevisiae is performed by 5' → 3' exoribonucleases. RNA 6: 1698-1703.

17. Petfalski E, Dandekar T, Henry Y (1998) Processing of the precursors to small Nucleolar RNAs and rRNAs requires common components. Mol Cell Biol 18: 1181-1189.

18. Miki TS, Grobhans H (2013) The multifunctional RNase XRN2. Biochem Soc Trans 41: 825-830.

19. Kim M, Krogan NJ, Vasiljeva L, Rando OJ, Nedea E, et al. (2004) The yeast Rat1 exonuclease promotes transcription termination by RNA polymerase II. Nature 432: 517-522.

20. Wang C, Zhao L, Lu S (2015) Role of TERRA in the regulation of telomere length. Int J Biol Sci 11: 316-323.

21. Shobuike T, Sugano S, Yamashita T, Ikeda H (1995) Characterization of cDNA encoding mouse homolog of fission yeast dhp1 gene: Structural and functional conservation. Nucleic Acids Res 23: 357-361.

22. Goebels C, Thonn A, Gonzalez-hilarion S, Rolland O, Moyrand F, et al. (2013) Introns Regulate Gene Expression in *Cryptococcus neoformans* in a Pab2p Dependent Pathway. PLoS Genet 9: e100368.

23. Consortium TU (2017) UniProt : The universal protein knowledgebase. Nucleic Acids Res 45: D158-D169.

24. Edgar RC (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32: 1792-7.

25. Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies. Syst Biol 52: 696-704.

26. Kim DE, Chivian D, Baker D (2004) Protein structure prediction and analysis using the Robetta server. Nucleic Acids Res 32: W526-W531.

27. Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, et al. (2018) Swiss-model : Homology modelling of protein structures and complexes. Nucleic Acids Res 46: W296-W303.

28. Xiang S, Cooper-Morgan A, Jiao X, Kiledjian M, Manley JL, et al. (2009) Structure and function of the 5' → 3' exoribonuclease Rat1 and its activating partner Rai1. Nature 458: 784-788.

29. Schneidman-Duhovny D, Inbar Y, Nussinov R, Wolfson HJ (2005) PatchDock and SymmDock : servers for rigid and symmetric docking. Nucleic Acids Res 33: W363-W367.

30. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, et al. (2004) UCSF Chimera — A visualization system for exploratory research and analysis. J Comput Chem 25: 1605-1612.

31. Chang JH, Xiang S, Xiang K, Manley JL, Tong L (2011) Structural and biochemical studies of the 5' → 3' exoribonuclease Xrn1. Nat Struct Mol Biol 18: 270-276.

32. Maldonado-Bonilla LD (2017) In Silico identification of potentially functional conserved motifs in two plant biochemistry & physiology in silico identification of potentially functional conserved motifs in two components of the 5 ' to 3 ' mRNA decay pathway of plants. J Plant Biochem Physiol 5: 1000194.

33. Dichtl B, Stevens A, Tollervey D (1997) Lithium toxicity in yeast is due to the inhibition of RNA processing enzymes. EMBO J 16: 7184-95.

34. Nel B, Steinberg C, Labuschagne N, Viljoen A (2007) Evaluation of fungicides and sterilants for potential application in the management of Fusarium wilt of banana. Crop Prot 26: 697-705.

35. Ting ASY, Mah SW, Tee CS (2011) Detection of potential volatile inhibitory compounds produced by endobacteria with biocontrol properties towards *Fusarium oxysporum* f. sp. *cubense* race 4. World J Microbiol Biotechnol 27: 229-235.

36. Yuan J, Raza W, Shen Q, Huang Q (2012) Antifungal activity of bacillus amyloliquefaciens NJN-6 volatile compounds against *Fusarium oxysporum* f. sp. *cubense*. Appl Environ Microbiol 78: 5942-5944.

37. Getha K, Vikineswary S (2002) Antagonistic effects of Streptomyces violaceusniger strain G10 on *Fusarium oxysporum* f. sp. *cubense* race 4: Indirect evidence for the role of antibiosis in the antagonistic process. J Ind Microbiol Biotechnol 28: 303-310.

38. Chan YC, Ali ASM, Salleh B, Rosli H, Quah CK (2014) Cu(II) complexes of 2-(diphenylmethylene)hydrazinecarboxamide derivatives: Synthesis, characterization and antifungal activity against *Fusarium oxysporum* f. sp. *cubense* tropical race 4. Arab J Chem 10: S3493-S3500.

39. Luke B, Panza A, Redon S, Iglesias N, Li Z, Lingner J (2008) Telomeric repeat-containing RNA and promotes telomere elongation in *Saccharomyces cerevisiae*. Mol Cell 32: 465-477.

40. An H, Lee K, Kim J (2018) Identification of an exoribonuclease homolog, CaKEM1/CaXRN1, in *Candida albicans* and its characterization in filamentous growth. FEMS Microbiol Lett 235: 297-303.

41. Johnson AW (1997) Rat1p and Xrn1p are functionally interchangeable exoribonucleases that are restricted to and required in the nucleus and cytoplasm, respectively. Mol Cell Biol 17: 6122-6130.

42. Nedea E, He X, Kim M, Pootoolal J, Zhong G, et al. (2003) Organization and function of APT, a sub-complex of the yeast cleavage and polyadenylation factor involved in the formation of mRNA and small nucleolar RNA 3'-ends . J Biol Chem 278: 33000-33010.