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Abstract
Gene expression is a multi-faceted phenomenon, governed not only by the sequence of nucleotides, but also by the extent to which a particular gene gets transcribed, how the transcript is processed, and whether or not the transcript ever makes it out of the nucleus. Rrp6p is a 5'-3' exonuclease that can function independently and as part of the nuclear exosome in Saccharomyces cerevisiae (Portin, 2014). It degrades various types of aberrant RNA species including small nuclear RNAs, small nucleolar RNAs, telomerase RNA, unspliced RNAs, and RNAs that have not been properly packaged for export (Butler & Mitchell, 2010). This exosome mediated degradation is important as the accumulation of unprocessed mRNA transcripts can be harmful to the cell. These experiments sought to quantify changes in RNA levels in rrp6-Δ strains grown in glucose versus those grown in galactose compared to wild type strains grown in each of the carbon sources. The goal was to determine if there was an interplay between the genes regulated by Rrp6p and the genes involved in the switch from glucose metabolism to galactose metabolism. Based on the data, it appears that the absence of Rrp6p increases transcription in glucose while, in galactose, the absence of Rrp6p increases transcription to a significantly lesser degree than in glucose and in some cases decreases transcription.

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ABSTRACT

Gene expression is a multi-faceted phenomenon, governed not only by the sequence of nucleotides, but also by the extent to which a particular gene gets transcribed, how the transcript is processed, and whether or not the transcript ever makes it out of the nucleus. Rrp6p is a 5'-3' exonuclease that can function independently and as part of the nuclear exosome in Saccharomyces cerevisiae (Portin, 2014). It degrades various types of aberrant RNA species including small nuclear RNAs, small nucleolar RNAs, telomerase RNA, unspliced RNAs, and RNAs that have not been properly packaged for export (Butler & Mitchell, 2010). This exosome mediated degradation is important as the accumulation of unprocessed mRNA transcripts can be harmful to the cell. These experiments sought to quantify changes in RNA levels in rrp6-Δ strains grown in glucose versus those grown in galactose compared to wild type strains grown in each of the carbon sources. The goal was to determine if there was an interplay between the genes regulated by Rrp6p and the genes involved in the switch from glucose metabolism to galactose metabolism. Based on the data, it appears that the absence of Rrp6p increases transcription in glucose while, in galactose, the absence of Rrp6p increases transcription to a significantly lesser degree than in glucose and in some cases decreases transcription.

Introduction

DNA was first discovered by Friedrich Miescher in human leukocytes in 1869. However, the importance of DNA was not known until 1944 when the work of Collin MacLeod, Oswald Avery, and Maclyn McCarty revealed that DNA was the transformative agent. This was accomplished by isolating and purifying the cell-free substance capable of causing the genetic transformation demonstrated in Frederick Griffith's well-known work with pneumococcus and showing that it was in fact DNA (Portin, 2014). Much progress has been made since then. It is well understood that DNA is replicated during cell division, that RNA is transcribed from DNA, and that mRNA is translated into amino acids specified by the triplet codons of the mRNA strand. This translation of amino acids yields the proteins that are ultimately responsible for the expression of information encoded by the DNA. The sequencing of the entire human genome in 2004 marked a turning point in the attempt to understand the genetic code and generated many more questions regarding the role of DNA (Portin, 2014).

An area of genetics that has not been elucidated to quite as great of an extent yet is that of epigenetics, the change in gene expression without the change in DNA sequence. There are many factors responsible for whether or not a certain gene is expressed and to what extent a gene is expressed that go beyond the simplistic Central Dogma of biology, such as post-transcriptional and post-translational genetic regulation. Rrp6p, found in the nucleus of *Saccharomyces cerevisiae*, is an example of a protein that mediates post-transcriptional genetic regulation. There is evidence that this protein may also be present in the cytoplasm of *T. brucei*, *A. thaliana* and humans (Butler & Mitchell, 2010). Rrp6p functions in association with the nuclear exosome and plays a key role in the regulation of RNA levels in two ways: it is involved in transcript elongation and proper packaging of RNA transcripts for export from the nucleus, and it degrades aberrant RNA transcripts (Butler & Mitchell, 2010; Kilchert & Vailjeva, 2013). This study focuses on the latter of these two functions. Rrp6p, together with the exosome core, Exo9, and the ribonuclease Dis3,

forms the nuclear exosome, Exo11 (Butler & Mitchell, 2010).

Types of aberrant RNA targeted by the exosome include small nuclear RNAs (snRNAs), small nucleolar RNAs (snoRNAs), telomerase RNA, unspliced RNAs, and RNAs that have not been properly packaged for export. This exosome mediated degradation is important as the accumulation of unprocessed mRNA transcripts can be harmful to the cell (Figure 1) (Kilchert & Vailjeva, 2013). Rrp6p can also act independently of the exosome complex and serves to regulate concentrations of specific mRNAs, thus contributing to feedback control and regulation of cell cycle events. For instance, Rrp6p works with the polyadenylating TRAMP complex to degrade histone mRNAs at the end of the S-phase of the cell cycle to ensure that histone synthesis does not continue beyond S-phase and is involved in a pathway that degrades mRNA transcripts that are exported out of the nucleus at slow rates (Butler & Mitchell, 2010).

As Rrp6p is involved in the degradation of mRNA transcripts, it would be expected that deletion of the *RRP6* gene would result in increased levels of mRNA corresponding to the genes normally regulated by Rrp6p. This study investigated the effects on mRNA levels of deletion of the *RRP6* gene in *S. cerevisiae* as well as the effects of an alternate carbon source on mRNA levels in an *rrp6-Δ* strain compared to wild type. Glucose is the preferred carbon source of *S. cerevisiae* and is metabolized to pyruvate and ATP via the process of glycolysis. However, in the absence of glucose, other carbon sources may be utilized (Demir & Kurnaz, 2006). This experiment used galactose as an alternative carbon source. Galactose undergoes a series of enzymatic reactions that eventually convert it to glucose-6-phospate (G6P) which can then enter glycolysis to yield ATP. Both galactose-induced upregulation and repression induced by the absence of glucose are necessary for the expression of galactose-metabolizing enzymes (Demire & Kurnaz, 2006). This study sought to determine if the absence of Rrp6p combined with the introduction of galactose as the carbon source elicits novel changes in mRNA levels that are not elicited by either factor individually.

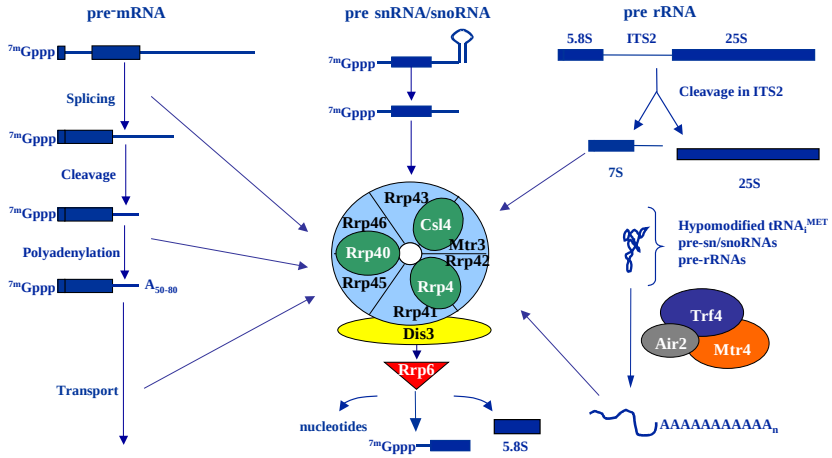

Figure 1. Functions of the Nuclear Exosome. For pre-mRNAs the diagram implies that defects occurring at the indicated steps result in degradation of the transcript by the nuclear exosome. For pre-sn/snoRNA and 7S pre-rRNA, the core exosome and Rrp6p catalyzes 3' end formation in a two-step reaction.

Materials and Methods

Growth of yeast cultures

Two different strains of *Saccharomyces cerevisiae*, wild type (WT) and an *rrp6*-Δ strain, were each cultured in YPD at 37 °C and YP-Gal at 37 °C. Cells were collected at an optical density at 600 nm between 0.6 and 1.5, when cells were in log phase.

RNA isolation

Pelleted cells were resuspended with lysis buffer containing Zymolase (Thermo Fisher Scientific) prior to a 30 minute incubation at 30 °C. A second lysis buffer was then added, followed by Proteinase K diluted according to manufacturer instructions. Cells were incubated for 20 minutes and then centrifuged for 10 minutes at 13,000 rpm. Supernatant was removed into a sterile tube and mixed with ethanol. This was then transferred to the GeneJet Purification Column and centrifuged for 1 minute at 13,000 rpm. The column was washed once with Wash Buffer 1 and twice with Wash Buffer 2, with centrifuging between washes. RNA was eluted with water, and samples were quantified using the Nanodrop Lite instrument (Thermo Fisher Scientific).

cDNA Generation

RNA concentrations were standardized between samples through dilution with water and combined with Oligo(dT), RevertAid Reverse Transcriptase, Riboblock RNase Inhibitor, dNTP, and 5X reaction buffer, as specified by manufacturer (Thermo Fisher Scientific). Reverse transcriptase reactions were carried out at 42 °C for 30 minutes followed by 5 minutes at 70 °C to denature RevertAid Reverse Transcriptase.

Real time quantitative PCR

Real time quantitative PCR was carried out using SYBR Green mix (Thermo Fisher Scientific), forward and reverse primers for the genes of interest, and 4.4 µL cDNA for a total reaction volume of 10 µL. Primers included sequences for Actin, Nrd1, Nab2, SnR45, and Cut652. An annealing temperature of 95 °C was used for 5 minutes, followed by 40 cycles consisting of 1 minute at 60 °C and 30 seconds at 95 °C. Each qPCR reaction was run in triplicate, and ΔΔCt values, normalized to Actin, were used to quantify changes in RNA transcript levels.

Results

Genes of interest included Actin, Nrd1, NAB2, SnR45, and Cut652. Actin served as a control as its expression is known to be unaffected by the loss of Rrp6p. Nrd1 encodes an RNA-binding protein that, along with Nab3p, is involved in termination of promoter-associated cryptic unstable transcripts, intergenic regions which are degraded after transcription. While Nrd1p's need to associate with polymerase II to induce termination somewhat restricts its function to terminating shorter transcripts rather than longer ones, simply the upregulation of recruitment of Nrd1p during transcription appears to be sufficient to prime an aberrant transcript for destruction. Nrd1p has been found ubiquitously at introns in the *S. cerevisiae* genome eliciting the hypothesis that Nrd1p may recruit the exosome complex to RNA that has not been properly spliced or packaged (Demire & Kurnaz, 2006). Nab2 encodes another RNA-binding protein that binds to polyadenylated nuclear RNA. It is involved in poly(A) tail length control as well as the packaging of mRNA for export out of the nucleus. Nab2p autoregulates its mRNA levels by interacting with an oligoadenylate sequence in the 3' untranslated region of its

mRNA to destabilize the transcript. This autoregulation is dependent on the activity of Rrp6p (Kilchert & Vailjeva, 2013). SnR45 is a nonsplicing C/D box small nucleolar RNA. Studies show that it does not appear to have an essential function within the cell (Balakin et al., 1996). Cut652 is a cryptic untranslated transcript of unknown function.

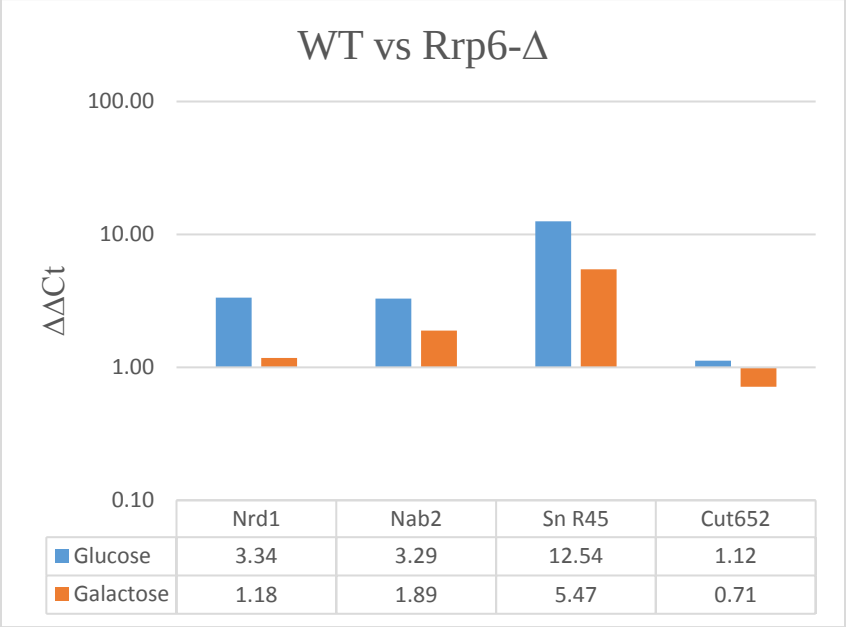

Figure 2. Averaged ΔΔCt values from two biological replicates. Data is presented using a logarithmic scale. A ΔΔCt value below 1.00 indicates a decrease in RNA levels in wild type cells compared to *rrp6*-Δ cells for the specified carbon source whereas a ΔΔCt value above 1.00 represents an increase in RNA levels. A value of 1.00 indicates no change.

Figure 2 shows that the *rrp6*-Δ strain grown in glucose contained higher levels of RNA transcripts for Nrd1, Nab2, SnR45, and Cut652 than the wild type strain grown in glucose as the ΔΔCt values for all genes in glucose were greater than 1.00. SnR45 exhibited the greatest change with a 12.54-fold increase in RNA transcripts. Nrd1 and Nab2 showed less drastic but still noteworthy changes with 3.34-fold and 3.29-fold increases, respectively. The change in Cut652 transcript levels was more modest with a 1.12-fold increase.

Trends for strains grown in galactose were less uniform with Nrd1, Nab2, and SnR45 transcripts showing increases but Cut652 showing a decrease in RNA transcript levels. As in glucose, SnR45 had the largest change with a 5.47-fold increase, with Nrd1 and Nab2 changes similar again with 1.18-fold and 1.89-fold increases, respectively. Cut652 had a ΔΔCt value of 0.71, indicating a 0.29-fold decrease.

Discussion

Previous studies have shown that deletion of *RRP6* in *S. cerevisiae* grown in glucose leads to increased RNA transcripts of Nrd1 and Nab2 (Houalla et al., 2006; Roth et al., 2005). Such findings are consistent with those of this experiment. When grown in glucose, RNA transcript levels for Nrd1 and Nab2, as well as SnR45 and Cut652, increased in the WT cells compared to the *rrp6*-Δ cells. The effect of deletion of *Rrp6* on transcript levels for *S. cerevisiae* grown

in galactose has not been as thoroughly explored. The data from this experiment suggests that utilization of galactose, rather than glucose, as a carbon source leads to considerably smaller increases, or in the case of Cut652, a decrease in RNA transcript levels. It appears that galactose has a novel effect on how these genes are regulated in the absence of rrp6p.

A potential area of future study could be to separately assess the effect of galactose on the activity of the nuclear exosome and the activity of rrp6p independent of the exosome. This could be achieved by generating different mutant strains of *S. cerevisiae*, in addition to WT and *rrp6*-Δ controls. In order to study the effect of galactose on the nuclear exosome, a strain containing mutant *RRP6* that will give rise to a protein that is able to bind to the exosome core but is incapable of executing independent functions could be utilized. To investigate the effect of galactose on rrp6p action independent of the exosome, a strain could be prepared in which the resultant rrp6p is fully functional in its independent endonuclease activities but is unable to bind to the exosome core.

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