A Cis-Acting tRNA Gene Imposes the Cell Cycle Progression Requirement for Establishing Silencing at the HMR Locus in Yeast

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A

YSH811 GAL-SIR3

cycling

nocodazole blocked, uninduced

nocodazole blocked, following five hours of galactose induction
B

**YSH829 sir3-8ts**

- Cycling (23°C)

- Nocodazole blocked, shifted to 37°C for one hour

- Nocodazole blocked, shifted from 37°C to 23°C for six hours
C

YSH829 sir3-8ts

cycling (23°C)

Blocked with α-factor and hydroxyurea; at 37°C for one hour

Blocked with α-factor and hydroxyurea; at 37°C for one hour, then shifted to 23°C for five hours
FIGURE S1.—FACS analysis of cell cultures. FACS analysis was carried out to confirm cell-cycle blocks described in the main text. Samples were withdrawn at the indicated time points throughout the experiment, and DNA content was measured by propidium iodide staining of DNA followed by flow cytometry. The y axis denotes cell count and the x axis represents DNA content. In all cases cells were collected for FACS at the start of the experiment (log phase), after cell cycle blocks were achieved (G1, G2/M), and at the final time points reported in the main text figures following galactose addition or temperature shift. A) Strain YSH811 was grown to log phase (first panel), when nocodazole was added; following sufficient time for >90% of the cells to arrest in the cell cycle (second panel) galactose was added to the culture for five hours to induce Sir3 production (third panel). B) Strain YSH829 was grown to log phase at 23°C (first panel), blocked at G2/M with nocodazole and shifted to 37°C for one hour (second panel), then shifted back to 23°C for six hours (third panel). C) Strain YSH829 was grown to log phase at 23°C (first panel), then arrested at G1/S with α-factor plus hydroxyurea and shifted to 37°C for one hour (second panel), then shifted back to 23°C for an additional five hours (third panel). D) Strain YSH967 was grown to log phase at 23°C (first panel), shifted to 37°C for one hour, and then shifted back to 23°C for six hours (second panel).
**FIGURE S2.**—Sir3 protein levels in *GAL-SIR3* strains. A) Strains YSH831 and YSH832 were grown to steady state in YPraffinose media with or without galactose. The endogenous *SIR3* and *pGAL-SIR3* are myc-tagged in these strains. Sir3 protein levels were monitored by western blot analysis. Tubulin was used as an internal control. Levels of Sir3 protein were quantified by determining the ratio of the Sir3p band to the control tubulin band. Values are given below each lane and expressed relative to the appropriate uninduced (no galactose) control containing endogenous *SIR3*. A Coomassie stained gel is shown in the upper panel of Figure 2A. B) Cumulative results from three independent determinations are shown.
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Establishment of silencing at *HMR*

![Graph showing the ratio of *a1*/*ACT1* over time for different conditions.]

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*ACT1*
**Figure S3.** — The establishment of silencing at the mating type loci in Sir3<sup>ts</sup> strains. A) Kinetics of repression at the HMR locus. A culture of strain YSH829 was grown to log phase in YPD media at the permissive temperature (PT, 23°C). Cells were harvested at time 0 and half the culture shifted to the non-permissive temperature (NPT, 37°C) for one hour. Cells were then shifted back to the permissive temperature (PT, 23°C) and cells were harvested every hour for five hours. RNA was collected from the samples at the indicated time points and the levels of α1 and ACT1 message were measured by RT-PCR. B) Kinetics of repression at the HML locus. α1 message was measured from RNA obtained from the same cell cultures described in panel A.