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Sir2 and the Acetyltransferase, Pat, Regulate the Archaeal Chromatin Protein, Alba*[S]

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The DNA binding affinity of Alba, a chromatin protein of the archaeon *Sulfolobus solfataricus* P2, is regulated by acetylation of lysine 16. Here we identify an acetyltransferase that specifically acetylates Alba on this residue. The effect of acetylation is to lower the affinity of Alba for DNA. Remarkably, the acetyltransferase is conserved not only in archaea but also in bacteria where it appears to play a role in metabolic regulation. Therefore, our data suggest that *S. solfataricus* has co-opted this bacterial regulatory system to generate a rudimentary form of chromatin regulation.

The process of packaging genomic DNA into cells presents problems in accessing the genetic material. In eukaryotic cells, the fundamental unit of chromatin is the nucleosome composed of roughly 150 base pairs of DNA wrapped around two copies of each of four histone proteins (1). The covalent modification of histones is a major feature of eukaryotic gene regulation, DNA repair, and DNA replication (2). More specifically, tails that extend from the central histone fold act as the primary sites for a range of modifications, including phosphorylation, ubiquitination, methylation, and acetylation (3). The reversible acetylation of lysine residues has been particularly well studied, and a range of histone acetyltransferases and deacetylases has been identified. Distinct histone acetyltransferases and deacetylases have defined substrate preferences, and the combinatorial modification of histone tails has been proposed to form a "histone code" (3).

Intriguingly, although some Archaea also possess histones (4), they do not possess the tail domains and no evidence has been found for post-translational modification of archaeal histones (5). Furthermore, members of the Crenarchaeal kingdom of Archaea, such as *Sulfolobus solfataricus*, do not possess histones (6). The best characterized *Sulfolobus* chromatin proteins are the Sul7d family and Alba (reviewed in Ref. 7). Interestingly, Sul7d proteins are differentially monomethylated on specific lysine residues (8). However, the consequences of this modification remain unclear and the identity of the enzyme responsible for methylation is currently unknown. More recently, Alba (formerly known as Sso10b (9, 10)) was found to be acetylated on lysine 16 (11), lowering the affinity of Alba for DNA. A *Sulfolobus* homolog of the conserved Sir2 NAD-dependent deacetylase was found to interact with, and deacetylate, Alba (11).

The Sir2 proteins (or sirtuins) form a family of broadly conserved deacetylases found in all three domains of life (12, 13). They have been demonstrated to deacetylate a range of substrates including histones, p53, and tubulin in eukaryotes (13). In the bacterium *Salmonella enterica*, a sirtuin, CobB, deacetylates and regulates acetyl-CoA synthetase (ACS)[1] (14). The enzyme responsible for acetylating ACS, Pat, has recently been identified (15).

In this work, we demonstrate that a *Sulfolobus* homolog of Pat specifically acetylates Alba on lysine 16 and lowers its DNA binding affinity. These results indicate that the Sir2/Pat pairing has been conserved in prokaryotic evolution. Furthermore, our results suggest that *Sulfolobus* has co-opted these bacterial metabolic regulators to generate a rudimentary form of chromatin modification system.

EXPERIMENTAL PROCEDURES

Identification of ssPat—The sequence of the final 95 amino acid residues of *S. enterica* Pat was used to perform a Blast search (16) of the *S. solfataricus* P2 genome sequence.

Cloning and Purification of ssPat—The SSO2813 open reading frame was amplified using the PCR with oligonucleotides Pat5, 5′-GGGATCC**CATATG**AATGACCAGATAAAGATAAG-3′, and Pat3, 5′-GAATT**CTCGAG**TGGGGCGGAGAAAGTTGCTAG-3′. The PCR introduced NdeI and XhoI restriction sites (*underlined* and in *boldface*) at the 5′ and 3′ ends of the open reading frame. The PCR product was digested with NdeI and XhoI and ligated into pET30a linearized with the same enzymes. The identity of the resultant pET30-Pat plasmid was confirmed by DNA sequencing. pET30-Pat expresses ssPat with a C-terminal hexahistidine tag. Rosetta cells (Novagen) were transformed with pET30-Pat and plated, and a 50-ml overnight culture grown of which 20 ml was used the following morning to inoculate 1 liter of fresh L broth was supplemented with kanamycin and chloramphenicol. Cells were grown shaking at 37 °C until $A_{600\,nm} = 0.6$, and then the expression of ssPat was induced with 1 mM isopropyl 1-thio-β-D-galactopyranoside for 4 h. Cells were harvested by centrifugation, resuspended in 25 ml of 20 mM Tris, pH 8.0, 300 mM NaCl (2× TBS), and lysed in an EmulsoFlex-C5 cell cracker (Glen Creston, Stanmore, United Kingdom). The lysate was clarified by centrifugation (35,000 × *g*, 30 min, 4 °C). The supernatant was heated to 75 °C for 30 min and then clarified by centrifugation. The heat-stable ssPat remained soluble and was purified by passage over a 2 ml of nickel-nitrilotriacetic acid-agarose column. Samples were applied in 2× TBS, washed sequentially with 25 ml of 2× TBS and then with 15 ml of 2× TBS with 15 mM imidazole followed by elution in 2× TBS + 500 mM imidazole. Positive fractions were identified by SDS-PAGE, pooled, and dialyzed overnight against 1000 volumes of 10 mM Tris, pH 8.0, 150 mM NaCl. One volume of dialysate was diluted next with 2.3 volumes of water and centrifuged at 35,000 × *g* for 30 min. ssPat remained soluble and was applied to a

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[S] The on-line version of this article (available at http://www.jbc.org) contains Supplemental Fig. 1.

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[1] The abbreviations used are: ACS, acetyl-CoA synthetase; ssPat, *S. solfataricus* Pat; TBS, Tris-buffered saline; mut, mutated; EMSA, electrophoretic mobility shift assay; ChIP, chromatin immunoprecipitation.

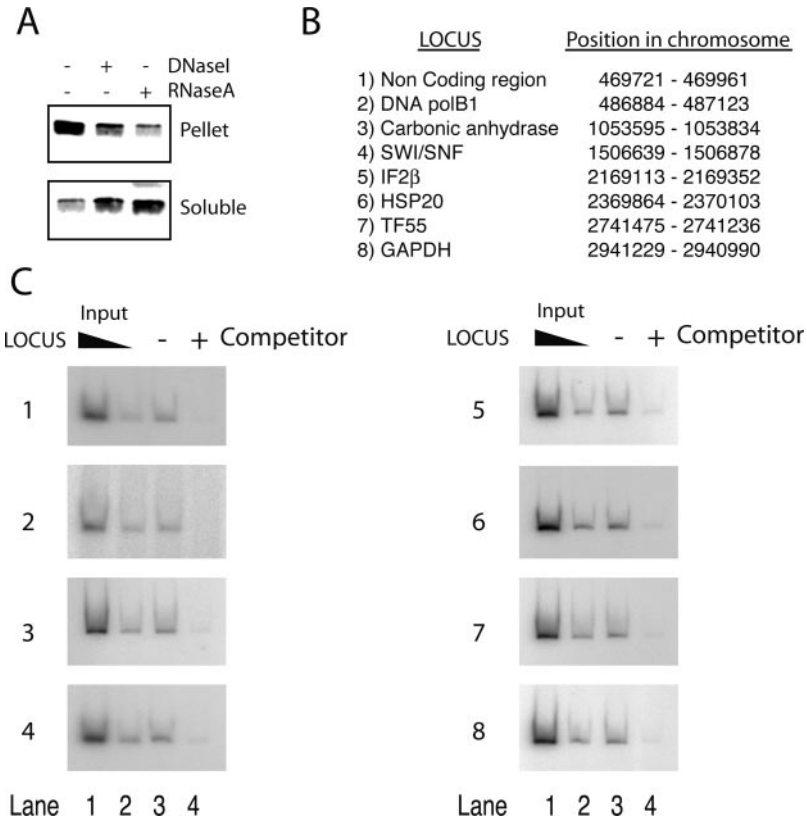

FIG. 1. **Alba is a chromatin protein.** *A*, nucleic acid association of Alba. *S. solfataricus* cells were lysed and separated into soluble and insoluble fractions. The ability of RNase A and DNase I to solubilize Alba was tested as detailed under "Experimental Procedures." *B*, location of loci used in the ChIP analysis. *C*, result of ChIP using the primer sets described in Fig. 1*B*. PCR reactions in *lanes 1* and *2* contained 10 and 1% input DNA. *Lanes 3* and *4* show PCR product from DNA recovered from ChIP in the absence (−, *lane 3*) and presence (+, *lane 4*) of an excess of recombinant Alba as competitor.

HiTrap Q XL column equilibrated in 3 mM Tris, pH 8.0, 45 mM NaCl. The column was developed with a 20-column volume linear gradient to 100 mM Tris, pH 8.0, 1.5 M NaCl. ssPat eluted as a sharp peak at roughly 800 mM NaCl. The peak sample was dialyzed against 100 volumes of 10 mM Tris, pH 8.0, 150 mM NaCl and stored at 4 °C. The ssPat-mut was purified by the same procedure.

Acetylation Assays—Acetylation assays containing the indicated amounts of ssPat and Alba, purified as described previously (17), with 1 μl (1.85 kBq) of [^{14}C,C1]acetyl-CoA (2.11 GBq/mmol) were performed in a 10-μl reaction containing 10 mM Tris, pH 8.0, 150 mM NaCl at 65 °C for 1 h. Reactions were terminated by the addition of 10 μl of 2× SDS-PAGE-loading buffer, boiled, and electrophoresed on 15% polyacrylamide gels. After staining with Coomassie Brilliant Blue, gels were destained, dried, and exposed to a storage phosphorimaging screen between 8 and 15 h. For acetylation of Alba for use in EMSA and mass spectrometry, the reaction volume was scaled up to 100 μl containing 5 μg of Alba and 1 μg of Pat. After mixing, two 50-μl aliquots were withdrawn and unlabeled acetyl-CoA was added to one aliquot to a final concentration of 100 μM. Both reactions, with and without acetyl-CoA, were incubated at 60 °C for 1 h.

Stoichiometry of Acetylation—Acetylation reactions were set up containing [^{14}C,C1]acetyl-CoA and 23 pmol of Alba in the presence or absence of 500 ng of Pat. Alba was quantified by measuring the $A_{280\,nm}$ using the molar extinction coefficient 1280 cm^{-1} M^{-1}. Reactions were incubated for 60 min at 60 °C and then terminated by the addition of SDS-PAGE-loading buffer and electrophoresed on a 4–20% gradient SDS-PAGE gel. Following staining with Coomassie Brilliant Blue, the Alba band was excised from the gel, crushed, and placed in scintillation fluid. Background levels were obtained from a blank lane that lacked Alba in the reaction. The value obtained from scintillation counting was corrected for background and compared with a standard curve of counts from [^{14}C,C1]acetyl-CoA. This revealed that 20 pmol of acetyl-CoA had been incorporated.

Mass Spectrometry—The Alba gel band was excised from a Coomassie Brilliant Blue-stained gel, washed, and in-gel-digested with chymotrypsin overnight at 25 °C, essentially as described (18). A portion of the extracted chymotryptic peptides mixture was desalted and concentrated using a Gel-loader tip filled with Poros oligo R3 sorbent (Perseptive Biosystems, Framingham, MA). The bound peptides were eluted with 50% acetonitrile, 2% formic acid directly into a nanoelectrospray capillary and then introduced into a Q-STAR hybrid tandem mass spectrometer (MDS Sciex, Concord, Ontario, Canada) equipped with a nanoelectrospray source (MDS Proteomics, Odense, Denmark). The MS survey scan for peptides from *m/z* 450 to 1000 was measured. Selected ions were fragmented by collision-induced dissociation with nitrogen in the collision cell, and the spectra of fragment ions produced were recorded in the time-of-flight mass analyzer.

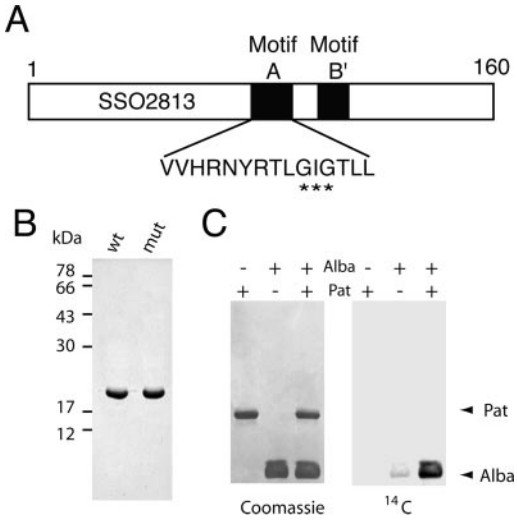

FIG. 2. *A*, cartoon of the ssPat open reading frame. Position in amino acid residues is shown above the *rectangle*. The positions of the conserved motifs A and B′ are indicated by *black rectangles*, and the sequence of part of motif A is shown. *Asterisks* indicate the residues mutated to alanine in ssPat-mut. *B*, Coomassie Brilliant Blue-stained gel of 5 μg of ssPat (*wt*) and ssPat-mut (*mut*). The positions of molecular mass markers and their sizes in kilodaltons are shown to the *left* of the figure. *C*, Pat acetylates Alba. The *left panel* shows a Coomassie Brilliant Blue-stained gel of resolved reactions containing ssPat, Alba, or ssPat and Alba in the presence of [^{14}C]acetyl-CoA. Reactions contained 3 μg of ssPat and/or 5 μg of Alba as indicated. The dried gel was exposed to a phosphorimaging storage screen to detect ^{14}C-labeled protein (*right panel*).

EMSA—The indicated amounts of proteins were incubated in 20 μl of 10 mM Tris, pH 8.0, 150 mM NaCl for 10 min at 60 °C with a 20-base

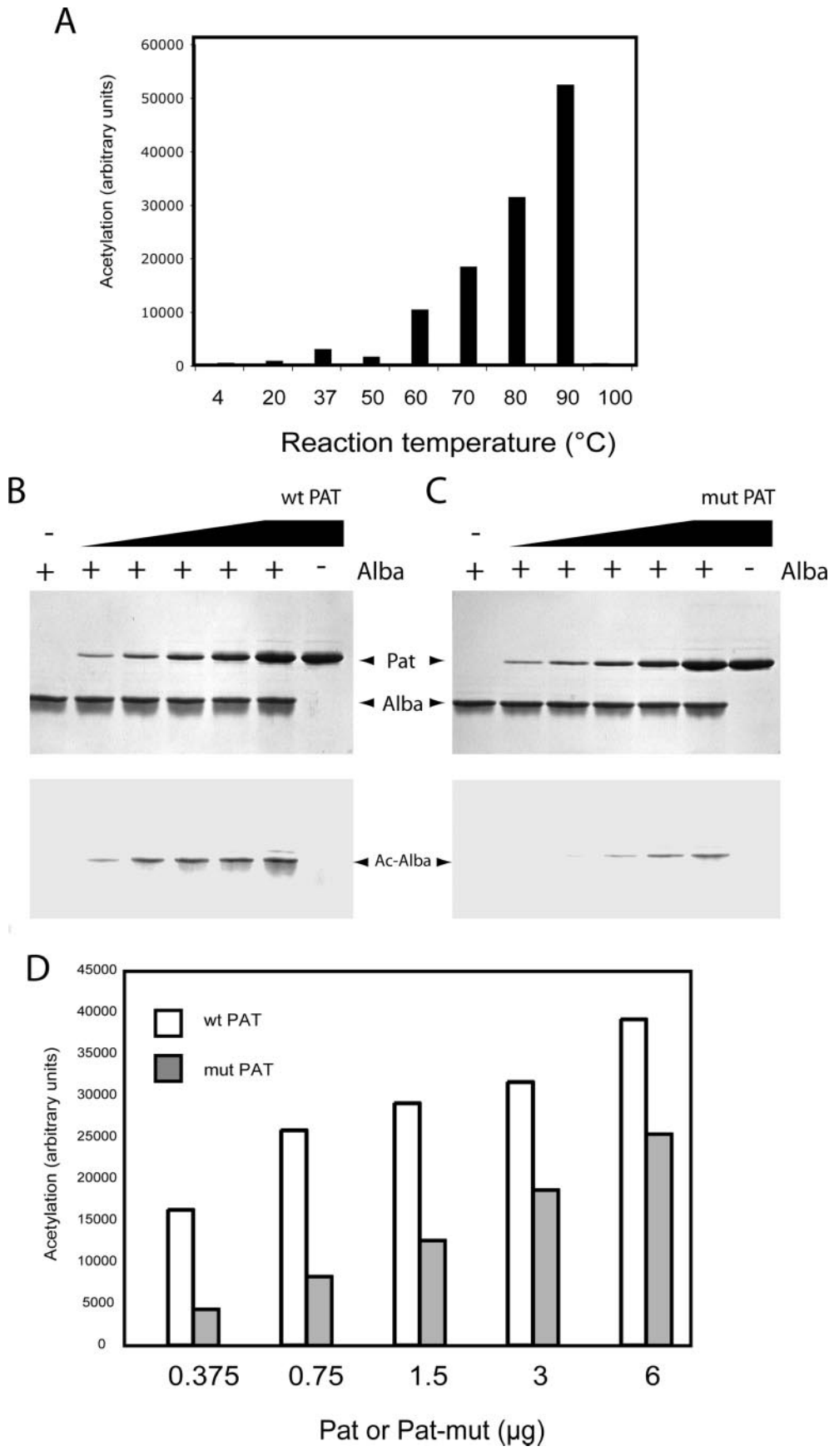

FIG. 3. *A*, effect of temperature on Alba acetylation. Reactions containing 5 μg of ssPat and 3 μg of Alba were incubated at the indicated temperatures for 60 min. Proteins were then resolved by SDS-PAGE, the dried gel was exposed to a phosphorimaging storage screen to detect ^{14}C-labeled protein, and the results were quantified by using the ImageQuant program. Values indicated have had the acetylation level seen in the absence of Pat subtracted. *B*, Pat acetylates Alba. Acetylation reactions were performed with 3 μg of Alba where indicated and with 0, 0.375, 0.75, 1.5, 3, or 6 μg of ssPat. *wt*, wild type; *mut*, ssPat mutated. The reactions were subjected to SDS-PAGE on a 15% resolving gel. The Coomassie

pair duplex DNA or RNA oligonucleotide prior to separation on a 8% polyacrylamide gel running in 0.5× Tris-buffered EDTA. The DNA oligonucleotide was prepared by annealing 5′-GATTTGTGACTTTGGTTACA-3′ with its complement 5′-TGTAACCAAAGTCACAAATC-3′ and the RNA duplex by annealing 5′-GAUUUGUGACUUUGGUUACA-3′ with 5′-UGUAACCAAAGUCACAAAUC-3′. Both DNA and RNA oligonucleotides were purchased from MWG Biotech.

ChIP—The ChIP experiments were performed as described previously (19) using antisera raised by immunization of sheep with recombinant Alba. ChIPs contained 50 μg/ml ethidium bromide. The control ChIP additionally contained 20 μg of pure recombinant Alba as competitor. The antiserum was a kind gift from Malcolm White (St. Andrews University). DNA recovered from immune or preimmune precipitates was amplified using oligonucleotide primers specific for the genomic regions indicated in Fig. 1*B*. Sequences of the oligonucleotides are available on request.

Nucleic Acid Association of Alba—Three O.5-ml aliquots of mid-logarithmic *S. solfataricus* culture were pelleted at 13,000 rpm for 2 min and then resuspended in 200 μl of Bugbuster™ protein extraction reagent (Novagen). One of these aliquots was supplemented with 10 mM $Mgcl_2$ and treated with 200 units of RNasin® RNase inhibitor (Promega) and 100 units of DNase 1 (Roche Applied Science). A second aliquot was supplemented with 5 mM EDTA and treated with 5 μg of RNase A (Mp Biomedicals UK). The third aliquot remained untreated. All three aliquots were incubated at 37 °C for 30 min and subsequently centrifuged at 13,000 rpm for 10 min at 4 °C. The supernatants were withdrawn, and the pellets were resuspended in an equal volume. 10 μl of each of these samples were loaded onto a 15% polyacrylamide gel and visualized via Western blotting using antisera previously raised against recombinant Alba by sheep immunization (a kind gift of Malcolm White).

RESULTS

Although initially characterized as an abundant chromatin protein in *Sulfolobus* species, recent work has indicated that Alba also possesses RNA binding activity. Furthermore, UV-cross-linking studies revealed that Alba can associate with RNA *in vivo* (20). Although a number of laboratories have demonstrated that Alba possesses DNA binding activity *in vitro*, we wished to determine whether Alba was associated with chromosomal DNA *in vivo*. To this end, we employed the chromatin fractionation technique described by Matsunaga *et al.* (21). As can be seen in Fig. 1*A*, Alba is associated with the insoluble chromatin-containing fraction. Treatment of the pellet fraction with DNase I solubilized a significant proportion of Alba. Interestingly, the treatment of the pellet with RNase A also liberated Alba. Thus, these data indicate that Alba is associated with both DNA and RNA *in vivo*. We were concerned that the association of Alba with DNA in the chromatin pellet observed in Fig. 1*A* may have arisen during preparation of the material. To take a snap shot of the location of Alba in living cells, we treated mid-logarithmic cells with formaldehyde to introduce protein-DNA cross-links and then performed chromatin immunoprecipitation with an anti-Alba antibody. To confirm the specificity of the immunoprecipitation, we performed a control ChIP in which an excess of pure recombinant Alba was added as competitor. We then tested for co-precipitation of DNA from several loci around the *S. solfataricus* genome by PCR amplification (Fig. 1*B*). As can be seen in Fig. 1*C*, DNA corresponding to the eight randomly chosen regions was co-immunoprecipitated with Alba and significantly reduced levels of DNA were immunoprecipitated in the presence of the competitor. Taken together with Fig. 1*A*, these data strongly support a role for Alba as a *bona fide* chromatin protein *in vivo*.

Identification of ssPat as an Alba Acetyltransferase—The C-terminal 95 residues of the predicted protein product of *S. enterica pat* show homology to the Gcn5 acetyltransferase superfamily. We performed a BLAST search (16) of the genome sequence of *S. solfataricus* P2 (6) for sequences homologous to this region of Pat. This search revealed that the 160 amino acid open reading frame encoded by the SSO2813 gene is homologous to the C-terminal Gcn5 acetyltransferase homology region of *S. enterica* Pat (Fig. 2*A* and Supplemental Fig. 1). The predicted polypeptide sequence of the product of the SSO2813 gene (hereafter referred to as ssPat) possesses regions with high sequence homology to the domains A and B′ of the Gcn5 acetyltransferase superfamily. In particular, the domain A region involved in acetyl-CoA binding is readily identifiable.

In *S. enetrica*, Pat and Sir2 work antagonistically to regulate ACS (15). We have previously demonstrated a functional interaction between *S. solfataricus* Sir2 and the chromatin protein Alba. For this reason, we speculated that ssPat might have a role in acetylating Alba. To test this possibility, recombinant ssPat was expressed in *Escherichia coli* as a C-terminally hexahistidine-tagged protein and purified (Fig. 2*B*). ssPat was tested for its ability to use [^{14}C,C1]acetyl-CoA to acetylate recombinant Alba. As can be seen in Fig. 2*C*, ssPat acetylates Alba. Interestingly, only very low levels of auto-acetylation by ssPat were observed upon prolonged exposure of the gel (data not shown), indicating a degree of substrate selectivity by the enzyme (see below). Note that a low level of ssPat-independent acetylation of Alba is detected. This is probably due to the intrinsic instability of acetyl-CoA at elevated temperatures and was never >20% of the level of acetylation detected in the presence of ssPat. Because *S. solfataricus* is a hyperthermophile, we examined the optimal temperature for the acetylation reaction. As can be seen in Fig. 3*A*, the reaction is optimal at temperatures between 70 and 90 °C, in agreement with the optimal growth temperatures of *Sulfolobus*, making it unlikely that acetylation is due to a contaminating protein from *E. coli*. We next prepared a derivative of ssPat (ssPat-mut) in which key conserved residues involved in binding acetyl-CoA have been mutated to alanine (indicated by *asterisks* in Fig. 2*A*). Mutations of analogous residues in the yeast histone acetyltransferase Gcn5 significantly reduced but did not abrogate its enzymatic activity (22). Correspondingly, ssPat-mut was found to have substantially reduced activity compared with wild type (Fig. 3, *B–D*). Thus, it appears that ssPat is capable of acetylating Alba. We measured the stoichiometry of acetylation of Alba by ssPat and found that 88% (±5%) Alba molecules possessed an acetyl group. The non-enzymatic thermal-mediated acetylation of Alba was 18% (±3%).

Specificity of ssPat for Alba Lysine 16—Alba isolated from *S. solfataricus* cells is acetylated on lysine 16 (11). Therefore, we next wished to test whether ssPat displayed selectivity in the residue(s) of Alba it acetylated. Thus, we prepared two mutant derivatives of Alba (Alba K16E and Alba K17E) and tested these in [^{14}C,C1]acetyl-CoA acetylation assays (Fig. 4*A*). Strikingly, whereas wild-type Alba and Alba K17E were acetylated to similar levels by ssPat, acetylation of Alba K16E was greatly reduced, indicating the importance of this residue for acetylation of Alba by Pat.

To determine whether Lys-16 is the site of acetylation by Pat, we performed an acetylation reaction with non-radiolabeled acetyl-CoA and isolated the resultant acetylated Alba by gel electrophoresis. Alba was in-gel-digested with chymotrypsin and analyzed by electrospray tandem mass spectrometry. A

Brilliant Blue-stained gel is shown in the *upper panel*, and the *lower panel* shows [^{14}C]acetyl-CoA-labeled species corresponding to acetylated Alba detected by a phosphorimaging storage screen. *C*, mutation of the ssPat motif A reduces acetylation. Acetylation reactions were performed as in *B*; however, ssPat-mut was used in place of ssPat. *D*, quantitation of results shown in parts *B* and *C*. Phosphorimagery was used to obtain values for acetylation by the various concentrations of Pat or Pat-mut. Values have had the background non-enzymatic acetylation subtracted.

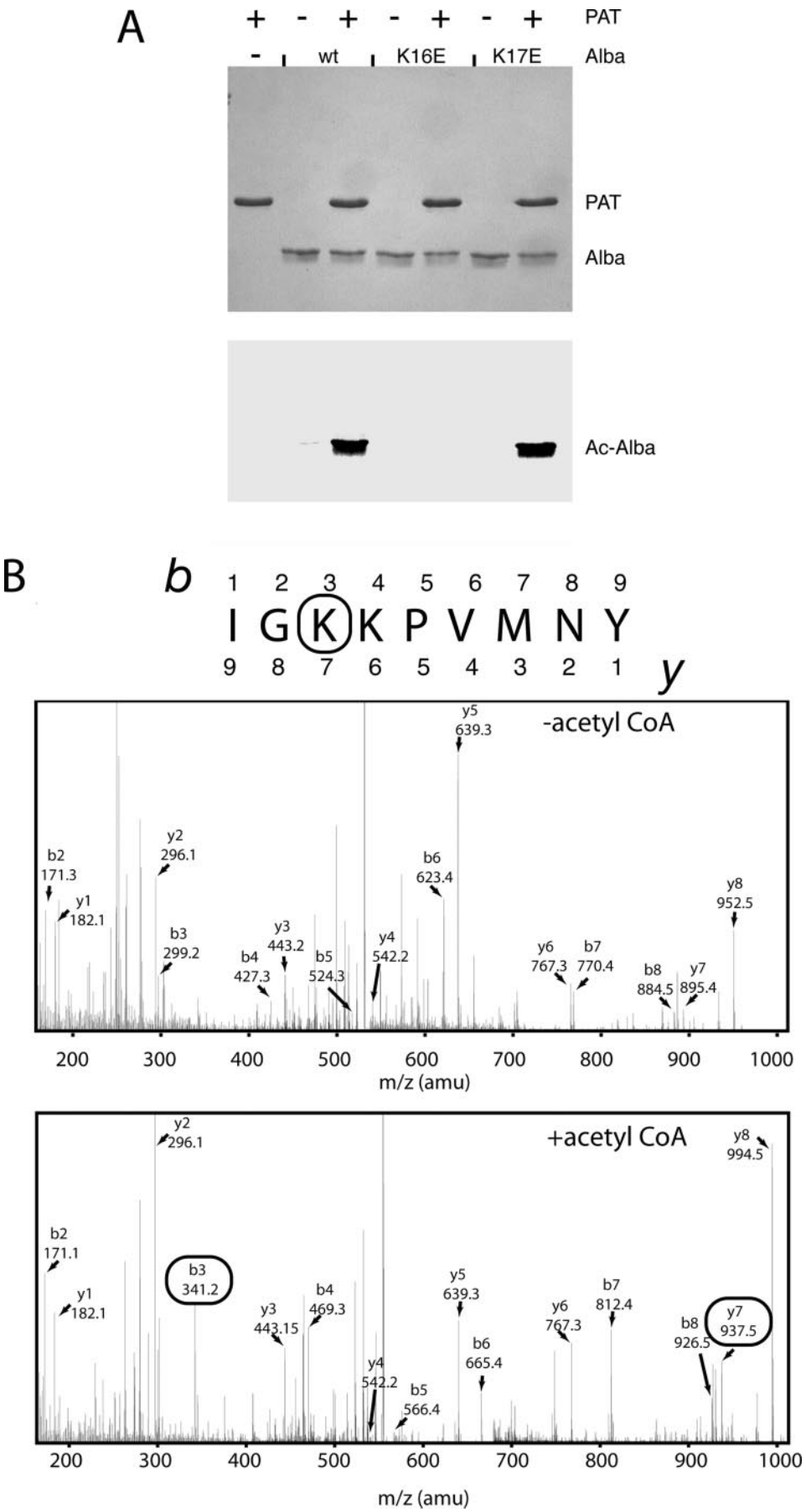

FIG. 4. *A*, lysine 16 of Alba is required for acetylation. Acetylation reactions were assembled containing 3 μg of Pat and 2 μg of Alba, Alba K16E, or Alba K17E as indicated. The reactions were subjected to SDS-PAGE on a 15% resolving gel. The Coomassie Brilliant Blue-stained gel is shown in the *upper panel*, and the *lower panel* shows [^{14}C]acetyl-CoA-labeled species corresponding to acetylated Alba and Alba K17E detected by

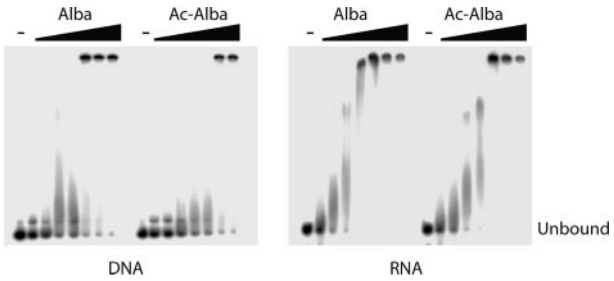

FIG. 5. **Effect of PAT-mediated acetylation of Alba in EMSA.** Alba was incubated with PAT in the presence (*Ac-Alba*) or absence (*Alba*) of acetyl-CoA as detailed under "Experimental Procedures." Reactions were then incubated with ~1 fmol of 20-nucleotide double-stranded DNA (*left panel*) or RNA (*right panel*). The amount of mock-treated or acetylated Alba in the binding reactions was 0, 0.32, 0.63, 1.25, 2.5, 5, or 10 pmol. Unbound DNA is indicated.

doubly charged ion at *m/z* 554.3 corresponding to Alba ^{14}IGKKPVMoNY^{22} (Mo is an oxidized methionine) incorporating a single acetyl group of 42 daltons was detected. This ion was selected and fragmented by collision-induced dissociation with nitrogen, and fragment ion spectra were recorded. Peaks within the fragmentation series were identified with reference to the known peptide sequence of this chymotryptic fragment of Alba. As can be seen in the Fig. 4*B*, *lower panel*, the boxed b3 and y7 ions have a mass of 42 greater than the corresponding ions in the mock-treated sample. This indicates the presence of an acetyl group on lysine 16. Subsequent ions in the series (b4–8) and y8 all show a mass increased by 42 daltons in the acetylated sample. Thus, the tandem mass spectrometry confirmed the identity of the peptide and reveals that the residue in Alba acetylated by Pat was Lys-16 of Alba (Fig. 4*B*).

ssPat-mediated Acetylation of Alba Lowers Its Nucleic Acid Binding Affinity—Acetylated Alba purified from *S. solfataricus* cells was initially observed to have a substantially (at least 10-fold) lower binding affinity for DNA than recombinant protein. More recent studies have suggested that the effect may not be as dramatic as first reported, with acetylated Alba having an ~3-fold reduced affinity for DNA.[2] In agreement with this more conservative estimate, treatment of acetylated Alba with the Sir2 deacetylase only stimulated DNA binding by ~3-fold, as measured by the effect of Alba on *in vitro* transcription assays (11).

For these reasons, we wished to test the effect on DNA binding activity of preincubation of recombinant, non-acetylated Alba with ssPat in the presence or absence of acetyl-CoA. Accordingly, Alba and ssPat were mixed in a 100-μl reaction, and two 50-μl aliquots were withdrawn. Acetyl-CoA was added to one of these aliquots to a final concentration of 100 μM. Both reactions, with and without acetyl-CoA, were incubated at 60 °C for 1 h. At the end of the incubation, Alba from the two reactions was used in electrophoretic mobility shift assays. As can be seen from the Fig. 5, *left panel*, Alba treated with acetyl-CoA and ssPat shows a 2-fold reduced affinity for DNA in the EMSA compared with Alba incubated with ssPat alone. Thus, ssPat-mediated acetylation of Alba reduces its DNA binding affinity. Recent work has suggested that, in addition to binding DNA, *in vivo* Alba can interact with RNA (Fig. 1) (20). We prepared a double-stranded RNA oligonucleotide and used this in EMSA with Alba as above. In agreement with the result observed with DNA, we see an ~2-fold reduction in the affinity of acetylated Alba for the double-stranded RNA (Fig. 5, *right panel*).

[2] C. Jelinska and M. F. White, personal communication.

DISCUSSION

Archaea possess machineries for DNA replication and transcription that are closely related to the analogous machineries of eukaryotes and distinct from those of bacteria (23, 24). However, in gross morphology, cellular organization, and genome size and structure, archaeal cells resemble bacteria. In eukaryotes, the dynamic modulation of the packaging of DNA by covalent modification of histones in the nucleus plays an active role in DNA-based processes (2). In bacteria, whereas nucleoid proteins play architectural roles in processes such as DNA replication and transcription (25), there is, to the best of our knowledge, no evidence of regulatory covalent modification of these proteins. Although histones are present in a significant subset of Archaea, they lack the N- and C-terminal tails that are the principal sites of modification in eukaryotic histones (26) and are not covalently modified (5). Even in very early diverging eukaryotes such as trypanosomes, histones possess tails and are covalently modified (27). Thus, the origins of these complex regulatory pathways and the evolutionary source of the participating enzymes remain unclear.

The Alba protein was initially identified as an abundant DNA-binding protein in *Sulfolobus* extracts (9, 10), a finding verified by several groups (11, 28–31) and was proposed to be a major chromatin protein. Recently, this view was challenged by the observation of Guo *et al.* (20) that, following UV treatment of *Sulfolobus* cells, little or no Alba was DNA bound and rather appeared to be primarily associated with RNA (20). However, the dose of UV used in that study was high, resulting in fragmentation of nucleic acids. Additionally, it has been observed that the cellular response of *Sulfolobus* to UV damage involves the recruitment of reverse gyrase to chromatin (32). This combination of DNA damage, cold shock during the 20-min irradiation, and possible topological alterations due to reverse gyrase recruitment may have reduced the levels of Alba bound to DNA. The findings of Guo *et al.* (20) differ markedly from our observations that, in bulk chromatin fractionation, a large proportion of Alba is DNA-bound and more importantly that formaldehyde treatment of cells leads to the covalent attachment of Alba to all of the genomic loci tested (Fig. 1). Taken together, our data strongly support the proposal that Alba is a chromatin protein.

Therefore, we have uncovered evidence for chromatin regulation by reversible acetylation in the archaeon, *S. solfataricus*, a crenarchaeote that lacks histones. Moreover, our data give a hint as to the derivation of eukaryotic-like chromatin regulation of non-histone proteins. Our previous work revealed that the chromatin protein, Alba, was acetylated *in vivo* on Lys-16 and deacetylated by Sir2 (11). Our current work establishes that the Pat acetyltransferase specifically acetylates Alba on Lys-16. Remarkably, studies by Starai *et al.* (14) and Starai and Escalante-Semerena (15) have revealed that the Sir2/Pat partnership is functionally conserved in the bacterium *S. enterica* where these enzymes regulate the modification and activity of acetyl-CoA synthetase. The lysine residue in ACS that is modified lies in the sequence motif P(X_4)GK. In Alba, Lys-16 is also preceded by a glycine in the motif ^{8}PSNVVLIGK^{16}. It is

phosphorimaging storage screen. *B*, the sequence of the chymotryptic peptide of Alba containing lysine 16 is shown with lysine 16 *boxed*. The b- and y-ion series are shown above and below the sequences, respectively. The *upper spectrum* shows the profile obtained from mock-treated Alba, and the *lower panel* shows the spectrum from acetylated Alba. The *boxed* b3 and y7 ions in the *lower panel* have a mass of 42 greater than the corresponding ions in the mock-treated sample. This indicates the presence of an acetyl group on lysine 16. Subsequent ions in the series (b4–8) and y8 all show a mass increased by 42 daltons in the acetylated sample.

possible that Gly-Lys preceded by a proline is a conserved recognition site for the Pat/Sir2 acetylation/deacetylation system. The observation that Pat is conserved in Archaea that lack Alba (Supplemental Fig. 1) suggests a further role for Pat in processes other than Alba modification, and we anticipate that this is most likely ACS regulation. This leads us to speculate that *S. solfataricus* has co-opted a general regulatory switch present in many bacteria and archaea to generate a rudimentary form of chromatin regulation. Our data suggest that the apparatus for regulation of chromatin by reversible acetylation may pre-date the acquisition of histone N- and C-terminal tails by eukaryotes. In this light, it is of great interest to note that many non-histone proteins are known to be acetylated in eukaryotes, including a number of non-histone chromatin proteins (33).

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