medical sciences forum

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Proceeding Paper

The Enigmatic Rid7C Protein Is an Endoribonuclease Involved in Differentiation and A40926 Production in *Nonomuraea gerenzanensis* †

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† Presented at the The 2nd International Electronic Conference on Antibiotics—Drugs for Superbugs: Antibiotic Discovery, Modes of Action and Mechanisms of Resistance, 15–30 June 2022; Available online: https://eca2022.sciforum.net/.

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Abstract: The protein subfamily YjgF/YER057c/UK114 (Rid) is widespread in all domains of life and includes proteins involved in detoxification, RNA maturation, and control of mRNA translation. The only member of this superfamily biochemically well-characterized is the archetypal RidA that is involved in cell detoxification function because it hydrolyzes the reactive intermediates enamine/imine generated from the PLP-dependent serine/threonine dehydrates. Besides RidA, seven families named Rid1 to Rid7 are identified in prokaryotes. A conserved arginine residue is shared by all Rid members with the detoxifying activity. Conversely, other members lack the Arg residue, and their role is mysterious. In this study, a step toward understanding the role of these proteins has been achieved by studying a protein, called Rid7C, in *Nonomuraea gerenzanensis*, a rare actinomycete industrially used to produce A40926, the precursor of the FDA-approved antibiotic dalbavancin.

Keywords: YjgF/YER057c/UK114 family proteins; rid endoribonuclease; RNase P; actinomycetes; secondary metabolism

Citation: Calcagnile, M.; Damiano , F.; Pasanisi, D.; Talà, A.; Tredici, S.M.; Giannotti, L.; Siculella , L.; Alifano , P. The Enigmatic Rid7C Protein Is an Endoribonuclease Involved in Differentiation and A40926 Production in *Nonomuraea gerenzanensis*. *Med. Sci. Forum* **2022**, *2*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s):

Published: date

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1. Introduction

YjgF/YER057c/UK114 (Rid family) is a large family of small proteins found across all domains of life and comprises enigmatic members. Eight subfamilies, RidA, Rid1 to Rid7, have been described [1]. Most of the Rid proteins, such as RidA, were involved in the detoxification function due to the ability to eliminate toxic enamine/imine intermediates. The pyridoxal 5′-phosphate (PLP) cofactor reacts with enamine/imines causing the inhibition of PLP-dependent enzymes and cellular damage [2–4]. In this study, we have characterized a YjgF/YER057c/UK114 family protein, Rid7C, with associated endoribonuclease activity in *Nonomuraea gerenzanensis*. *N. gerenzanensis* is an actinomycete [5] used in industry to produce the glycopeptide A40926, which is the precursor of dalbavancin, an FDA-approved antimicrobial effective against methicillin-resistant *Staphylococcus aureus* [6]. *N. gerenzanensis* exhibits two RNAP β chain-encoding genes: *rpoB(S)* (the wild-type *rpoB* gene) and *rpoB(R)* (a mutant-type *rpoB* gene) [7–9]. RpoB(R) controls the activation of secondary metabolisms, including the production of A40926 and a wide range of adaptive metabolic behaviors [7–9]. We reported computational, in vivo, and in vitro evidence that

Rid7C-associated endoribonuclease activity is involved in regulating the expression of *rpoB(R)* at the post-transcriptional level thereby driving morphological and biochemical differentiation in *N. gerenzanensis* [10].

2. Materials and Methods

We described materials and methods in detail in a previous publication [10].

3. Results

The *N. gerenzanensis* genome has 10 different families of YjgF/YER057c/UK114 proteins. We used two approaches to assign *N. gerenzanensis* Rid proteins to a subfamily: (i) molecular phylogeny and (ii) sequence conservation. We report the result of this analysis in Table 1. The R105 residue, associated with deaminase activity, is absent in the Rid7 protein. Based on the genomic background, Rid1 and Rid2 may have metabolic functions. In contrast, other Rid proteins do not have an assigned function.

Table 1. Rid proteins in *N. gerenzanensis*: ID of proteins and assigned subfamilies

Subfamilies	ID of Proteins in *N. gerenzanensis* Genome
RidA	SBO92579.1 SBO90862.1 SBO96592.1
Rid1	SBO91465.1
Rid3	SBO98760.1
Rid6	SBO94674.1
Rid7	SBO96935.1 (Rid7A) SBO95965.1 (Rid7B) SBP00267.1 (Rid7C) SBO92286.1

We have focused our attention on the proteins of the Rid7 subfamily, as their function was unknown. Specifically, we chose three proteins and named them Rid7A, Rid7B, and Rid7C. We expressed these proteins in *Escherichia coli* and then purified them. We hypothesized that these proteins might be involved in the post-transcriptional modulation of RpoB(R) mRNA. Translation of RpoB(R) mRNA is negatively regulated by a self-complementary loop in its 5′-UTR that hide the Shine & Dalgarno sequence. We synthesized an RNA (riboprobe) in vitro using the 5′ end of the *rpoB(R)* gene as a template, including the complementary untranslated end. We performed an in vitro ribonuclease digestion assay and showed that one of the proteins, Rid7C, has endoribonuclease activity. We implemented 5′ RACE to determine where this cleavage occurs. In addition, we expressed the *rpoB(R)* gene in *Streptomyces lividans*, with or without any of the three rid7 genes (A, B, or C). By measuring antibiotic production by *S. lividans*, we demonstrated that RpoB(R) increases the amount of antibiotic produced when co-expressed with Rid7C. During the experiments, we noticed an additional band in Rid7C that was not present in Rid7A or Rid7B. We verified that this band was RNA M1 (a component of RNase P) using an RT-PCR. However, this RNA is not required for Rid7C endoribonuclease activity. We used some 3D modeling software to get the Rid7A, Rid7B, and Rid7C protein models. The Rid7C protein was the only one that I-Tasser (Ligand Binding Site) [11] predicted as an antibiotic-binding protein. We tested the protein's ability to bind A40926, confirming how this binding occurred and was specific. We found that binding to A40926 negatively modulated the endoribonuclease activity of Rid7C. Furthermore, when the Rid7C protein was purified with A40296, the amount of RNA M1 bound to Rid7C decreased. The gene *rpoB(R)* is characterized by five amino acid substitutions located in the RNA polymerase fork domain. Two of these amino acid substitutions were associated with resistance to

rifamycins [7]. For this reason, we administered rifamycin B alone or with A40926. When the bacterium grew only with rifamycin it was resistant, while the presence of A40926 made the bacterium sensitive (Figure 1), confirming the hypothesis of a negative feedback mechanism (Figure 2). The full results of this research are fully described in the paper of Damiano et al. (2021) [10].

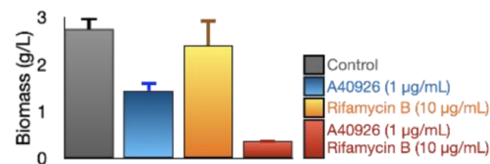

Figure 1. Growth of *N. gerenzanensis* with A40926 (1 μg/mL), with Rifamycin B (10 μg/mL) and with both A40926 (1 μg/mL) and Rifamycin B (10 μg/mL).

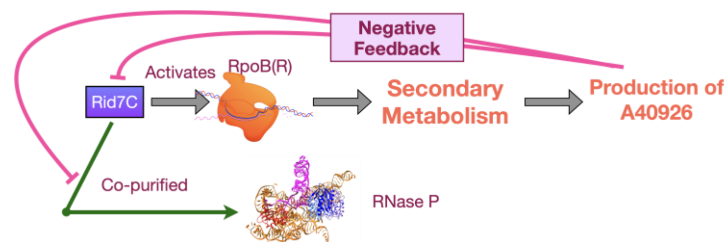

Figure 2. Schematic representation of negative feedback loop. The A40926 negative modulate both the endoribonuclease activity of Rid7C and the co-purification of Rid7C with RNA M1.

4. Discussion and Conclusions

Rid7C endoribonuclease is involved remotion of a ~80 nt segment that negatively modulated the translation of the RpoB(R) mRNA. Rid7C may be associated with ribonuclease P, although it is not required for *rpoB(R)* mRNA processing in vitro. Here we provide evidence that Rid7C activity is negatively regulated by A40926, consistent with Rid7C-mediated regulation of *rpoB(R)* expression by endogenous antibiotics. Computational data predict that A40926 can bind Rid7C. Furthermore, binding experiments using native agarose gels showed that the preincubation of Rid7C with A40926 resulted in a gradual increase in the mobility of the Rid7C complex with the A40926 concentration, suggesting that the interaction between A40926 and Rid7C affects the total charge or oligomeric state, and thus the enzymatic activity of Rid7C. Notably, we found that recombinant Rid7C was purified from *E. coli* extracts together with M1 RNA (the catalytic RNA subunit of *E. coli* RNase P), and we found that the addition of A40926 to bacterial lysates reduced M1 RNA binding by ~70%. These results raise the possibility that A40926 may disrupt M1 RNA binding to Rid7C. Furthermore, the computational data we produced with I-Tasser (not shown) and transcriptomics data suggest that Rid7 family proteins, which do not have the conserved R105 residue, may be associated with host adaptation in some bacteria (e.g., *Pseudomonas aeruginosa*). The PA4173 gene of *P. aeruginosa* (Rid7C family) is most expressed during human infection, suggesting a role for these proteins in host adaptation [12]. Similarly, the *Mycobacterium tuberculosis* Rv2704 gene is 8-fold more transcribed in pulmonary tuberculosis [13].

Author Contributions: Conceptualization, P.A.; investigation, F.D., L.G., D.P., S.M.T., M.C. and A.T.; writing—original draft preparation, P.A., M.C., F.D.; writing—review and editing, P.A., L.S.; project administration, P.A.; funding acquisition, P.A. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported partially by grants from the italian MIUR to P.A. (PRIN 2017, grant 2017SFBFER), and from CIB (Consorzio Interuniversitario Biotecnologie, grant N. 86/19) to P.A.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement:

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ernst, D.C.; Downs, D.M. Mmf1p couples amino acid metabolism to mitochondrial DNA maintenance in *Saccharomyces cerevisiae*. *mBio* **2018**, *9*, e00084-18.
2. Likos, J.J.; Ueno, H.; Feldhaus, R.W.; Metzler, D.E. A novel reaction of the coenzyme of glutamate decarboxylase with L-serine O-sulfate. *Biochemistry* **1982**, *21*, 4377–4386.
3. Ueno, H.; Likos, J.J.; Metzler, D.E. Chemistry of the inactivation of cytosolic aspartate aminotransferase by serine O-sulfate. *Biochemistry* **1982**, *21*, 4387–4393.
4. Lambrecht, J.A.; Schmitz, G.E.; Downs, D.M. RidA proteins prevent metabolic damage inflicted by PLP-dependent dehydratases in all domains of life. *mBio* **2013**, *4*, e00033-e13.
5. Dalmastri, C.; Gastaldo, L.; Marcone, G.L.; Binda, E.; Congiu, T.; Marinelli, F. Classification of *Nonomuraea* sp. ATCC 39727, an actinomycete that produces the glycopeptide antibiotic A40926, as *Nonomuraea gerenzanensis* sp. nov. *Int. J. Syst. Evol. Microbiol.* **2016**, *66*, 912–921.
6. Anderson, V.R.; Keating, G.M. Dalbavancin. *Drugs* **2008**, *68*, 639–651.
7. Vigliotta, G.; Tredici, S.M.; Damiano, F.; Montinaro, M.R.; Pulimeno, R.; di Summa, R.; Massardo, D.R.; Gnoni, G.V.; Alifano, P. Natural merodiploidy involving duplicated *rpoB* alleles affects secondary metabolism in a producer actinomycete. *Mol. Microbiol.* **2005**, *55*, 396–412.
8. D'Argenio, V.; Petrillo, M.; Pasanisi, D.; Pagliarulo, C.; Colicchio, R.; Talà, A.; de Biase, M.S.; Zanfardino, M.; Scolamiero, E.; Pagliuca, C.; et al. The complete 12 Mb genome and transcriptome of *Nonomuraea gerenzanensis* with new insights into its duplicated "magic" RNA polymerase. *Sci. Rep.* **2016**, *6*, 18.
9. Talà, A.; Wang, G.; Zemanova, M.; Okamoto, S.; Ochi, K.; Alifano, P. Activation of dormant bacterial genes by *Nonomuraea* sp. strain ATCC 39727 mutant-type RNA polymerase. *J. Bacteriol.* **2009**, *191*, 805–814.
10. Damiano, F.; Calcagnile, M.; Pasanisi, D.; Talà, A.; Tredici, S.M.; Giannotti, L.; Siculella, L.; Alifano, P. Rid7C, a Member of the YjgF/YER057c/UK114 (Rid) Protein Family, Is a Novel Endoribonuclease That Regulates the Expression of a Specialist RNA Polymerase Involved in Differentiation in *Nonomuraea gerenzanensis*. *J. Bacteriol.* **2022**, *204*, e0046221. https://doi.org/10.1128/JB.00462-21.
11. Yang, J.; Yan, R.; Roy, A.; Xu, D.; Poisson, J.; Zhang, Y. The I-TASSER Suite: protein structure and function prediction. *Nat. Methods* **2015**, *12*, 7–8.
12. Cornforth, D.M.; Dees, J.L.; Ibberson, C.B.; Huse, H.K.; Mathiesen, I.H.; Kirketerp-Møller, K.; Wolcott, R.D.; Rumbaugh, K.P.; Bjarnsholt, T.; Whiteley, M. *Pseudomonas aeruginosa* transcriptome during human infection. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, E5125–E5134.
13. Rachman, H.; Strong, M.; Ulrichs, T.; Grode, L.; Schuchhardt, J.; Mollenkopf, H.; Kosmiadi, G.A.; Eisenberg, D.; Kaufmann, S.H.E. Unique transcriptome signature of Mycobacterium tuberculosis in pulmonary tuberculosis. *Infect. Immun.* **2006**, *74*, 1233–1242.