Data in Brief

The conserved HDAC Rpd3 drives transcriptional quiescence in *S. cerevisiae*

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**ABSTRACT**

Quiescence is a ubiquitous cell cycle stage conserved from microbes through humans and is essential to normal cellular function and response to changing environmental conditions. We recently reported a massive repressive event associated with quiescence in *Saccharomyces cerevisiae*, where Rpd3 establishes repressive chromatin structure that drives transcriptional shutoff[6]. Here, we describe in detail the experimental procedures, data collection, and data analysis related to our characterization of transcriptional quiescence in budding yeast (GEO: GSE67151). Our results provide a bona fide molecular event driven by widespread changes in chromatin structure through action of Rpd3 that distinguishes quiescence as a unique cell cycle stage in *S. cerevisiae*.

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1. Direct link to deposited data

Strand-specific RNA sequencing for wild type and Δrpd3 yeast entering quiescence [RNA-seq].

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE67149.

Genome-wide maps of nucleosome positions in purified quiescent *S. cerevisiae* cells [MNase-seq].

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE67148.

Characterization of H3 density, H3 or H4 acetylation, Rpd3 binding, TFIIA binding, and Rpb3 (pol II) binding in wild type and rpd3 cells as they transition from logarithmic growth to diauxic shift to quiescence (ChIP-seq).

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE67150.

2. Experimental design, materials and methods

2.1. Methods

2.1.1. Growth conditions and quiescent cell isolation

Cells were grown in YPD from overnight cultures diluted to OD₆₀₀ = 0.02. Log cells were grown for ~8 h to OD₆₀₀ = 0.4–0.6; diauxic shift cells were grown for ~16 h and were harvested exactly 2 h after glucose starvation.
was no longer detected in the media by glucose test strips (Precision Labs, Inc.); Q cells were purified from stationary phase cultures after 7 days. For thiolutin or rapamycin treatment, cells were grown to OD_{600} = 0.6 and rapamycin (Millipore, 100 nM final) or thiolutin (abcam, 3 μg/mL final) was added for 60 min at 30 °C. Cells were separated by Percoll gradient as follows: 9 mL Percoll (GE Healthcare) was combined with 1 mL (1500 mM NaCl) and spun in 30 mL glass centrifuge tubes at 10,000 × g for 15 min at 4 °C to establish a density gradient. Stationary phase cultures (25 mL) were pelleted and resuspended in 1 mL 10 mM Tris pH 7.5. Resuspended cells were gently layered on top of the Percoll gradient and spun at 400 × g for 60 min at 4 °C [1]. The upper layer of NQ cells was removed and the bottom layer of Q cells was combined with 10 mM Tris pH 7.5, pelleted, and OD_{600} was measured to determine yield. For rpD3 Q cell isolation, up to 250 mL of cells were grown in 25 mL batches, separated individually, and combined after Q cell purification for further analyses.

2.1.2. RNA-seq

RNA was purified as follows: 100 OD_{600} units of cells were grown with chilled mortar and pestle in the presence of glass beads in liquid nitrogen until the bead/cell mixture was a fine white powder. Lysed cells were resuspended in 300 μL of TES buffer (10 mM Tris pH 7.5, 10 mM EDTA, 0.5% SDS) and combined with 300 μL acid phenol. Lysate was incubated at 65 °C for 30 min with vortexing every 10 min, then centrifuged 16,000 × g for 10 min at 4 °C. The aqueous layer was extracted once more with 300 μL acid phenol then once with 300 μL chloroform. RNA was precipitated by ethanol precipitation then quantified by NanoDrop. RNA was cleaned using the RNeasy kit with on-column DNase treatment (Qiagen) per the manufacturer protocol. Purified RNA (3 μg) was combined with 1.5 μL of a 1:10 dilution of ERCC Spike-in control mix 1 (Life Technologies) and de Adding second strand buffer (30 μL), 4 μL of 10 mM dNTP mix with dUTP replacing dTTP, 4 μL first-strand buffer, and 2 μL (100 mM) DTT and incubating on ice for 5 min. Then 1 μL RNase H (NEB) and 5 μL DNA Polymerase I (NEB) were added, mixed, and incubated for 2.5 h at 16 °C. DNA was purified by Qiaquick PCR purification (Qiagen) and eluted into 50 μL buffer EB prior to library construction. After adapter ligation, DNA was gel purified (excised between 200 and 300 bp size range) and resuspended in 50 μL buffer EB, then 19 μL of cDNA was incubated with 1 μL USER enzyme (NEB) for 15 min at 37 °C and heat-inactivated for 5 min at 95 °C. All 20 μL of USER-treated cDNA was subject to 15 cycles of amplification according to the TruSeq protocol, then a second gel extraction and selection between treated cDNA was subject to 15 cycles of amplification. Raw dyad counts were piled up into a 1 nucleosome for 200, the midpoint of each read was calculated as

2.1.3. MNase-seq

Cells were grown and isolated at the appropriate cell cycle stage, then crosslinked with 1% formaldehyde for 20 min at 30 °C with shaking. Crosslinking was quenched with glycine (125 mM final) then cells were pelleted and washed twice with H2O. Cells were resuspended in 20 mL of [1 M sorbitol, 10 mM beta-mercaptoethanol, 50 mM Tris pH 7.5] and treated with zymolyase (100 T, ambio). Cells harvested from exponentially-growing cultures in YPD (~1000OD_{600} units) or after diauxic shift (~1250OD_{600} units) were treated with 2 mg of zymolyase for approximately 20 min at 30 °C. Q cells were treated with ~10 mg of zymolyase for 60–90 min at 30 °C. Complete spheroplast production was determined microscopically. Spheroplasts were pelleted at 4000 × g for 20 min and resuspended in 2 mL [1 M sorbitol, 50 mM NaCl, 10 mM Tris pH 7.5, 5 mM MgCl_2, 1 mM CaCl_2, 0.075% (w/v) Igepal, 0.5 mM spermidine, 1 mM beta-mercaptoethanol]. Three separate digestion reactions (600 μL) were prepared with 30 units of Exonuclease III and 10, 20, or 40 units of Micrococcal Nuclease (MNase, Worthington) for 10 min at 37 °C. Reactions were quenched with 150 μL [5% (w/v) SDS, 50 mM EDTA] and incubated with 0.2 mg Proteinase K overnight at 65 °C. DNA was purified by phenol/chloroform extraction, ethanol precipitated, and resuspended in 10 μg RNAse A in 60 μL (1×) NEB Buffer 2. Reactions with desired digestion extents (eg 50% mononucleosomes, 80% mononucleosomes) were determined by 2% agarose gel electrophoresis and mononucleosomes were gel-purified for library preparation. Mononucleosomal DNA was resuspended in 50 μL of 1× NEB Buffer 3 and treated with 10 units alkaline phosphatase (CIP, New England Biolabs) for 1 h at 37 °C. DNA was purified by MinElute PCR Purification kit (Qiagen) and sequencing libraries were constructed using the Illumina TruSeq RNA Sample Prep Kit v2 beginning at the End Repair step according to the manufacturer protocol [8].

Paired end sequencing (50 cycles) was performed with an Illumina HiSeq 2500 on high output mode. Base calling was performed with Illumina CASAVA software and reads were mapped to the S. cerevisiae reference genome (Saccharomyces_cerevisiae.EF4.65.dna.toplevel.fa) with Bowtie2. Aligned reads were filtered for properly paired primary alignments with SAMtools (~f3 – F 256). For paired reads with an insert size 100 < insert < 200, the midpoint of each read was calculated as a single dyad position (assuming MNase digestion is roughly equal on each side of the nucleosome). Raw dyad counts were piled up into a single coverage file, then data sets were normalized such that the average dyad count at any genomic position (excluding the rDNA locus) was 1.0. The final processed data files are normalized dyad coverage file with an average per-base count of 1. For analysis at transcription start sites (TSS), data within 1 kb of annotated TSS [7] were binned while preserving gene orientation to give average nucleosome dyad signal at a given TSS. For calling of + 1 or − 1 nucleosomes, a 140 bp sliding window was passed through the genome to find local maxima within 100 base pairs of annotated TSS with a threshold average value of 1.0. These conservative criteria identified + 1 and − 1 nucleosomes for...
80% of transcription start sites. Nucleosome depleted region (NDR) ranking analysis was performed by calculating the difference in signal between Q cells and log cells from TSS-200 to TSS for each annotated transcription start site.

2.1.4. ChIP-seq

Cells were grown to the appropriate cell cycle stage (~70 OD_{600} units log cells, ~125 OD_{600} units DS cells, or ~250 OD_{600} units purified Q cells) and crosslinked with 1% formaldehyde for 20 min at 30 °C before quenching with a final concentration of 125 mM glycine. Crosslinked cells were pelleted and washed twice with ice cold TBS (150 mM NaCl, 20 mM Tris pH 7.5). Pellets were resuspended in 300 μL [100 mM Tris pH 8.0, 20% (v/v) glycerol] with 300 μL glass beads and subject to 5 min of bead beating (log, DS) or 2 rounds of 5 min bead beating (Q cells). Lysate was combined with 1 mL FA buffer [50 mM HEPES pH 7.6, 150 mM NaCl, 1 mM EDTA, 1% (v/v) Triton X100, 0.1% (w/v) sodium deoxycholate] and centrifuged 16,000 × g for 5 min. Pellet was resuspended in 1 mL FA buffer and sonicated with a Bioruptor sonication bath (Diagenode UCD-200) in a recirculating ice-water bath for 30 min (max output, 30 s on/30 s off). Sheared chromatin was centrifuged 16,000 × g for 30 min and the supernatant was used for chromatin immunoprecipitation. Chromatin immunoprecipitation was performed with 150 μL of sonicated chromatin with 30 μL Protein G-coupled magnetic beads conjugated to the appropriate antibody as detailed in [8]. For each chromatin sample, 50 μL of chromatin was separately sequenced without immunoprecipitation as input DNA.

Yield of samples was determined using the Quant-It picogreen dsDNA Assay (Life Technologies). Sequencing libraries were constructed using the NuGEN Ovation Ultralow System per the manufacturer protocol.

Single-end sequencing (50 cycles) was performed on an Illumina HiSeq 2500 on high output mode. Base calling was performed with Illumina CASAVA software and reads were mapped to the S. cerevisiae reference genome (Saccharomyces_cerevisiae.EF4.65.dna.toplevel.fa) using Bowtie2 on “very sensitive” mode. Aligned reads were filtered for primary alignments with SAMtools (− F 256) and converted to per base coverage files. Data were normalized such that the average signal at a genomic location was 1.0, then the ratio of normalized ChIP sample to sample-matched Input was calculated across the genome.

2.1.5. Data analysis

For analyzing features at predicted transcription factor (TF) binding sites, we obtained TF consensus sequences from the JASPAR CORE fungi database [5] (http://jaspar.genereg.net/). Instances of intergenic motifs (excluding the rDNA locus and mitochondrial genome) were obtained using the pattern matching tool from SGD (http://www.yeastgenome.org/cgi-bin/PATMATCH/nph-patmatch). Normalized coverage file data were binned within 500 bp of individual motifs and normalized to the number of instances for each motif to give the average signal (nucleosome dyads or ChIP) at a given motif. Motifs were then ranked based on difference between log and Q cells in within the 500 bp window (Fig. 1). Correlations between transcriptional shutoff, histone density, histone acetylation, and Rpd3 binding at transcription start sites were
determined as follows: average acetylation ChIP signal or Rpd3 ChIP signal from (TSS − 300) to (TSS + 400) was calculated or average H3 ChIP signal from (TSS − 300) to (TSS + 200) was calculated for each TSS. Difference in transcription, H3, acetylation, or Rpd3 binding was determined for different growth conditions and Pearson correlations between relevant variables were determined using R software.

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