Sir2 AND THE ACETYLTRANSFERASE, Pat, REGULATE THE ARCHAEAL CHROMATIN PROTEIN, Alba
Victoria L Marsh¹, Sew Yeu Peak-Chew² and Stephen D Bell¹,³
¹Medical Research Council Cancer Cell Unit, Hutchison MRC Research Centre, Hills Road, Cambridge CB2 2XZ, UK
²Medical Research Council, Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, UK
Running Title: Acetylation of archaeal chromatin
³Corresponding author Steve Bell sb419@hutchison-mrc.cam.ac.uk
Tel (44) 1223 763 311, Fax (44) 1223 763 296

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 acetyl transferase that specifically acetylates Alba on this residue. The effect of acetylation is to lower the affinity of Alba for DNA. Remarkably, the acetyl transferase is conserved not only in archaea but also in bacteria, where it appears to play a role in metabolic regulation. Our data suggest therefore that S. solfataricus has co-opted this bacterial regulatory system to generate a rudimentary form of chromatin regulation.

Keywords acetylation, archaea, chromatin, evolution, histone

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 2 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 acetyl transferases (HATs) and deacetylases (HDACs) identified. Distinct HATs and HDACs 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 characterised Sulfolobus chromatin proteins are the Sul7d family and Alba; reviewed in (7). Interestingly, Sul7d proteins are differentially mono-methylated 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) (14).
The enzyme responsible for acetylating ACS, Pat, has recently been identified (15). In the current 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. Further, 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. eneterica Pat was used to perform a Blast search(16) of the S. solfataricus P2 genome sequence (http://www-archbac.upsud.fr/projects/sulfolobus/).

**Cloning and purification of ssPat** - The SSO2813 open reading frame was amplified using the polymerase chain reaction (PCR) with oligonucleotides Pat5 GGGATCCCATATGAAATGACCAGATAAAGATAAG and Pat3 GAATTCTCGAGTGGGGCGGAGAAAGTTGCTAG. The PCR introduced NdeI and XhoI restriction sites (underlined and in bold) at the 5' and 3' ends of the open reading frame. The PCR product was digested with NdeI and Xhol restriction sites and ligated into pET30a linearised 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 hexa-histidine tag. Rosetta cells (Novagen) were transformed with pET30-Pat, plated, and 50 ml overnight culture grown of which 20 ml was used the following morning to inoculate 1 litre of fresh L broth supplemented with kanamycin and chloramphenicol. Cells were grown shaking at 37°C until the OD 600nm=0.6 then expression of ssPat induced with 1 mM IPTG for 4 hours. Cells were harvested by centrifugation, resuspended in 25 ml 20 mM Tris pH 8.0, 300 mM NaCl (2xTBS) and lysed in an EmulsoFlex-C5 cell cracker (Glen Creston, UK). The lysate was clarified by centrifugation (35,000 g, 30 minutes, 4°C). The supernatant was heated to 75°C for 30 minutes then clarified by centrifugation. The heat stable ssPat remained soluble and was purified by passage over a 2ml Ni-NTA agarose column. Samples were applied in 2xTBS, washed sequentially with 25ml 2xTBS then 15 ml 2xTBS with 15 mM imidazole followed by elution in 2xTBS + 500 mM imidazole. Positive fractions were identified by SDS-PAGE, pooled and dialysed overnight against 1000 volumes 10 mM Tris pH 8.0, 150 mM NaCl. Next, one volume of dialysate was diluted with 2.3 volumes of water and centrifuged at 35, 000 g for 30 minutes. ssPat remained soluble and was applied to a 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 dialysed against 100 volumes 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 [¹⁴C, 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 hour. Reactions were terminated by the addition of 10 µl of 2x SDS-PAGE loading buffer, boiled and electrophoresed on 15% polyacrylamide gels. After staining with Coomassie brilliant Blue, gels were destained, dried and exposed to storage phosphor screen for between 8 and 15 hours. For acetylation of Alba for use in EMSA and mass spectrometry the reaction volume was scaled up to 100 µl, containing 5 µg Alba and 1 µg Pat. After mixing, two 50 µl aliquots were withdrawn and unlabelled acetyl-CoA added to one aliquot to a final concentration of 100µM. Both reactions, with and without acetyl-CoA, were incubated at 60°C for 1 hour.

**Stoichiometry of acetylation** - Acetylation reactions were set up containing [¹³C, C¹] acetyl-CoA and 23 pmol of Alba in the presence
or absence of 500 ng of Pat. Alba was quantified by measuring the OD$_{280}$ nm using the molar extinction coefficient 1280 cm$^{-1}$ M$^{-1}$. Reactions were incubated for 60 minutes at 60°C and then terminated by addition of SDS-PAGE loading buffer and electrophoresed on a 4-20% gradient SDS-PAGE gel. Following staining with Coomassie blue the Alba band was excised from the gel, crushed and placed in scintillation fluid. Background levels were obtained from a blank lane which lacked Alba in the reaction. The value obtained from scintillation counting was corrected for background and compared to 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-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). The bound peptides were eluted with 50% acetonitrile / 2% formic acid directly into a nano-electrospray capillary and then introduced into a Q-STAR hybrid tandem mass spectrometer (MDS Sciex, Concord, Ontario, Canada) equipped with a nano-electrospray source (MDS Proteomics, Odense, Denmark). The MS survey scan for peptides from 450 to 1000 m/z were measured. Selected ions were fragmented by CID with nitrogen in the collision cell and spectra of fragment ions produced were recorded in the TOF mass analyzer.

**EMSA** - The indicated amounts of proteins were incubated in 20 μl of 10 mM Tris pH 8.0, 150 mM NaCl for 10 minutes at 60°C with a 20 basepair duplex DNA or RNA oligonucleotide prior to separation on a 8% polyacrylamide gel running in 0.5 x TBE.

The DNA oligonucleotide was prepared by annealing 5’ GATTTGTGACTTTGGTTACA 3’ with its complement 5’ TGTAACCAAGTCACAAAATC 3’ and the RNA duplex by annealing 5’ GAUUUGUACUUUGGUUACA 3’ with 5’ UGUAAACAAAGUCAACAAAC 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 immunisation of sheep with recombinant Alba. The control ChIP contained 20 μg of pure recombinant Alba as competitor. The antisera was a kind gift from Malcolm White, St Andrews. DNA recovered from immune or pre-immune precipitates was amplified using oligonucleotide primers specific for the genomic regions indicated in Figure 1B. Sequences of the oligonucleotides are available on request.

**Nucleic acid association of Alba** - Three 0.5 ml aliquots of mid logarithmic *S. solfataricus* culture were pelleted at 13 000 rpm for 2 mins and then re-suspended in 200 μl Bugbuster™ Protein Extraction reagent (Novagen). One of these aliquots was supplemented with 10 mM MgCl$_2$ and treated with 200 u Rnasin® RNase inhibitor (Promega) and 100 u DNase1 (Roche). A second aliquot was supplemented with 5 mM EDTA and treated with 5 μg RNaseA (Mp Biomedicals UK). The third aliquot remained untreated. All three aliquots were incubated at 37°C for 30 minutes and subsequently centrifuged at 13 000 rpm for 10 mins at 4°C. The supernatants were withdrawn and the pellets resuspended in an equal volume. 10 μl of each of these samples were loaded onto a 15% polyacrylamide gel and visualised via western blotting using antisera previously raised against recombinant Alba by sheep immunisation (a kind gift of Malcolm White).

**RESULTS**

Although initially characterised as an abundant chromatin protein in *Sulfolobus* species, recent work has indicated that Alba also possesses RNA binding activity. Further, UV crosslinking
studies revealed that Alba can associate with RNA in vivo (20). While 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 and colleagues (21). As can be seen in Fig. 1A, Alba is associated with the insoluble, chromatin-containing, fraction. Treatment of the pellet fraction with DNase I solubilised a significant proportion of Alba. Interestingly, 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. 1A may have arisen during preparation of the material. In order to take a snapshot of the location of Alba in living cells, we treated mid-logarithmic cells with formaldehyde to introduce protein-DNA crosslinks 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. 1B. As can be seen in Fig. 1C, DNA corresponding to the eight randomly chosen regions was co-immunoprecipitated with Alba, significantly reduced levels of DNA were immunoprecipitated in the presence of the competitor. Taken together with Fig. 1A, these data strongly support a role for Alba as a bona fide chromatin protein in vivo.

**Identification of ssPat as an Alba acetyl transferase**

The C-terminal 95 residues of the predicted protein product of *S. enterica* pat show homology to the Gcn5 acetyltransferase (GNAT) 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 GNAT homology region of *S. enterica* Pat (Fig. 2A and Supplemental Data). The predicted polypeptide sequence of the product of the SSO2813 gene (hereafter referred to as ssPat) possesses regions with high sequence homology to the domain A and B’ of the GNAT superfamily. In particular the domain A region involved in acetyl-CoA binding is readily identifiable.

In *S. enterica*, 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, recombinant ssPat was expressed in *E. coli* as a C-terminally hexa histidine tagged protein and purified (Fig. 2B). ssPat was tested for its ability to use [14C, C1] acetyl-CoA to acetylate recombinant Alba. As can be seen in Figure 2C, ssPat acetylates Alba. Interestingly, only very low levels of auto-acetylation by ssPat were observed on 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 likely due to the intrinsic instability of acetyl-CoA at elevated temperatures and was never more than 20% of the level of acetylation detected in the presence of ssPat. As *S. solfataricus* is a hyperthermophile, we examined the optimal temperature for the acetylation reaction. As can be seen in Fig. 3A, 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*. Next, we 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 2A). Mutations of analogous residues in the yeast HAT Gcn5 significantly reduced, but did not abrogate, its enzymatic activity (22). Correspondingly, ssPat-mut was found to have substantially reduced activity compared to wild-type (Fig. 3B, C and 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%) of 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). We therefore next wished to test whether ssPat displayed selectivity in which residue(s) of Alba it acetylated. Thus, we prepared two mutant derivatives of Alba; Alba K16E and Alba K17E and tested these in [14C, C1] acetyl-CoA acetylation assays (Figure 4A). Strikingly, while 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 K16 is the site of acetylation by Pat we performed an acetylation reaction with non-radiolabelled acetyl-CoA and isolated the resultant acetylated Alba by gel electrophoresis. Alba was in-gel digested with chymotrypsin and analysed by electrospray tandem mass spectrometry. A doubly charged ion at m/z 554.3 corresponding to Alba\(^{14}IGKKPV\text{MoNY}^{22}\) (Mo is an oxidized methionine), incorporating a single acetyl group of 42 daltons was detected. This ion was selected and fragmented by CID (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 the lower panel of figure 4B, the boxed b3 and y7 ions in 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 K16 of Alba (Fig. 4B).

**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 approximately three fold reduced affinity for DNA (C. Jelinska and M.F. White, pers. comm.). In agreement with this more conservative estimate, treatment of acetylated Alba with the Sir2 deacetylase only stimulated DNA binding by approximately three fold, as measured by the effect of Alba on *in vitro* transcription assays (11).

For these reasons we wished to test the effect on Alba’s DNA binding activity of pre-incubation 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 withdrawn. Acetyl-CoA was added to one of these to a final concentration of 100 μM. Both reactions, with and without acetyl-CoA, were incubated at 60°C for one hour. At the end of the incubation, Alba from the two reactions was used in electrophoretic mobility shift assays. As can be seen from the left panel of Figure 5, Alba treated with acetyl-CoA and ssPat shows a two-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, Figure 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 approximately 2-fold reduction in the affinity of acetylated Alba for the double stranded RNA (Fig. 5, right panel).

**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 organisation 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, while 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 remains 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 proposed to be a major chromatin protein. Recently, this view was challenged by the observation of Guo and colleagues 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 minute 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. 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 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, albeit of non-histone proteins. Our previous work revealed that the chromatin protein, Alba, was acetylated in vivo on K16 and deacetylated by Sir2 (11). Our current work establishes that the Pat acetyl transferase specifically acetylates Alba on K16. Remarkably, studies by Starai and Escalante-Semerena have revealed that the Sir2/Pat partnership is functionally conserved in the bacterium Salmonella enterica where these enzymes regulate the modification and activity of acetyl-CoA synthetase (14,15). The lysine residue in ACS that is modified lies in the sequence motif P(X4)GK. In Alba, K16 is also preceded by a glycine in the motif PSNVVLIGK16. It is possible that GK 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 data) 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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sharing data and antisera prior to publication. This work was funded by the Medical Research Council.

References
Figure Legends

Figure 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 RNaseA and DNaseI to solubilise Alba was tested as detailed in Experimental procedures. B Location of loci used in the ChIP analysis. C Result of ChIP using the primer sets described in Fig. 1B. PCR reactions in lanes 1 and 2 contained 10 and 1% of 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.

Figure 2 A Cartoon of the ssPat open reading frame. Position in amino acid residues is shown above the rectangle. The positions of the conserved motif A and B’ are indicated by black rectangles and the sequence of part of motif A shown. Asterisks indicate the residues mutated to alanine in ssPat-mut. B Coomassie stained gel of 5 µg of ssPat (wt) and ssPat-mut (mut). The positions of molecular weight markers, and their size in kilodaltons, are shown to the left of the figure. C Pat acetylates Alba. The left panel shows a Coomassie stained gel of resolved reactions containing ssPat, Alba or ssPat and Alba in the presence of 14C acetyl-CoA. Reactions contained 3 µg of ssPat and/or 5 µg of Alba as indicated. The dried gel was exposed to a phosphor storage screen to detect 14C labelled protein, right panel.

Figure 3 A Effect of temperature on Alba acetylation. Reactions containing 5 µg ssPat and 3 µg Alba were incubated at the indicated temperatures for 60 minutes. Proteins were then resolved by SDS-PAGE, the dried gel was exposed to a phosphor storage screen to detect 14C labelled 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 0, 0.375, 0.75, 1.5, 3 or 6 µg of ssPat. The reactions were subjected to SDS-PAGE on a 15% resolving gel. The Coomassie stained gel is shown in the upper panel and the lower panel shows 14C acetyl-CoA labelled species corresponding to acetylated Alba, detected by phosphor storage screen. C Mutation of the ssPat motif A reduces acetylation. Acetylation reactions were performed as in Fig. 3A, 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.

Figure 4 A Lysine 16 of Alba is required for acetylation. Acetylation reactions were assembled containing 3 µg of Pat and 2µg of Alba, Alba K16E or Alba K17E, as indicated. The reactions
were subjected to SDS-PAGE on a 15% resolving gel. The Coomassie stained gel is shown in the upper panel and the lower panel shows 14C acetyl-CoA labelled species, corresponding to acetylated Alba and Alba K17E, detected by phosphor 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, the lower panel 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.

**Figure 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 in Methods. Reactions were then incubated with ~1 fmol of 20 nt 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.
A
- + - DNasel
- - + RNaseA
Pellet
Soluble

B
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C

Marsh et al. Figure 1

Marsh et al. SFig. 1
Motif A

SSO2813

VHRNYRTLGIGTLL

Motif B'

Alba

Pat

Marsh et al. Fig. 2
Marsh et al. Fig. 3
Marsh et al. Fig. 4
Marsh et al. Fig. 5
Supplementary Fig. 1

Sequence alignment of Pat from the bacteria Salmonella enterica (Sen) Acc No. NC_003198.1 with
selected archaeal Pat homologues from *Methanopyrus kandleri* (Mka) Acc. No. NC_003551; *Methanosarcina mazei* (Mma) Acc. No. NC_003901.1; *Methanococcus jannaschii* (Mja) Acc. No. NC_000909.1; *Picrophilus torridus* (Pto) Acc. No. NC_005877.1; *Thermoplasma acidophilum* (Tac) Acc. No. NC_002578.1; *Ferroplasma acidarmanus* (Fac) Acc. No. NZ_AABC03000011.1; *Sulfotobus solfataricus* (Sso) Acc. No. NC_002754.1; *S. tokodaii* (Sto) Acc. No. NC_003106.2; *Archaeoglobus fulgidus* (Afu1) Acc. No. NC_000917.1; *Aeropyrum pernix* (Ape) Acc. No. NC_000854.1; *A. fulgidus* (Afu2) Acc. No. NC_000917.1; *Methanothermobacter thermautotrophicus* (Mth) Acc. No. NC_000916.1
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