The SAGA Subunit Ada2 Functions in Transcriptional Silencing

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FIG. S1. The Ada2 silencing function acts through SAGA, not SLIK/SALSA. Diagram of C-terminal spt7 mutant alleles (78) tested for telomeric silencing. spt7-200 lacks amino acids (aa) 1125-1150 (-200) containing the C-terminal proteolytic cleavage site (P); spt7-300 lacks aa 1151-1180 (-300) containing a domain mediating Spt8-association (S); spt7-400 lacks both these regions (-400). Wild type, spt7-200 (LPY 10057), spt7-300 (LPY 10328), spt7-400 (LPY 10061) strains harboring a telomeric URA3 gene were plated on synthetic complete medium (growth) or synthetic complete medium containing 5-FOA (silencing) and assayed as in Fig. 1A. The spt7-200 mutant fails to make the proteolytic cleavage that creates the truncated form of Spt7 and is severely depleted for SLIK/SALSA (78). The spt7-200 strain did not have a telomeric silencing defect, indicating that SLIK/SALSA was not required for telomeric silencing. The spt7-300 mutant has predominantly SAGA-related complexes lacking Spt8 that elute from a Mono-Q column in the same fractions as SLIK/SALSA (78). This mutant had an intermediate silencing defect. The spt7-400 mutant lacks both regions and is depleted for SAGA (78). This strain was the most severely defective for telomeric silencing. A gcns5Δ spt7-400 double mutant (LPY 10258) was also defective for silencing, further supporting the conclusion that the SLIK/SALSA complex was not causing this defect.

FIG. S2. Deletion of ADA2 does not cause a significant mating defect. WT, ada2Δ and control strains containing the single mutants sir2Δ, sir1Δ, sas2Δ, gcns5Δ, or spt8Δ (upper two panels) and double mutant combinations of these genes (lower panels) are shown as mating patches in MATα and MATα strains as described in Fig. 2A. Strain numbers are listed in order of their presentation in the panels in Supplemental Table 1.
FIG. S3. Ada2 occupancy at the telomere is not dependent on Sir2 or Sir3. Fold increase in Ada2 occupancy (dark bar) as a function of distance from the end of telomere VIR (in kb) analyzed by ChIP in wild type (LPY 10885), sir2Δ (dark grey bar, LPY 11044) and sir3Δ (light gray bar, LPY 11049) cells. Occupancy of Ada2 in wild type cells for each location surveyed was assigned a value of 1.0 and fold changes in mutant cells were calculated as in Materials and Methods.

FIG. S4. Ada2 and Sas2 promote telomeric silencing by similar but distinct mechanisms (A) Increased gene dosage of Sir3 selectively rescued the ada2Δ telomeric silencing defect. Cells were assayed as described in Fig. 1A. An ada2Δ or sas2Δ strain was transformed with a high copy plasmid expressing the protein indicated in the middle column, and plated on selective medium (growth) or medium containing 5-FOA (silencing). (B) Sir1 targeted to the telomere rescues the ada2Δ and sas2Δ silencing defect. Top: diagram of GBD-Sir1 fusion protein targeted to the Gal4 UASGal site adjacent to a telomereic URA3 reporter gene (15). Cells were assayed as described in Fig. 1A. Cells were plated on selective medium (growth) or medium containing 5-FOA (silencing). Strains are labeled according to gene deletion present followed by the plasmid transformed into the strain (GBD or GBD-Sir1). GBD is a control plasmid expressing only the Gal4 DNA binding domain (aa 1-147). Strain numbers are listed in order of their presentation in the panels in Supplemental Table 1.

Fig S5. Quantitation of RNA expressed from the subtelomeric genes HXK1 (15 kb from Tel VIR) and HXK2 (25 kb from TelVIII) by RT-PCR in glucose-grown wild type (WT, light grey bar, LPY 8726), ada2Δ (dark grey bar, LPY 8723) or sas2Δ (hatched bar, LPY 8712) strains. RNA abundance in wild type cells was assigned a value of 1.0, from which fold changes in mutant cells was calculated as described in Materials and Methods.
Fig S6. Ada2 telomeric occupancy at Tel IXR mimics that of VIR. Occupancy of Ada2 (dark grey bar, LPY 10885) and Gcn5 (light grey bar, LPY 12169) was calculated as fold increase over untagged samples at Tel IXR under glucose conditions (Glc; upper panel) or under inducing conditions, galactose as the sole carbon source (Gal; lower panel) and plotted as a function of distance from the end of telomere IXR. Primers used are listed in Supp. Table 2.

Fig. S7 (A) Telomeric H3 K14 acetylation levels are similar in wild type cells grown in glucose or galactose. Wild type (lighter boxes, LPY 8726) or sir3Δ (dark box, LPY 10) strains were grown in glucose (Glc, light grey box) or induced with galactose (Gal, dark grey box) and assayed by ChIP for telomeric H3 K14 acetylation levels. The level of H3 K14 acetylation in wild type cells grown in glucose was assigned a value of 1.0, from which fold changes in telomeric H3 K14 acetylation was calculated for wild type cells grown in glucose or galactose, and for sir3Δ cells grown in glucose. Fold changes in H3 K14 acetylation were plotted as a function of distance from the end of telomere VIR as in Fig. 5.

(B) Deletion of GCN5 does not suppress the galactose induced telomeric silencing defect. Cells were assayed as described in Fig. 1A. Cells were plated on synthetic complete medium containing 2% galactose (growth) or synthetic complete medium containing 2% galactose and 5-FOA (silencing). Note that there is a range of severity in the telomeric silencing defect in wild type cells grown on galactose. The silencing assay shown here illustrates a more severe silencing defect than in Fig. 7E. Although more severe, loss of GCN5 does not relieve the telomeric silencing defect upon growth in galactose, either as the sole mutation or in combination with an ada2 null mutation. An ada2Δ gcn5Δ control strain (LPY 8833) that is ura3- was included as a control for growth on 5FOA medium. Other strains plated to assess telomeric silencing that contain a telomeric URA3 reporter gene (labeled Tel URA3 at left) are: wild type (LPY 8037), ada2Δ (LPY 8039), gcn5Δ (LPY 8719), and ada2Δ gcn5Δ (LPY 8805).
WT
spt7-400
WT
spt7-200
WT
spt7-300
WT
spt7-400
gcn5Δ spt7-400

growth
silencing
Distance from telo (kb)

Fold Increase Ada2p

Ada2p WT
Ada2p sir2Δ
Ada2p sir3Δ
A. \(\text{ada2}\Delta\)

- Vector
- Sir1p
- Sir2p
- Sir3p
- Sir4p
- Sas2p
- Ada2p

\[\text{growth} \quad \text{silencing}\]

B. \(\text{sas2}\Delta\)

- Vector
- Sir1p
- Sir2p
- Sir3p
- Sir4p
- Sas2p
- Ada2p

\[\text{growth} \quad \text{silencing}\]

B. \(\text{sas2}\Delta\)

- GBD-Sir1p
- UAS
- GAL
- URA3
- T

\[\text{growth} \quad \text{silencing}\]
WT  ada2Δ  sas2Δ

fold change mRNA

1.0

HXK1

Tel VIR

1.2

HXK2

Tel VIII

Tel VIR  Tel VIII

HXK1

Tel

15 kb

Tel

25 kb

HXK2
Fold increase Ada2p, Gcn5p

Distance from Tel IXR (kb)

Glc

Fold increase Ada2p, Gcn5p

Distance from Tel IXR (kb)

Gal
A.

![Graph showing H3 K14 Ac levels over different glucose concentrations and distances from telo (kb).](image)

B.

**SC-Gal**

- **TEL URA3**
- **ura3- control**

**Gal 5-FOA**

- **WT**
- **ada2Δ**
- **gcn5Δ**
- **ada2Δ gcn5Δ**

**Silencing**

**Growth**
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Supplemental Table 2

Oligonucleotides used in ChIP and RT-PCR analysis:

Tel VIR:
200 bp:
5’ AAATGGCAAGGGTAAAAACCAG 3’
5’ TCGGATCCTACACACCGGAAAT 3’

1 kb AND YFR057W:
5’ GGACCTACTAGTGTCATAGTAAGTG 3’
5’ CTCTAACTAATTGGATCCTTTACTCG 3’

5 kb:
5’ GGCTAGAAAAGCTTCAACATGGCCTTAC 3’
5’ CTCCAGCCTGCTAAGACAAGCTATAG 3’

20 kb:
5’ GAATGTAACGTACTATTGGACAAGATATTC 3’
5’ GAACCTCCATTAAGATAGCAAAATTCG 3’

GAL1 core promoter region:
5’ ATAGGATATAATGCGATTAGTTTTTTAGCCTT 3’
5’ GAAAATGTTTGAAGATATTAGTAAAGTGTTATGCA 3’

rDNA array (25S):
5’ TGCGACGTAAGCAGGATG 3’
5’ CTGGCTTACCCCTATTCAGG 3’

rDNA array (5S):
5’ CATGGAGCAGTTTTTTCCGC 3’
5’ TACAAGCACTCATGTTTGCCG 3’

ACT1:
5’ GGTGGTTCTATCTTGAGCCTTC 3’
5’ ATGGACACTTTTGGTCTAT 3’

HXK1:
5’ GACCTCCGTTACTACTTTGGAAGG 3’
5’ CCTGAGTTCTGCTGGGTAGG 3’

HXK2:
5’ GCTAGAGCCTGACTAGATTGTCCG 3’
5’ GTGAGGTTTTGAGCTCCAGCCG 3’

TEL IXR:
2 kb:
5’ GCAGGCTTCTGGAGGAGGCTGTC 3’
5’ GTTTTTCACAGGCAATCTACGC 3’

4 kb:
5’ TACGCACCTCGCAGATTTG 3’
5’ TGTAAGCCAGCATAACAGCTTC 3’

9 kb:
5’ ATTTGCTACCTGCTGCTCA 3’
5’ GAAGAGTAGCTTGAGAGG 3’