Sas4 and Sas5 Are Required for the Histone Acetyltransferase Activity of Sas2 in the SAS Complex*

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The SAS2 gene is involved in transcriptional silencing in Saccharomyces cerevisiae. Based on its primary sequence, the Sas2 protein is predicted to be a member of the MYST family of histone acetyltransferases (HATs). Sas2 forms a complex with Sas4 and Sas5, which are required for its silencing function. Here we show that recombinant Sas2 has HAT activity that absolutely requires Sas4 and is stimulated by Sas5. The recombinant SAS complex acetylates H4 lysine 16 and H3 lysine 14.

Furthermore, a purified SAS complex from yeast shows similar activity and specificity. In contrast to other MYST HATs, neither the recombinant nor the native SAS complex acetylated nucleosomal histones under conditions that were optimum for acetylating free histones. Finally, although the SAS subunits interact genetically and physically with Asf1, a histone deposition factor, association of H3 and H4 with Asf1 blocks their acetylation by the SAS complex, raising the possibility that the SAS HAT complex may acetylate free histones prior to their deposition onto DNA by Asf1 or CAF-I.

Sas2 encodes a putative member of the MYST family of histone acetyltransferases (HATs), which also includes human MOZ, MORF, TIP60, and HBO1; Drosophila MOF and Chm; and S. cerevisiae Sas3 and Esa1. Although many of these proteins have been shown to possess HAT activity (8), no enzymatic activity had previously been detected for Sas2. However, recent work suggests that acetyltransferase activity may be important for Sas2 function. We and others (5, 7) have shown that the conserved acetyl-CoA binding domain of Sas2 is required for HML and telomeric silencing, as mutations in this motif cause the same silencing defects as does deletion of SAS2 (5, 7). Furthermore, a point mutation (K16R) in the histone H4 N-terminal tail phenocopies the effects of sas2 mutants on silencing (5). The H4-K16R mutation causes complete loss of telomeric silencing and improves silencing at a mutated HMR to the same extent as deletion of SAS2. Combination of a SAS2 gene deletion with the H4-K16R mutation leads to no additional increase in HMR silencing (5). Very recently, two groups (9, 10) have demonstrated that lysine 16 of histone H4 is hypoacetylated in sas2 mutants. Together, these data are consistent with the possibility that histone H4 lysine 16 is a direct substrate for acetylation by Sas2.

The SAS complex has also been functionally linked to the histone deposition proteins Cac1 and Asf1. Sas4 was isolated in a two-hybrid screen with Asf1 as bait (11), and Cac1 was isolated in a two-hybrid screen with Sas2 as bait (5). Co-immunoprecipitation analyses further showed that the SAS complex associates with both Asf1 (7) and Cac1 in yeast (5). Cac1 is a subunit of the yeast CAF-I complex (12). Both Asf1 and CAF-I bind to histones H3 and H4 and are suggested to function in non-overlapping pathways for histone deposition and chromat in assembly (13–18). asf1 mutants display the same effects on silencing at HML and HMR as sas2, 4, or 5 mutants (5, 7), but unlike sas mutants, asf1 mutants have little or no defect in telomeric silencing (19). cac mutants, like sas mutants, have defects in telomeric and HM silencing although the magnitudes of these defects differ (12, 20). Furthermore, cac mutations cause silencing defects at HML and HMR that are only partially epistatic with sas mutations (5). Together, these data suggest that the interactions among the SAS complex, CAF-I, and Asf1 may be complex and locus-specific.

In this study we show that co-expression of Sas2, Sas4, and Sas5 in Escherichia coli leads to formation of a stable SAS complex, which is required for histone acetyltransferase activity of Sas2.

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1 The abbreviations used are: HAT, histone acetyltransferase; IPTG, isopropyl β-D-thiogalactoside; TAP, tandem affinity purification; CBP, calmodulin binding protein.
complex that acetylates histones. Sas4 is essential for the acetyltransferase activity of Sas2, and Sas5 is important. The preferred in vitro substrates for the recombinant SAS complex are lysine 16 of histone H4 and lysine 14 of histone H3. In contrast to the Sas3-containing NuA3 and the Esa1-containing NuA4 complexes, the SAS complex shows no activity toward nucleosomes or (H3-H4) tetramers deposited onto DNA under conditions of our assays. We have also purified enzymatically active SAS complex from yeast, which contains Sas2, Sas4, and Sas5. This native enzyme shows the same substrate specificity as the recombinant enzyme, including the preference for free histones.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains—**Yeast strains used in this study were derived from YJW228 (SAS4–13MyclchanMX6) described previously (7). Strains YJLA58 and YJW495 were constructed by integration into YJW228 of the sequences encoding the tandem affinity purification (TAP) tag immediately before the stop codon of the SAS2 and SAS5 genes, respectively, as described elsewhere (21). Standard yeast manipulations were performed as described (22).

**Plasmids—**Plasmids for expression of Sas proteins in *E. coli* were created as follows. pAS131 (Sas2-His6) was made by PCR amplification of the SAS2 open reading frame (ORF) from yeast genomic DNA. The primers were designed so that the SAS2 sequence contained a 5′ NcoI site and a 3′ EcoRI site. The PCR product was cloned into pET28b (Novagen) so that the SAS2 was under control of an inducible T7 promoter and was in-frame with sequences for a C-terminal Hisα tag. pDB16 (sas2m1–His6) was made exactly as described for pAS131 except that pS139 was used for the template for the PCR reaction. pS139 was previously described (7) and encodes a mutated version of Sas2 in which amino acids 219–221 are changed to alanines. pDB5 (Sas4) was made by cloning a PCR fragment containing the SAS4 gene with a 5′ NcoI site and a 3′ BamHI site into pET22b. pDB6 (Sas5) was made by cloning a PCR fragment containing the SAS5 ORF with a 5′ NcoI site and a 3′ BamHI site into pET22b. pDB7 (Sas4,Sas5) was made by cloning a BglII–BamHI fragment containing SAS4 from pDB5 into the BglII site of pDB6. Both SAS4 and SAS5 are transcribed in the same direction, each under control of a separate T7 promoter. PCR was used to amplify a fragment from pAS131, which contained SAS2-His6, the T7 promoter and terminator, and SpH1 restriction sites on each end. The fragment was cloned into the SpH1 site of pDB7 to make pAS132 (Sas2-His6,Sas4,Sas5), into pDB5 to make pDB18 (Sas2-His6,Sas4), and into pDB6 to make pDB17 (Sas2-His6,Sas5). A similar SpH1 fragment was made, using pDB16 as template and cloned into pDB7 to make pAS134 (sas2m1–His6,Sas4,Sas5). All coding regions were sequenced to check for PCR errors.

**Protein Expression and Purification in Yeast—**Sas proteins were expressed in *E. coli* strain BL21 (DE3) codon plus with 0.4 mM IPTG. The proteins were purified from 100-ml cultures with 1 mM IPTG. Protein was purified as described above for the SAS complex that acetylates histones and, recombinant yeast core histones and 40 μM H4 N-terminal peptides. The amount of enzyme used was 1% of that obtained from a 12-liter preparation (5% of that shown on the silver-stained gel in Fig. 6A). Reactions were incubated at 30 °C for 45 min and then spotted onto Whatman paper before air-dried. The dried papers were washed three times for 5 min each with 50 mM sodium carbonate, pH 9.0 and once with acetone. Radioactivity was quantitated in a liquid scintillation counter.

**SDS-PAGE of Acetylated Histones—**For the experiment in Fig. 3A, chicken histones were acetylated with SAS complexes or Esa1 as described above except that the final concentration of histones was 0.4 mg/ml. For the experiment in Fig. 6, reactions were carried out exactly as described above for the native SAS enzyme. Reactions were terminated by addition of 5 μl of 5× protein loading dye. Samples were heated to 100 °C for 5 min, centrifuged at 16,000 × g for 2 min, and resolved on 15% SDS-polyacrylamide gels. Proteins were stained with Coomassie Blue, destained, saturated with ENHANCE (PerkinElmer Life Sciences), dried under vacuum, and exposed to film.

For Fig. 5, reactions were performed with 150 nCi of [14C]acetyl coenzyme A (57 μCi/mmol). Reactions were performed in a 20-μl reaction volume in the same buffer described above, plus 10 mM sodium butyrate and 1 mM phenylmethylsulfonyl fluoride. Reactions were assembled on ice, incubated at 30 °C for 30 min, and stopped with the addition of 5 μl of 5× SDS-PAGE loading buffer. Reactions were heated at 100 °C for 1 min and loaded onto 15% SDS-PAGE gels, which were silver-stained, photographed, treated with 1 M sodium salicylate/30% MeOH, dried, and subjected to fluorography.

**Amino Acid Sequence Analysis—**HAT assays were done as described above using the recombinant SAS complex and either the H3 or H4 peptide. Four reactions were pooled, applied to ProSorb membranes (PerkinElmer Life Sciences) and processed for sequencing according to instructions from the manufacturer. The samples were subjected to Edman degradation. Eluates from each cycle were collected, dried, resuspended in 50 mM sodium acetate, pH 5.2, and counted in a liquid scintillation counter.

**RESULTS**

Recombinant preparations of most MYST family proteins identified to date function as HATs in vitro in the absence of other factors (8). Although Sas2 displays a high degree of homology to MYST family proteins, we and others (5) have been unable to detect HAT activity using recombinant Sas2. Because Sas2 is part of a complex with Sas4 and Sas5 in vitro, and because mutation of SAS4 or SAS5 causes the same phenotype as mutation of SAS2, we reasoned that Sas2 might require these additional subunits for activity. Furthermore, Sas2 expressed in *E. coli* is to a large extent insoluble (data not shown). It has been shown that, in some cases, co-expression of several subunits of a multiprotein complex can increase the solubility of the individual proteins (28, 29). Therefore, we designed a plasmid for co-expression of SAS2, SAS4, and SAS5 in *E. coli*. Plasmid pAS132 contains SAS2 (including sequences for a C-terminal Hisα tag), SAS4 and SAS5, with each gene under control of an inducible T7 promoter. Upon induction, all three proteins were expressed, although only a small fraction of Sas2-His6 and Sas5 was soluble (data not shown). When the soluble fraction was purified using nickel-affinity chromatography by virtue of the His tag on Sas2, substantial amounts of Sas4 and Sas5 coeluted with Sas2, indicating that the proteins formed a complex in *E. coli* (Fig. 1B). Remarkably, this complex had robust HAT activity using chicken histones as substrate (Fig. 1A). In contrast, equivalent amounts of recombinant Sas2-His6 expressed and purified in the same manner in the absence of Sas4 and 5 displayed no activity (Fig. 1, A and B).

To determine whether all three Sas proteins were required for HAT activity, or whether the activity resulted from either Sas2, Sas2,5 subcomplexes, additional expression plasmids were constructed that encoded Sas2-Hisα and either Sas4 or Sas5. In both cases, equivalent amounts of the Sas4 or Sas5 proteins copurified with Sas2-Hisα as when all three proteins were present.
were expressed in the same cell (Fig. 1D). These data demonstrate that both Sas4 and Sas5 can bind directly to Sas2. When these complexes were tested in a HAT assay using chicken histones as substrate, the Sas2,4 complex had activity but the Sas2,5 complex did not (Fig. 1C). However, the specific activity of the Sas2,4,5 complex was consistently 2–3 fold lower than that of the Sas2,4,5 complex, even though these complexes contained comparable amounts of Sas2 and Sas4 proteins (Fig. 1, C and D). Both the Sas2,4,5 complex and the Sas2,4 complex were also able to acetylate a peptide that corresponds to the first 28 amino acids of histone H4 as substrate. Sas2-His6 and associated proteins were purified from E. coli expressing only Sas2-His6; lane 2 is from E. coli in which Sas2-His6, Sas4, and Sas5 are co-expressed from the same plasmid. C. HAT assays as in A with either chicken histones or a synthetic peptide corresponding to the first 25 amino acids of histone H4 as substrate. Sas2-His6 and associated proteins were purified from E. coli cells containing a plasmid co-expressing Sas2-His6 and Sas4, or Sas2-His6 and Sas5, or all three proteins. D. Coomassie Blue-stained SDS-PAGE gel of the purified proteins used in C. Lane 1, Sas2-His6, Sas4; lane 2, Sas2-His6, Sas5; lane 3, Sas2-His6, Sas4, Sas5.

Fig. 1. The recombinant SAS complex has HAT activity. A. In vitro HAT assay using purified recombinant proteins from E. coli expressing either Sas2-His6 or Sas2-His6, Sas4, and Sas5 with chicken histones as substrate. The bar graph represents the amount of [3H]acetyl groups (cpm) transferred from [3H]acetyl-CoA to chicken histones for each enzyme. B. Coomassie Blue-stained SDS-PAGE gel of the purified proteins used in A showing positions of Sas2-His6, Sas4, and Sas5. Lane 1 has ~150 ng of purified protein from E. coli expressing only Sas2-His6; lane 2 is from E. coli in which Sas2-His6, Sas4, and Sas5 are co-expressed from the same plasmid. C. HAT assays as in A with either chicken histones or a synthetic peptide corresponding to the first 25 amino acids of histone H4 as substrate. Sas2-His6 and associated proteins were purified from E. coli cells containing a plasmid co-expressing Sas2-His6 and Sas4, or Sas2-His6 and Sas5, or all three proteins. D. Coomassie Blue-stained SDS-PAGE gel of the purified proteins used in C. Lane 1, Sas2-His6, Sas4; lane 2, Sas2-His6, Sas5; lane 3, Sas2-His6, Sas4, Sas5.

the silencing phenotypes caused by the sas2-M1 mutation correlate with the loss of HAT activity.

To further characterize the substrates of the SAS complex, we separated the products of a HAT reaction on a polyacrylamide gel to resolve the individual histones. Fluorography revealed that histone H4 is the major substrate for the SAS complex. However, histone H3 is also acetylated by this enzyme (Fig. 3A). This acetylation pattern is similar to that of Esa1, another member of the MYST family of acetyltransferases (Fig. 3A). The Sas2,4 complex showed a similar substrate specificity but had lower specific activity (data not shown). We conclude that the Sas5 subunit does not alter which histone polypeptides are acetylated by Sas2.

To determine which residues within the histone H3 and H4 tails are acetylated by the SAS complex, we used as substrates synthetic peptides corresponding to the first 28 amino acids of H4 or the first 21 amino acids of H3. Both peptides were efficiently acetylated when incubated with the SAS complex, but not with Sas2 alone (Figs. 1 and 2 and data not shown). In vitro 3H-acetylated peptides were subjected to N-terminal sequencing, and the amount of radioactivity in each residue was determined. We observed that the primary site of acetylation on histone H4 was lysine 16 and that the primary site of acetylation on the H3 peptide was lysine 14 (Fig. 3B).

Interestingly, when the products of the HAT assays using the wild-type SAS complex were analyzed on a protein gel, Sas2, as well as histones H3 and H4, was radioactively labeled (Fig. 3A). Because the labeling of Sas2 survived SDS-PAGE and because no radioactivity was incorporated into the sas2M1 mutant protein (Fig. 3A), the labeling most likely resulted from autoacetylation of Sas2 rather than non-covalent trapping of acetyl-CoA. Similar autoacetylation was observed for Esa1 (Fig. 3A), but not for Hat1, a histone acetyltransferase that is not a member of the MYST family (data not shown). Interestingly, the autoacetylation of Sas2 was dependent upon the presence of histones; when histones were omitted from the reaction, very little radioactivity was incorporated into Sas2 (data not shown). Whether this autoacetylation is intermolecular or intramolecular, and whether it is important for the function of the enzyme, is not known.

Most MYST family members studied to date exist in multiprotein complexes and interact with chromatin in order to acetylate histones (8). In S. cerevisiae, these include Sas3, a component of the NuA3 complex (30), and Esa1, the HAT for the NuA4 complex (31). Both the NuA3 and NuA4 complexes acetylate histones in a nucleosomal context. Therefore, we hypothesized that SAS would display a similar activity. Consistent with this idea, a Gal4-Sas2 fusion protein, when tethered...
via Gal4 binding sites placed at HMR, functioned as an effective barrier to the spread of silencing (32). This was proposed to result from Sas2-mediated histone acetylation counteracting Sir2-mediated histone deacetylation. These data suggested that when artificially tethered, Sas2 can acetylate histones in nucleosomes.

To test whether the SAS complex acetylates nucleosomal histones, nucleosomes and core histones derived from human HeLa cells were compared as substrates of the SAS complex. Strikingly, we detected no acetylation of nucleosomes using assay conditions that were optimum for acetylating free histones (Fig. 4). However, the SAS complex did acetylate free histones derived from these nucleosomes (Fig. 4), demonstrating that these HeLa histones were not already fully acetylated on histone H3 lysine 14 and H4 lysine 16. Furthermore, the Sas3-containing NuA3 complex, which acetylates H3 lysine 14 on nucleosomes, had robust activity using the HeLa nucleosomes as substrate (Fig. 4). We also purified nucleosomes from wild-type and Δsas2 yeast strains and tested those nucleosomes as substrates for the SAS complex. The assays were done using several different buffer conditions that are optimal for other HATs, with a salt range from 0–150 mM. The SAS complex showed no activity on nucleosomes from either strain (data not shown). In contrast, the Esa1-containing NuA4 complex was active on both (data not shown). We also tested whether SAS could acetylate histones H3 and H4 in the form of (H3/H4)2 tetramers deposited onto DNA, in the absence of H2A/H2B dimers. Again, no modification of the histones was observed, although the same preparation of histones used to form the tetramer-DNA complexes was efficiently acetylated (Fig. 5). Thus, under our reaction conditions, free histones H3 and H4, but not those deposited onto DNA, are substrates of the recombinant SAS complex in vitro.

Neither Esa1 nor Gcn5 acetylate nucleosomes in vitro unless they are part of a multiprotein complex (NuA4 for Esa1, Ref. 31 and SAGA or ADA for Gcn5, Ref. 33). Therefore, the inability of the recombinant SAS complex to acetylate nucleosomes may result because a targeting subunit is missing. To address this, we purified active SAS complex from yeast. We created strains in which either Sas2 or Sas5 contained at their C terminus a tag for TAP (21). This tag enabled us to purify native SAS complex consisting of Sas2, Sas4, and Sas5 (Fig. 6A). We tested these purified complexes for HAT activity using HeLa nucleosomes, HeLa core histones and recombinant yeast histones. Just as for the recombinant SAS complex, the native yeast complex could acetylate free histones, but not the same histones when incorporated into nucleosomes (Fig. 6B). The native complex, like the recombinant SAS complex, acetylated histones H3 and H4, with a stronger preference for H4 than for H3 (Fig. 6B).

To further characterize the specificity of the yeast enzyme, we used as substrates a series of peptides corresponding to amino acids 1–20 of the histone H4 N terminus. These peptides were either unacetylated or tri- or tetra-acetylated at lysines 5, 8, 12, and 16. Only peptides in which lysine 16 was not acetylated were substrates for the native SAS complex (Fig. 7). Therefore, under our reaction conditions the recombinant and native SAS complexes show similar substrate specificities; both acetylate free histone H4 at lysine 16, and to a lesser extent histone H3, and neither acetylates histones that are packaged into nucleosomes.

![Image](https://example.com/image.png)

**Fig. 3.** The recombinant SAS complex acetylates lysine 16 of histone H4 and lysine 14 of histone H3. A, products of HAT assays similar to those in Figs. 1 and 2 were separated by 15% SDS-PAGE to resolve the histones. The gels were stained with Coomassie Blue to visualize histones, and then dried and subjected to fluorography. The reaction shown in lane 1 contained the Sas2-His6,4,5 complex; lane 2 contained the sas2M1,4,5 complex; lane 3 had no enzyme; and lane 4 had recombinant Esa1. B, synthetic peptides corresponding to the first 28 amino acids of histone H4 or the first 21 amino acids of histone H3 were acetylated by the SAS complex in the presence of [3H]acetyl-CoA. The peptides were subjected to N-terminal sequencing, and the radioactivity in each fraction determined by scintillation counting.

**Fig. 4.** The recombinant SAS complex does not acetylate nucleosomes. HAT assays using 120 μg/ml HeLa nucleosomes or HeLa core histones as substrate were performed as described in the legend to Fig. 1 using Sas2-His6,4,5 or purified NuA3 complex.
Because of the phenotypic similarity of asf1 and sas2 mutants for HM silencing and the physical interactions between Asf1 and SAS subunits (5, 7), we tested the hypothesis that SAS acetylates histones that are bound to Asf1. In this experiment, Asf1/H3/H4 complexes were purified (14) and then used as substrate for recombinant SAS in a HAT assay. Surprisingly, we observed that association of histones with Asf1 efficiently blocked their acetylation by SAS (Fig. 5).

DISCUSSION

Sas2 was predicted to be a histone acetyltransferase because of its sequence similarity to members of the MYST family. However, until this report, no in vitro activity was detected either from recombinant Sas2 or from Sas2 purified from yeast. In this analysis, we show that recombinant Sas2 can acetylate histones, but only when associated with the Sas4 subunit. Although the in vitro specificity of other HATs is altered by associated proteins (8), this is the first case in which enzymatic activity of a histone acetyltransferase absolutely depends upon additional subunits. Furthermore, maximal activity of Sas2,4 required the Sas5 subunit. Sas5 may be required to help stabilize the complex, or to help in substrate recognition. Sas5 has homology to tf2f domain-containing proteins including yeast TAF2, which is a component of a number of transcription/chromatin remodeling complexes (34–36). Perhaps the tf2f domain of Sas5 is involved in histone binding. Because sas5 mutants are as defective in silencing as sas2 mutants, Sas5 may be crucial for HAT activity in cells in a manner not reflected in our in vitro assays.

Previous attempts by this group and others (5, 7) to purify enzymatically active SAS complex from yeast were unsuccessful, although the complexes that were purified contained Sas2, Sas4, and Sas5. In this report, we show that using the tandem affinity purification method (21) to isolate the SAS complex results in active enzyme. Perhaps the previously isolated complexes co-purified with an inhibitor, or the purification schemes used inactivated the enzyme. For all substrates tested, the recombinant enzyme and yeast native enzyme show similar substrate specificities. Both enzymes acetylate histone H4 and to a lesser extent, histone H3. Furthermore, both enzymes acetylate free histones, but not those in nucleosomes.

We showed that both the recombinant and native enzymes acetylate lysine 16 of histone H4. Previous genetic data showed that mutating histone H4 lysine 16 to a nonacetylatable residue (K16R) confers silencing defects similar to those of sas2 null cells (5) and that H4 lysine 16 is underacetylated in sas2 mutants (9, 10). Our data strongly suggest that these genetic effects are a direct consequence of acetylation of histone H4 lysine 16 by the SAS complex. Both the recombinant and native
enzyme complexes also acetylate histone H3 in vitro, and we determined that the recombinant enzyme that the target was lysine 14. It has not been determined whether histone H3 lysine 14 is also an in vivo substrate for the SAS complex. Acetylation of H3 lysine 14 is reduced in a sas2 mutant, but this effect may be indirect (10). Since the histone H4 K16R mutation causes the same reduction in silencing as deletion of SAS2 at telomeres and at HML (in a ΔafI strain) (5), it may be that acetylation of histone H3 lysine 14 by Sas2 either does not occur in vitro or is not important for silencing.

Almost all HAT enzyme complexes studied to date except for Hat1 acetylate both free and nucleosomal histones. However, neither the recombinant nor native SAS complex could acetylate histones in HeLa nucleosomes; whereas using the same assay conditions, both enzymes could acetylate free histones derived from these nucleosomes. Furthermore, the recombinant enzyme had no activity on nucleosomes purified from wild-type and sas2 mutant cells. We considered four possible explanations for these results. First, it is possible that a subunit required to target SAS to nucleosomal histones was lost during the purification of the enzyme from yeast. Second, SAS may only work on histones that have been previously modified in some way, and these modified histones are not present in the HeLa or yeast nucleosomes we used. If true, then this modification must only be required when histones are part of nucleosomes, since once released from the HeLa nucleosomes the HeLa histones were good substrates for the SAS enzyme. Third, acetylation of nucleosomes by SAS may require assay conditions, both enzymes could acetylate free histones in HeLa nucleosomes; whereas using the same assay conditions, neither the recombinant nor native SAS complex could acetylate histones in HeLa nucleosomes; whereas using the same assay conditions, both enzymes could acetylate free histones in HeLa nucleosomes. Furthermore, the recombinant enzyme had no activity on nucleosomes purified from wild-type and sas2 mutant cells. We considered four possible explanations for these results. First, it is possible that a subunit required to target SAS to nucleosomal histones was lost during the purification of the enzyme from yeast. Second, SAS may only work on histones that have been previously modified in some way, and these modified histones are not present in the HeLa or yeast nucleosomes we used. If true, then this modification must only be required when histones are part of nucleosomes, since once released from the HeLa nucleosomes the HeLa histones were good substrates for the SAS enzyme. Third, acetylation of nucleosomes by SAS may require assay conditions different from those that we used. A fourth explanation is that in vitro SAS does acetylate only free histones and not those in nucleosomes. Sas proteins physically interact with subunits of the Asf1 and CAF-I histone deposition factors. Furthermore, asf1 mutations and sas mutations cause similar effects on silencing at the HM loci, and cac mutants and sas mutants both display reduced telomeric silencing. Asf1 and CAF-I bind to free histone H3 and H4, and are believed to function in histone deposition. SAS may function in these two pathways to participate in the deposition process. However, we have demonstrated that recombinant SAS does not acetylate H3 or H4 when they are bound to DNA as tetramers or associated with Asf1. A model to explain the biochemical data and genetic interactions between SAS and Asf1 (and CAF-I) would be that SAS acetylates newly synthesized H3 and H4 and then passes these histones on to Asf1 (or CAF-I) for deposition onto chromatin. SAS has been implicated in formation of a barrier between silenced and unsilenced regions of the chromosome (32, 9, 10). Perhaps Asf1 and CAF-I function downstream of SAS in the formation of these barriers by depositing acetylated histones adjacent to regions of silent chromatin.

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SAS Complex Has HAT Activity
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