The enigmatic Rid7C protein is an endoribonuclease involved in differentiation and A40926 production in *Nonomuraea gerenzanensis*

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Background

The Rid superfamily proteins are widespread in all organisms. The RidA is the only biochemically well-characterized member and is involved in the hydrolysis of the reactive intermediates generated from the PLP-dependent serine/threonine dehydrates. Besides RidA, seven families named Rid1 to Rid7 are identified in prokaryotes. 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. This actinomycete is characterized by the presence of duplicated genes encoding β-subunit of RNA polymerase: *rpoB(S)* and *rpoB(R)*. RpoB(R) isoform controls the morphological differentiation and the activation of secondary metabolism. Translation of the RpoB(R) mRNA is negatively modulated by a self-complementary hairpin loop in its 5'-UTR which hides the Shine & Dalgarno sequence.

Aims

- ✔ Reveal post-transcriptional control of *rpoB(R)* mRNA.
- ✔ Reveal the function of Rid7C in *N. gerenzanensis*
- ✔ Reveal the effect of A40926 on the expression of *rpoB(R)*

Results

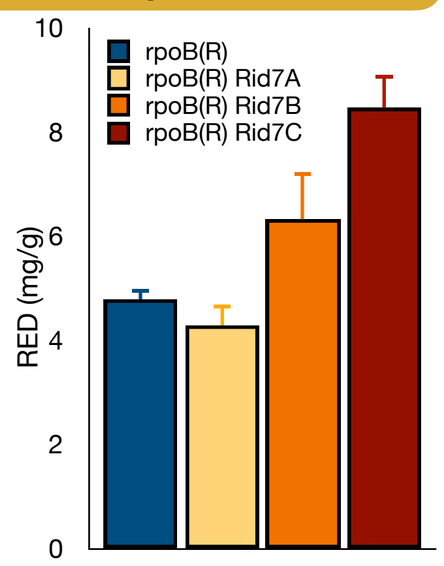
1. *N. gerenzanensis* show ten distinct YjgF/YER057c/UK114 family proteins

Rid Family	Genes
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

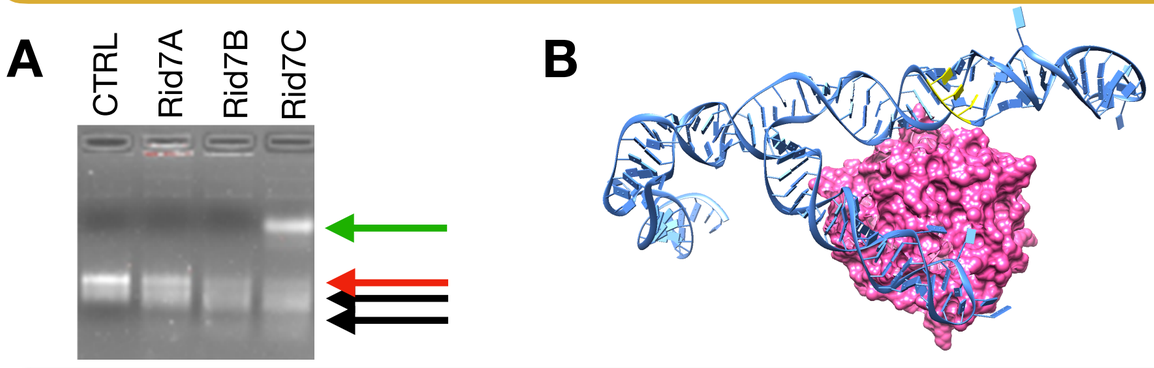
Two approaches have been used to assign Rid proteins to a subfamily: i) molecular phylogeny and ii) sequence conservation. Residue R105, which is associated with deaminase activity, is absent in the Rid7 protein. Based on the genomic background, Rid1 and Rid2 may have metabolic functions. In contrast, other Rid proteins (e.g., Rid7) have no function.

2. Expression of *rid7C* in *S. lividans* harboring *N. gerenzanensis rpoB(R)* boosts antibiotic production

The production of undecylprodigiosin (RED) at 96 h from transconjugants *Streptomyces lividans* grown in R4 broth. RED production did not change significantly in S. lividans *rid7A*, slightly increased in *S. lividans rid7B*, and significantly increased with *rid7C*. This discovery demonstrated the ability of *rid7C* to increase antibiotic production in the presence of *rpoB(R)*.



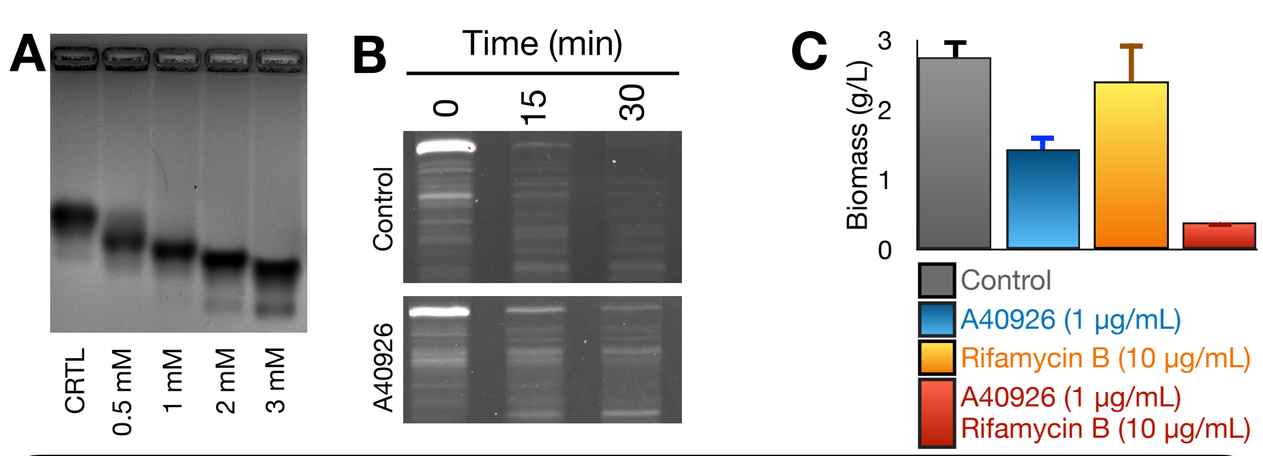
3. N. gerenzanensis Rid7C performs site-specific processing of *rpoB(R)* 5'-UTR mRNA in vitro



The purified Rid7A, Rid7B, and Rid7C proteins were incubated in the presence of the RNA substrate (riboprobe, red arrow) (**A**). The reaction products (black arrows) were analyzed by electrophoresis on the non-denaturing agarose gel. The presence of an additional band migrating slower than that of the RNA substrate could be noted with Rid7C (green arrow, identified subsequently as RNA M1). This band was not visible in the samples incubated with Rid7A or Rid7B.

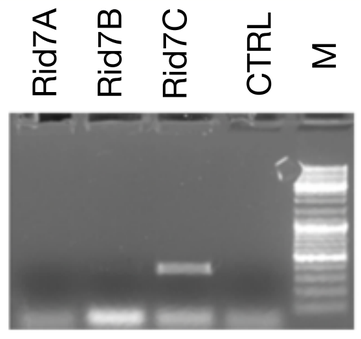
We identified the sites where the probe was cut by Rid7C using the a 5'-RACE. Docking simulations (**B**) confirmed that Rid7C bound the riboprobe at the points where the RNA was cut. Molecular modeling of Rid7C indicated that Rid7C could bind antibiotics, as we verified subsequently.

4. A40926 binding to Rid7C and negatively modulates its ribonuclease activity in vitro



A) A40926 (but not other antibiotics, e.g., Rifamycin B) bind Rid7C protein **B)** and negatively modulates its ribonuclease activity *in vitro*. **C)** *In vivo* inhibition of rifamycin B resistance by A40926 in *N. gerenzanensis*

5. Recombinant Rid7C co-purifies with *E. coli* ribonuclease P RNA component



Recombinant Rid7A, Rid7B, and Rid7C proteins were subject to (RT)-PCR analysis RNA M1-specific primers. RT-PCR product size is consistent with the M1 RNA component of *E. coli* RNAse P.

Conclusion

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. Computational, in vitro, and in vivo evidence suggest that Rid7C activity is inhibited by A40926 suggesting the existence of a negative feedback loop on A40926 production.

References

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