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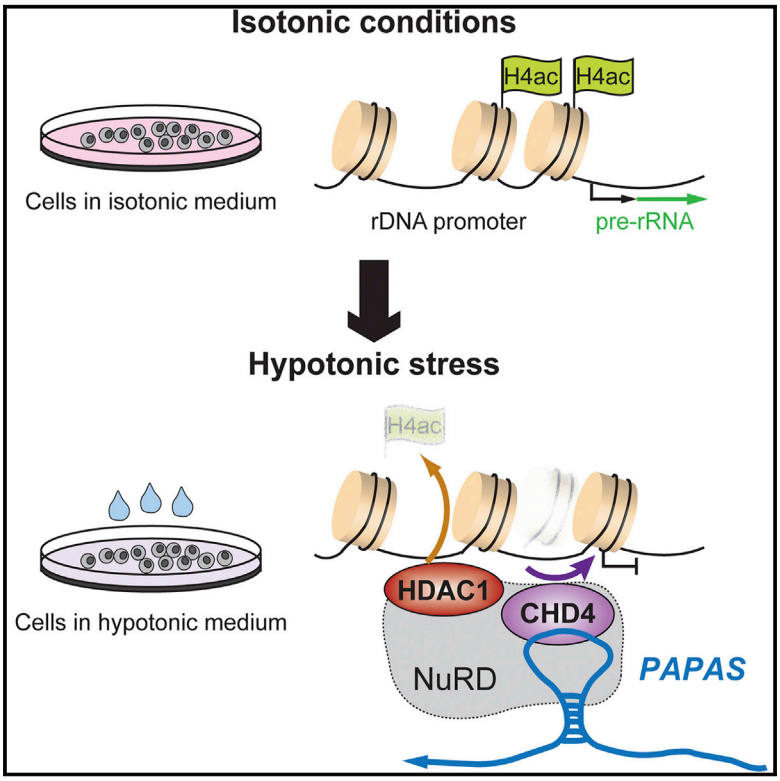
Report

lncRNA-Induced Nucleosome Repositioning Reinforces Transcriptional Repression of rRNA Genes upon Hypotonic Stress

Graphical Abstract



Highlights

- Synthesis of pre-rRNA is shut down under hypotonic conditions
- Hypotonic stress induces *PAPAS*, a transcript that is antisense to pre-rRNA
- *PAPAS* recruits the chromatin remodeling complex NuRD to rDNA
- NuRD repositions the promoter-bound nucleosome

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In Brief

The lncRNA *PAPAS* directs Suv4-20h2 to rDNA for repressive H4K20 trimethylation upon quiescence. Zhao et al. find that, upon hypotonic stress, *PAPAS* recruits the chromatin remodeling complex NuRD to rDNA. NuRD induces a nucleosomal "off" position, thereby reinforcing stress-dependent rDNA silencing.

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lncRNA-Induced Nucleosome Repositioning Reinforces Transcriptional Repression of rRNA Genes upon Hypotonic Stress

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SUMMARY

The activity of rRNA genes (rDNA) is regulated by pathways that target the transcription machinery or alter the epigenetic state of rDNA. Previous work has established that downregulation of rRNA synthesis in quiescent cells is accompanied by upregulation of *PAPAS*, a long noncoding RNA (lncRNA) that recruits the histone methyltransferase Suv4-20h2 to rDNA, thus triggering trimethylation of H4K20 (H4K20me3) and chromatin compaction. Here, we show that upregulation of *PAPAS* in response to hypoosmotic stress does not increase H4K20me3 because of Nedd4-dependent ubiquitinylation and proteasomal degradation of Suv4-20h2. Loss of Suv4-20h2 enables *PAPAS* to interact with CHD4, a subunit of the chromatin remodeling complex NuRD, which shifts the promoter-bound nucleosome into the transcriptional "off" position. Thus, *PAPAS* exerts a "stress-tailored" dual function in rDNA silencing, facilitating either Suv4-20h2-dependent chromatin compaction or NuRD-dependent changes in nucleosome positioning.

INTRODUCTION

The nucleolus has emerged as a central hub for coordinating the stress response, regulating cell growth, and promoting survival and recovery from stress. Environmental cues, including virtually any type of stress, have been shown to feed into the tight regulation of rRNA synthesis as part of ribosome biogenesis surveillance and growth control (Boulon et al., 2010; Grummt, 2013). As ribosome biogenesis consumes a tremendous amount of cellular energy, rRNA synthesis is tightly regulated to be responsive to specific environmental challenges. Actually, almost all signaling pathways that affect cell growth and proliferation directly regulate rRNA synthesis, their downstream effectors converging at the RNA polymerase I (Pol I) transcription machinery and at the chromatin structure of rRNA genes (Kusnadi et al., 2015).

We have recently discovered an epigenetic pathway that attenuates pre-rRNA synthesis in growth-factor-deprived or density-arrested cells, which is orchestrated by a long noncoding RNA (lncRNA) that is transcribed by RNA polymerase II (Pol II) from a fraction of rRNA genes in antisense orientation (Bierhoff et al., 2010, 2014). This antisense RNA, dubbed *PAPAS* (promoter and pre-rRNA antisense), is upregulated in quiescent cells and guides the histone methyltransferase Suv4-20h2 to rDNA, leading to trimethylation of histone H4 at lysine 20 (H4K20me3) and chromatin compaction. Thus, *PAPAS* reinforces transcriptional repression by inducing a chromatin environment that is incompatible with binding of Pol I to the rDNA promoter.

In this study, we have investigated whether *PAPAS*-mediated changes in chromatin structure is a general mechanism that contributes to shutdown of rDNA transcription under different stress conditions. Similar to growth factor deprivation, we observed a marked decrease in pre-rRNA and a strong increase in *PAPAS* upon hypotonic stress. In contrast to serum deprivation, however, hypotonicity increased the interaction of Suv4-20h2 with the E3-ubiquitin ligase Nedd4, leading to enhanced ubiquitinylation and degradation of Suv4-20h2. Depletion of Suv4-20h2 facilitates the interaction of *PAPAS* with CHD4, a subunit of the nucleosome remodeling and deacetylation complex (NuRD) (Zhang et al., 1998; Tong et al., 1998; Xue et al., 1998), which shifts the promoter-bound nucleosome into a position that is refractory to transcription initiation (Xie et al., 2012). The results reveal that *PAPAS* triggers epigenetic silencing of rDNA in different ways, emphasizing the versatility of lncRNAs in adapting the chromatin landscape to environmental cues.

RESULTS

Hypoosmotic Stress Leads to Upregulation of *PAPAS*

To investigate whether *PAPAS*-mediated rDNA silencing is induced by stress conditions other than growth factor deprivation and density arrest, we monitored the levels of pre-rRNA and *PAPAS* in mouse NIH 3T3 fibroblasts, comparing standard