Characterization of an RNase III mutant of *Rhodobacter sphaeroides*

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Abstract. In this work an RNase III deletion mutant of the facultative phototrophic α-proteobacterium *Rhodobacter sphaeroides* was characterized. The aim of this study was to identify differences in phenotype and RNA physiology between wild-type and mutant, which can be explained by a loss of RNase III activity. We analyzed the growth behaviour of the mutant and the wild type under different growth conditions, including microaerobic and phototrophic mode of growth. As *Rhodobacter sphaeroides* is known for its metabolic versatility, we were also interested in regulation of the transcriptome. The results suggest that the deletion of the RNase III coding gene (*rnc*) strongly impacts phenotype and transcriptome of *Rhodobacter sphaeroides*.

1. Introduction
As bacteria are exposed to an ever-changing environment, they have evolutionarily developed complex regulatory networks to adapt their proteome to different environmental conditions. At the same time, protein biosynthesis is one of the most resource- and energy intensive biological processes in all living organisms [1]. Therefore, bacteria use different exo- and endoribonucleases to regulate gene expression quickly by degrading transcripts that are no longer needed.

RNase III is a highly conserved dsRNA processing endoribonuclease, which is present in nearly every living organism, but homologues are missing in most archaeal species [2]. The bacterial RNase III is structurally classified as an RNase III class I enzyme and consists of an N-terminal catalytic active RNase III domain and a C-terminal dsRNA binding domain. The well-studied eukaryotic RNase III enzyme Drosha is classified as a class II RNase III enzyme and harbours two RNase III domains, whereas eukaryotic Dicer enzymes are classified as class III RNase III enzymes and harbour not only two RNase III domains, but also an additional helicase domain at the N-terminal part of the protein [3, 4]. Previous studies showed that in both, *Rhodobacter sphaeroides* and in the phylogenetic closely related species *Rhodobacter capsulatus*, RNase III recognizes RNA molecules with structured regions as a substrate. While the binding to the substrate is facilitated by the C-terminal dsRNA binding domain, the hydrolysis of the phosphodiester bond is catalyzed by the N-terminal RNase III domain. Although RNase III regulates the abundance of rRNAs, structured mRNAs and regulatory ncRNAs and therefore is known as a global regulator of gene expression, the substrate specificity of the enzyme is still not fully understood [5-7].

In our studies we worked with the model organism *Rhodobacter sphaeroides*, which is a gram negative purple non sulphur α-proteobacterium. Interestingly, *R. sphaeroides* is capable of multiple different metabolic modes of growth and can use aerobic or anaerobic respiration, fermentation or anoxygenic photosynthesis for chemical energy conservation. In the last decades *R. sphaeroides* found numerous applications in biotechnology, for example in vitamin B12 synthesis [8], biological hydrogen...
production [9], *in vitro* carbon dioxide fixation [10], bioremediation of waste water and soil [11, 12] or synthesis of polyhydroxyalkanoates [13], which can be used as a resource for production of biodegradable plastics.

The photosynthetic apparatus of *R. sphaeroides* consists of three photosynthetically active pigment-protein complexes, the so called light-harvesting complex I (LHC I), the light-harvesting complex II (LHC II) and the reaction center (RC), which are located in membrane invaginations of the inner cell membrane. Structurally, these pigment-protein complexes are organized in a defined manner, where a single RC is surrounded by the LHC I and moreover encircled by multiple LHC II. The production and assembly of the photosynthetic apparatus allows *R. sphaeroides* to conserve chemical energy in form of ATP, by absorbing the photons of light [14].

When light is present and the oxygen tension in the environment drops under a certain threshold, synthesis of the compounds of the photosynthetic apparatus gets induced [15]. This tight regulation is advantageous for *R. sphaeroides*, because the photosynthetically active pigment-protein complexes can act as photosensitzers by transferring energy not only to the RC, where the photo-chemical energy conversion happens, but also to molecular oxygen, which can then react to singlet oxygen and cause formation of reactive oxygen species (ROS) *in vivo*. ROS are harmful for all kind of living cells, because they can oxidize essential biomolecules like DNA, RNA or lipids and can cause protein aggregation. Therefore, the formation of ROS causes a stress response on RNA and protein layers of gene expression in *R. sphaeroides* [16-19].

2. Results

The deletion mutant and the wild type of *R. sphaeroides* were cultivated under microaerobic and phototrophic conditions. Interestingly, a weaker pigmentation of the mutant was observed during microaerobic cultivation. To investigate the differences in pigmentation level, the pigments of both strains were extracted via formaldehyde/methanol extraction and photometrically quantified at \( \lambda=770 \) nm (Bchl a) and \( \lambda=484 \) nm (carotenoids). For normalization the measured absorbance values were divided by the absorbance values measured at \( \lambda=660 \) nm.

![Figure 1(a, b).](attachment:image1.png)

**Figure 1(a, b).** (a) Photo of microaerobically grown culture samples during mid exponential growth; (b) Extracted pigment amounts of wild type (dots) and \( \Delta rnc \) mutant (squares).

Full cell absorbance spectra were measured under microaerobic conditions of biological triplicates of wild type and the \( \Delta rnc \) mutant strain. The data was normalized to the absorbance at \( \lambda=660 \) nm after calculating mean values of the triplicates. Characteristic absorbance peaks of the light harvesting complexes I & II, as well as for the RC, are marked with arrows.
Our data shows less absorbance in the mutant samples in the area around 750–900 nm, where the photosynthetically active pigment-protein complexes have their specific absorbance maxima. Additionally, decreased absorbance values for carotenoids were measured, which indicates that pigment and pigment-protein complex production in general is impaired in the Δrnc mutant strain of *R. sphaeroides*.

### 3. Discussion

RNase III is a highly conserved dsRNA processing endoribonuclease, which is known as a global regulator of gene expression in nearly every living species. To investigate the *Rhodobacter sphaeroides* RNase III enzyme a deletion mutant was characterized. Our results showed clear differences in phenotype of pigment and pigment-protein complex production in the Δrnc mutant strain of *R. sphaeroides* 2.4.1 in comparison to the wild type. In particular, the production of the photosynthetically active pigment-protein complexes seems to be diminished in the Δrnc strain, which could probably be explained by a reduced amount of bacteriochlorophyll a, which is an essential compound of the LHC I, the LHC II and the RC [20]. Moreover, an alteration in the steady state level of transcripts coding for photosynthesis genes was observed in this mutant (data not shown). Especially the *puf* genes, which encode for proteins of the LHC I and the RC, were shown to be down regulated on mRNA level in the mutant strain, which could also be responsible for the impaired production of photosynthetically active pigment-protein complexes.

In contrast, an RNase E deletion mutant of *Rhodobacter sphaeroides* did not showed differences in phenotype of pigmentation under microaerobic growth conditions, but under phototrophic conditions [21]. Since the underlying molecular mechanisms behind the observed phenotype of the Δrnc mutant of *Rhodobacter sphaeroides* still remain unclear, it will be subject of our further investigations.

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