Figure outline / packet

Measuring the depth and breadth of Fkh1-FHA-dependent chromatin structure and replication function at replication origins

2022-11-07

Materials and methods

SORT-seq

Cutluring and sorting

Followed protocol as outlined in [@Batakou2020] with slight modifications. Briefly, 30 mL YPD cultures grown to 0.5 ODs/mL at 30°C . Harvested and fixed yeast in 70% EtOH, storing overnight at 4°C .

DNA extraction, library prep, and sequencing

Data processing and analysis

FKKH1 motif mapping

MNase-seq

Benchwork

DNA extraction, library prep, and sequencing

Data processing and analysis

Results

The loss of functional Fkh1FHA impacts replication at up to 25% of yeast origins.

Figure 1 (establishing the big Q and our SORT-seq experiment)

Take-home: Qualitatively and quantitatively, the loss of a functional Fkh1FHA changes replication dynamics at replication origins and perhaps even at termination events). Thus, more origins than the our original target PC cohort are affected by fkh1-R80A.

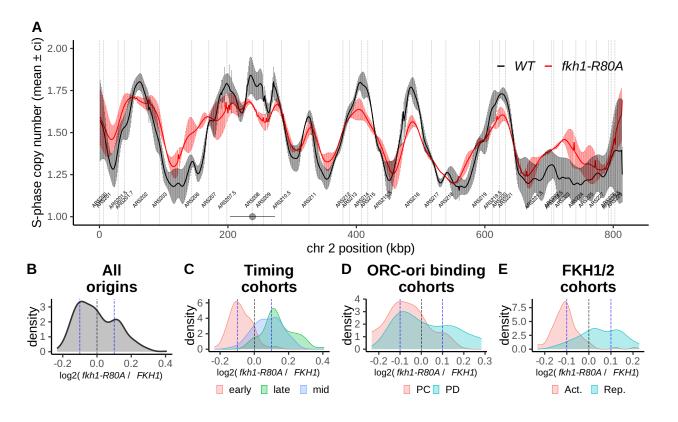


Figure 1: Measuring replication in multiple FKH1 (3) and fkh1-R80A (2) meiotic independents in unperturbed yeast cultures. A. S-phase copy numbers as means and 95% confidence intervals measured across chromosome 2 in FKH1 (black) and fkh1-R80A (red) cells. Sites of origins are labeled, with the position of the T-rich start of the ORC site indicated by vertical lines. The a 30 kbp window centered on the centromere is marked by a horizontal line with a central grey dot denoting the centromeric sequence. B-E. Smoothed density estimates of fkh1-R80A / FKH1 S-phase copy number ratios for all confirmed origins (n = 392) and then selected cohorts defined by Trep (C - [@Yabuki2002]), ORC-origin binding cohorts (D - [@Hoggard2013]), and FKH1/2-regulation (E - [@Knott2012]).

Supplemental 1 (all chromosomal scans)

Figure 2 (CEN sequence and CEN origins are FHA-SORT-dependent / fkh1-R80A-SORT-negative)

Take-home: In contrast to a previous study that measured replication through BrdU-chip (Knott et al, 2012), our study suggests that normal replication through yeast centromeres is dependent on a functional Fkh1FHA.

- a) Example scans
- b) Illustration of how CEN ratios were determined.
- c) Distribution of CEN ratios for all sixteen cens.

Supplemental 2 (all cen scans)

Figure 3 (FKH1 match frequency by SORT-seq cohorts)

Take-home: In contrast to the PC origins sensitive to fkh1-R80A in the NAR paper, fkh1-R80A-negative / FHA-SORT-dependent origins defined by the SORT-seq are characterized by a FKH1 match that overlaps the ORC binding site

Figure 4 (MNase titration reveals global change in accessibility in fkh1-R80A)

Data Tk May save for reviewers or as a "compromise" for resubmission

Thought(s): A figure to address the "extent" of chromatin accessibility governed by Fkh1-FHA. If no change between FKH1* and fkh1-R80A, then we will reserve for supplemental. We will start with asynchronous cells and then move to G1-arrested cells. If chromatin accessibility parallels the MNase-seq data, we may see opposing phenotypes between asynchronous and G1-arrested cells.*

Figure 5 (MNase-seq experiment validation)

Take-home: We present data that suggest the Fkh1-FHA regulates chromatin structure as measured by MNase-seq experiments to such an extent that changes in MNase protection is evidenced for all confirmed origins and is G1-specific.

- a) heats comparing confirmed to likely origins
- b) Quantification of heats
- c) Heats by region
- d) Quantification of heat regions.

Supplemental 3

Comparison of experimental replicates for the two genotypes...

Figure 6 (MNase-seq experiment with cohorts of interest)

Take-home: As expected, Fkh1-FHA-dependent MNase protection is most evident in G1-phase and at FHA-sort-positive origins relative to FHA-sort-negative origins.

As in figure 5, but with fkh1-R80A-SORT-negative and fkh1-R80A-SORT-positive.

Supplemental 4

Comparison of experimental replicates for the two genotypes...

Figure 7 (MNase-seq experiment with single locus plots & FKH1-matches)

Analysis Tk