

Physical and functional interaction of the archaeal single-stranded DNA-binding protein SSB with RNA polymerase

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Received December 1, 2003; Revised and Accepted January 9, 2004

ABSTRACT

Archaeal transcription utilizes a complex multisubunit RNA polymerase and the basal transcription factors TBP and TF(II)B, closely resembling its eukaryal counterpart. We have uncovered a tight physical and functional interaction between RNA polymerase and the single-stranded DNA-binding protein SSB in *Sulfolobus solfataricus*. SSB stimulates transcription from promoters *in vitro* under TBP-limiting conditions and supports transcription in the absence of TBP. SSB also rescues transcription from repression by reconstituted chromatin. We demonstrate the potential for promoter melting by SSB, suggesting a plausible basis for the stimulation of transcription. This stimulation requires both the single-stranded DNA-binding domain and the acidic C-terminal tail of the SSB. The tail forms a stable interaction with RNA polymerase. These data reveal an unexpected role for single-stranded DNA-binding proteins in transcription in archaea.

INTRODUCTION

Archaea are prokaryotic and resemble bacteria in their gross morphology and many of their metabolic pathways, whilst also possessing unique features such as ether-linked, branched membrane lipids (1). The core processes concerned with the flow of information from DNA through RNA to proteins, including DNA replication (2,3), transcription and translation (4–6), are, however, strikingly similar to those in eukarya, suggesting a shared evolutionary heritage between the archaeal and eukaryal domains (7). The first suggestion of this relationship came from the molecular phylogenetic studies of Woese and Fox (8,9) based on rRNA sequences. Some of the first biochemical evidence confirming this unexpected connection came from studies of the archaeal RNA polymerase, which was shown to consist of multiple subunits with a complexity comparable with the eukaryal RNA polymerases (10). In the mid 1990s, the completion of archaeal genome sequences accumulated further evidence supporting the relationship of archaea and eukarya.

Concurrently, a series of biochemical studies of archaeal transcription demonstrated the functional requirement for the general transcription factors TBP (TATA-binding protein) and TFB (TFIIB) (11–13), whilst archaeal promoters were also shown to resemble eukaryotic RNA polymerase II promoters, with a highly conserved TATA box sequence and adjacent TFB-responsive element (BRE) (14,15). This biochemical evidence, coupled with structural studies of archaeal TBP and TFB (16–18), confirmed beyond any remaining doubt that the archaea and eukarya share a common basal transcription apparatus.

Studies of archaeal transcription have thus provided many insights applicable to the much more complex eukaryal transcriptional apparatus. Clearly, in addition to its utility as a model system, archaeal transcription is a fundamental process worthy of study in its own right, and is likely to have unique features that may also point to the evolution of information processing pathways. Many archaeal species do have true histone homologues that form nucleosomes, wrapping chromosomal DNA (19). In addition to histone proteins, many archaeal species also possess the DNA-binding protein Alba (Sso10b) (20). Reversible acetylation of Alba has been demonstrated, and this is a potential mechanism for the regulation of transcription at the level of chromatin, analogous to the histone modification systems in eukaryotes (21). *In vitro* transcription using purified RNA polymerase, TFB and TBP is active when using a naked DNA template, but is severely inhibited in the presence of recombinant Alba (21). This suggests that transcription from archaeal chromatin may require protein accessory factors additional to the core set identified to date, and parallels have been drawn between the covalent modification of the Alba protein by acetylation and the covalent modification of histone tails in eukaryotic chromatin (21).

All cellular organisms and many viruses encode an abundant single-stranded DNA (ssDNA)-binding protein [SSB in bacteria, replication protein A (RPA) in eukarya]. SSBs have vital functions in many different DNA processing pathways, including DNA replication, recombination and repair, whenever ssDNA is generated. In *Sulfolobus solfataricus* and other crenarchaea, SSB has a domain organization strikingly similar to that of *Escherichia coli* SSB, with a single OB-fold (oligonucleotide-binding fold) domain for ssDNA binding, followed by a flexible spacer and

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acidic C-terminal tail (22). Whilst this domain organization is reminiscent of the bacterial protein, the structure of the OB-fold domain of *Sulfolobus* SSB shows its close relationship with the OB-folds in the eukaryotic RPA protein (23). As the tail of *Sulfolobus* SSB plays no part in ssDNA binding, it has been thought to function in the recruitment of other proteins to ssDNA regions, as has been observed for the *E.coli* SSB tail (24,25).

In this study, we report a specific interaction of the RNA polymerase from *S.solfataricus* with the SSB, mediated by the C-terminal tail of SSB. We demonstrate that SSB stimulates transcription under TBP-limiting conditions, and in the presence of the chromatin protein Alba. We provide evidence that SSB can melt AT-rich promoter sequences specifically, and postulate a model whereby SSB has a role in promoter opening and RNA polymerase recruitment. We further show that SSB is capable of functionally replacing TBP in transcription assays *in vitro*.

MATERIALS AND METHODS

Cloning and expression of GST fusion proteins

The full-length SSB gene (Sso2364; protein accession number Q97W73) was amplified from *S.solfataricus* chromosomal DNA by PCR (Pfu polymerase, Promega) using the primers 5'-CGT CGG ATC CCC ATG GAA GAA AAA GTA GGT AAT CTA AAA CC and 5'-CCG GGG ATC CGT CGA CTC ACT CCT CTT CAC CTT CTT CGT TTT. A fusion of full-length SSB to the C-terminus of the GST protein was constructed by cloning the PCR product into the BamHI-SalI sites of pGEX5x-3 (Amersham Pharmacia) to create vector pGEX5x-SSB.

A C-terminally truncated version of the GST-SSB fusion protein was constructed by site-directed mutagenesis of the pGEX5x-SSB plasmid using the primers 5'-GCTCCTCAGCAAATGCGTTAAGGAGGAAGAGG and 3'-CCTCTTCC-TCCCTAACGCATTTGCTGAGGAGC to introduce a stop codon in place of residue 120, leading to expression of GST-SSB lacking the final 29 residues of the C-terminal tail of SSB (named GST-SSB Δ C).

A GST fusion with the last 22 residues of the SSB C-terminal tail was constructed by annealing the following two complementary oligonucleotides and ligation into the BamHI and SalI sites of pGEX-5x3 (creating plasmid pGEX5x-tail, sequence: GST-GGRRYGRRGRRQENEE-GEEE); SSB Tail 5'-GATCCCCGGTGGGGGAAGAAGG-TATGGAAGAAGAGGTGGTAGAAGACAAGAAAACGA-AGAAGGTGAAGAGGAGTAAG; SSB Tail 3'-TCGACT-TACTCCTCTTCACCTTCTTCGTTTTCTTGTCTTCTACC-ACCTCTTCTTCCATACCTTCTTCCCCCACC GGG.

Protein purification

TBP, TFB, SSB and Alba were expressed and purified as described previously (11,12,22,26). TBP and TFB proteins were from *Sulfolobus acidocaldarius*, expressed in *E.coli*. They display 82–88% sequence identity with the equivalent proteins from *S.solfataricus*. RNA polymerase was prepared from a cell extract of *S.acidocaldarius* as described previously (27). SSB and Alba were from *S.solfataricus*, expressed in *E.coli*. SSB lacking the C-terminal tail (SSB Δ C) was prepared

by limited trypsinolysis and gel filtration and checked by mass spectrometry, as described previously (28). Cell pellets containing GST fusion proteins were resuspended in 35 ml of buffer G [20 mM Tris pH 7.6, 1 mM dithiothreitol (DTT), 15% glycerol, 1 M NaCl]. This was sonicated for 4 \times 2 min on ice then centrifuged at 48 000 *g* for 30 min. The supernatant was mixed with 2 ml of glutathione-agarose beads (Sigma) with rotation at 4°C for 2 h. The beads were washed in 4 \times 20 ml of buffer G for 10 min rotating at 4°C to remove unbound proteins.

GST affinity chromatography

Columns (5 ml disposable polypropylene columns; Pierce) were prepared using purified GST, or GST fusion proteins bound to glutathione-agarose beads (prepared as described above). Protein levels of GST fusions were compared on Coomassie R250-stained NuPAGE gels. Protein levels were equalized for each column by addition of agarose beads to give a uniform protein concentration. Columns with a 1 ml bed volume were equilibrated with 10 vols of column buffer [50 mM HEPES pH 7.5, 150 mM KCl, 0.2 mM EDTA, 5 mM MgCl₂, 10% glycerol, 1 mM DTT, 0.05% NP-40, 0.2 mM phenylmethylsulfonyl fluoride (PMSF)]. Whole-cell extracts of *S.solfataricus* were prepared from 6 g of cell paste. Cells were resuspended in 4 vols of column buffer. This was sonicated for 4 \times 2 min on iced water then centrifuged at 48 000 *g* for 30 min (4°C). Cell lysate was then passed through a 0.45 μ m filter. Cell lysate was allowed to pass through a GST-agarose pre-clearing column by gravity flow before being applied to the affinity column and again allowed to pass through by gravity flow. All experiments were performed at 4°C. Columns were then washed with 10 column volumes of column buffer containing 250 mM KCl. Interacting proteins were eluted with column buffer containing 500 mM KCl. Higher concentrations of KCl did not result in elution of different proteins from the columns.

Western blotting and immunoprecipitation

Whole-cell extracts were prepared from *S.solfataricus* cell paste in 10 vols of buffer A (20 mM Tris pH 7.6, 150 mM NaCl, 5 mM MgCl₂, 10% glycerol and 1 mM DTT), as described above. Immunoprecipitation of RNA polymerase and interacting proteins was carried out by the addition of 5 μ l of anti-RNA pol B' sera (13) to 100 μ g of cell extract in a total volume of 100 μ l of buffer A. This mixture was incubated on a rotating wheel at room temperature for 30 min prior to the addition of 10 μ l of protein A-agarose (Sigma) followed by incubation for a further 30 min at room temperature with rotation. Beads were allowed to settle for 10 min and the supernatant was removed. Beads were subsequently washed a further four times in buffer A (100 μ l). After the final wash, beads were resuspended in 20 μ l of NuPAGE loading buffer (Invitrogen). For western blotting, proteins were transferred from NuPAGE gels to nitrocellulose membranes (Amersham) by semi-dry blotting. These membranes were probed with SSB antibodies diluted 1:2000 according to standard procedures.

DNA pull-down assays

DNA pull-down assays were performed using the EcoRI-HindIII T6 promoter fragment from pBluescriptT6 (13). The pBluescriptT6 plasmid (100 μ g) was digested with EcoRI and

HindIII (MBI fermentas) to release the T6 promoter fragment. The T6 promoter fragment was purified on a 1% agarose TBE gel followed by elution using a QIAquick Gel extraction kit (Qiagen). The purified T6 promoter DNA was then end-labelled with biotinylated dUTP using Klenow fragment (MBI fermentas) before being bound overnight at room temperature to 200 μ l of streptavidin-agarose beads (Sigma). DNA pull-down assays were performed in a total of 50 μ l of buffer S (20 mM MES pH 6.5, 1 mM MgCl₂, 100 mM potassium glutamate, 10% glycerol, 0.05% NP-40) containing 10 μ l of T6 streptavidin-agarose beads. The proteins indicated were incubated with T6 streptavidin beads for 30 min at 4°C on a rotating wheel. After incubation, beads were allowed to settle on ice for 10 min. Supernatant was removed and beads were washed a further three times in 250 μ l of buffer S. Each time, beads were placed on ice and allowed to settle before the supernatant was removed. Interacting proteins were detected by western analysis following separation on NuPAGE gels.

***In vitro* transcription assays**

In vitro transcription assays were performed essentially as described previously (11). Transcripts were detected by primer extension analysis using a large molar excess of T7 primer. Both the T6 and 16S rRNA promoters were cloned into the pBluescript vector as described previously (29). All assays presented here were performed on negatively supercoiled DNA template (100 ng of plasmid DNA per reaction). Protein concentrations were as used previously (30 nM TFB, 50 nM TBP). In TBP limiting assays, TBP was reduced from 20 to 4 ng (10 nM) per reaction. Transcripts were analysed by denaturing polyacrylamide-urea gel electrophoresis. Following electrophoresis, gels were exposed to phosphorimage plates (Fuji), the plates were scanned using a Fuji FLA-5000 imaging system and transcripts were quantified using Imagegauge software (Fuji).

Single round transcription assays

Assays were performed essentially as previously described (29). Re-initiation of transcription was blocked by the addition of sarkosyl to a final concentration of 1%, as described for eukaryal transcription (30). Sarkosyl has been shown to have the same effect as heparin in preventing re-initiation of archaeal transcription *in vitro* (S.D.Bell, unpublished). Transcripts were analysed by denaturing polyacrylamide-urea gel electrophoresis. Following electrophoresis, gels were exposed to phosphorimage plates (Fuji), the plates were scanned using a Fuji FLA-5000 imaging system and transcripts were quantified using Imagegauge software (Fuji).

DNA melting assays

Radiolabelled duplex DNA was prepared by end-labelling one strand (100 pmol) using polynucleotide kinase (MBI Fermentas) and [γ -³²P]ATP (10 μ Ci, Amersham). The kinase reaction was stopped by heating to 90°C for 15 min. Equimolar amounts of the radiolabelled strand and the complementary strand were added to buffer M and heated to 90°C in a water bath before being allowed to cool overnight to room temperature. Duplex DNA was purified using a G50 spin size exclusion column (Amersham).

Four different duplex DNA species were constructed using the following pairs of oligonucleotides: rRNA promoter

upstream duplex, 5'-GCCGTTTATATGGGATTCAGAA-CAATATGTATAATGCGG and 5'-CCGCATTATACATA-TTGTCTGAAATCCCATATAAACGGC; rRNA promoter downstream duplex, 5'-GCCGGCGGATGCCCCCGGG-AGAAACTCCCGCCGCGG and 5'-CCGCGGCGGGA-GTGTCTCTCCCGGGGGGCATCCGCGCGC; T6 promoter upstream duplex, 5'-GCCGTTTAAATACTTATATAGATAGATAGATAGCGG and 5'-CCGCTATCTATACTCTATCTATATAAGTATTTAAACGGC; and T6 promoter downstream duplex, 5'-GCCGGAGGGTTCAAAAATGG-TTTCACCCCAAACGCGG and 5'-CCGCGTTTGGGGTG-AAACCATTTTTGAACCCTCCGGC.

The melting assays were performed at the indicated temperatures in buffer M (20 mM MES pH 6.5, 1 mM MgCl₂, 100 mM potassium glutamate). In experiments where Alba protein was included, it was added 5 min prior to addition of SSB. Following addition of SSB, samples were incubated for the indicated times and then loaded on to native polyacrylamide gels (8% polyacrylamide, TBE buffer, 130 V for 3 h) to separate double- and single-stranded species. Following electrophoresis, gels were dried and phosphorimaged as described above.

Mass spectrometry

Peptide mass fingerprint data were obtained by in-gel trypsin digests of Coomassie-stained gel slices using a ProGest Investigator robot (Genomic solutions), using a method based on Shevchenko *et al.* (31). Half of the digested sample was concentrated and purified by reverse phase chromatography and analysed by MALDI-TOF mass spectrometry (Top Spec ZE, Micromass, UK) and BioLynx Software.

RESULTS

***Sulfolobus* SSB interacts with RNA polymerase**

To identify proteins interacting with *S.solfataricus* SSB, we constructed and expressed a fusion of GST with full-length SSB (Fig. 1a). Soluble protein extract from *S.solfataricus* was passed over an affinity column with immobilized GST-SSB (as well as a GST control column), and washed with increasing concentrations of KCl to elute interacting proteins. Proteins eluting from the column were trichloroacetic acid (TCA) precipitated, separated by SDS-PAGE (Fig. 1b), and identified by in-gel trypsin digestion and MALDI-TOF mass spectrometry followed by interrogation of the complete *S.solfataricus* sequence database. A number of proteins were observed to interact with *Sulfolobus* SSB and to elute from the affinity column at high ionic strength (Fig. 1b). Five of the strongest bands were identified as subunits of RNA polymerase. These five proteins were identified from three independent experiments. The results were identical when the affinity chromatography was carried out in the presence of ethidium bromide (100 μ g ml⁻¹) (Fig. 1b, lane 4), with the same five RNA polymerase subunits identified by mass spectrometry. As ethidium bromide prevents the interaction of RNA polymerase with DNA (32), this suggests a direct interaction between SSB and RNA polymerase. Immunoprecipitation of *Sulfolobus* cell extracts using anti-RNA polymerase B' antibodies followed by western blotting to identify SSB in the immune precipitates confirmed that the interaction occurs under physiological conditions (Fig. 1c).

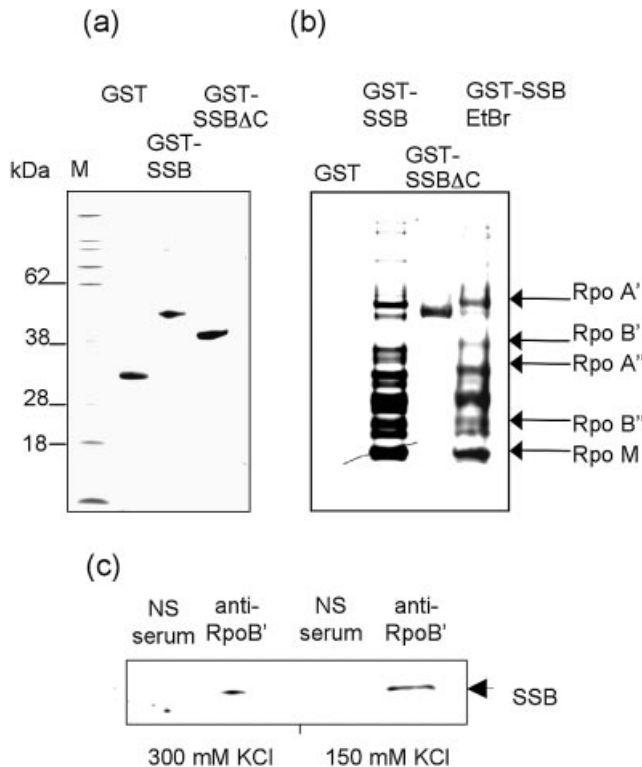


Figure 1. SSB interacts with RNA polymerase. (a) SDS-polyacrylamide gel showing purified recombinant GST, GST-SSB and GST-SSB Δ C, and molecular weight markers (M). (b) Affinity purification of proteins interacting with SSB. Coomassie R250-stained NuPAGE gel of proteins from a *Sulfolobus* total cell extract that interact with the following affinity columns: GST control, GST-SSB, GST-SSB Δ C and GST-SSB in the presence of 100 μ g ml⁻¹ ethidium bromide. Interacting proteins were eluted with a 500 mM KCl wash. Arrows indicate RNA polymerase (Rpo) subunits identified (at least three times by mass spectrometry in independent experiments) after elution from the columns containing full-length SSB. (c) SSB and RNA polymerase co-immunoprecipitate from *Sulfolobus* cell extracts. Immune precipitations using anti-RNA polymerase B' antibodies (or pre-immune serum controls) were carried out from *S.solfataricus* cell extracts (10 mg) in buffers containing 150 or 300 mM KCl. The protein samples obtained on precipitation were analysed by SDS-PAGE, and probed for the presence of SSB using a sheep polyclonal antibody raised against recombinant SSB protein. Bands corresponding to SSB are indicated with an arrow.

SSB interacts with RNA polymerase via its C-terminal acidic tail

To confirm that SSB interacts with RNA polymerase via the C-terminal tail of SSB, we constructed a GST-SSB fusion lacking the SSB C-terminal 29 amino acids, GST-SSB Δ C (Fig. 1a, lane 4). GST affinity chromatography with this construct gave dramatically different results, with no RNA polymerase subunits detectable (Fig. 1b, lane 3). A single strongly interacting protein was observed which has not yet been identified. Similarly, a GST fusion containing only the last 22 amino acids of the SSB C-terminal tail also resulted in the identification of the same subunits of RNA polymerase (experiment repeated twice, data not shown). Thus, SSB appears to interact specifically with RNA polymerase via the C-terminal tail of SSB.

SSB activates transcription *in vitro*

The tight physical interaction observed between *Sulfolobus* SSB and RNA polymerase raised the possibility that SSB plays an active role in transcription. This was tested using the well characterized *in vitro* transcription system comprising the *Sulfolobus* T6 SSV promoter and the 16S rRNA promoter (Fig. 2a), purified RNA polymerase and recombinant TFB and TBP (13). Addition of SSB to a standard reconstituted transcription assay had little effect on transcription levels (Fig. 2b, top panels). However, when TBP levels were reduced from 20 ng per reaction to 4 ng, resulting in a reduction in basal transcription levels, addition of SSB resulted in a 5-fold (T6) and an 8-fold (16S) activation of transcription (Fig. 2b, middle panels), assessed by quantitative phosphorimaging of transcription assays. This is a more dramatic effect than that observed for the transcription factor TFE under similar conditions, where a 2-fold enhancement was observed (33). The activation requires the presence of the C-terminal tail of SSB (Fig. 2b, bottom panel), indicating that activation is dependent on the physical interaction of SSB with RNA polymerase and is not a consequence simply of ssDNA binding by SSB. Higher levels of SSB appear to have an inhibitory effect on transcription.

SSB acts at the level of recruitment/initiation

The transcription assays described above measure transcript levels following multiple rounds of initiation. Therefore, the stimulation of transcription detected could be a consequence of either enhanced initiation rates within a given cycle of transcription or an increase in premature termination, allowing RNA polymerase to re-initiate repeatedly during an assay. To discriminate between these possibilities, we carried out transcription assays in the presence of sarkosyl, which prevents re-binding of RNA polymerase to the promoter and thus limits transcription to a single round (30). As SSB still activated transcription strongly under these conditions (Fig. 2c), this suggests a role for SSB in the stimulation of RNA polymerase recruitment and/or initiation, rather than an artificial increase in transcription levels by increasing the frequency at which RNA polymerase dissociates from the DNA, allowing re-initiation.

SSB supports transcription in the absence of TBP

TBP is usually considered an essential component for transcription initiation. In the absence of added TBP, there was no transcription on the T6 promoter in the presence of TFB and RNA polymerase (Fig. 3a, lane 1). However, addition of SSB (0.5–5 μ M) restored transcriptional activity to levels equivalent to those observed when TBP was present at non-limiting conditions. The presence of TBP in the RNA polymerase sample used for these experiments was undetectable by western blotting with an anti-TBP antibody (data not shown), suggesting TBP is present at only very low levels, if at all. Thus SSB appears able to replace TBP to support initiation of transcription. This phenomenon requires the presence of TFB. Removal of both TBP and TFB resulted in a complete loss of transcriptional activity that could not be restored by addition of SSB (Fig. 3b). This suggests that SSB can substitute for TBP at the promoter and support TFB-dependent transcriptional initiation.

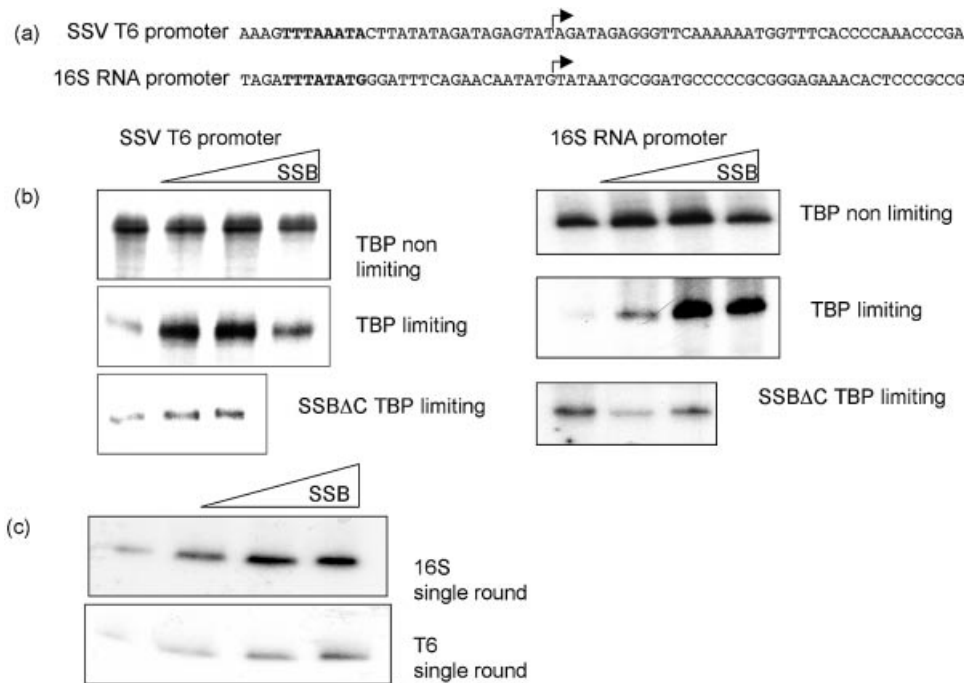


Figure 2. *In vitro* transcription assays show stimulation of transcription by SSB. (a) DNA sequences of the SSV T6 promoter and the 16S rRNA promoter used in these studies. The transcription start sites are indicated by arrows and the TATA boxes are in bold. (b) The effects of full-length SSB and C-terminally truncated SSB (SSBΔC) on transcription from the T6 (left panels) and 16S rRNA (right panels) promoters. Final concentrations of SSB used in the assays were 0.5, 1 and 5 μ M. Top panels: TBP present at 20 ng per reaction; middle panels, TBP levels at 4 ng per reaction. At limiting concentrations of TBP, transcriptional activity was severely reduced (compare the first lanes in the top and middle panels for each promoter) and is rescued by addition of SSB (0.5, 1 and 5 μ M SSB). The bottom panels show that addition of the SSBΔC protein (0.5 and 1 μ M) had no stimulatory effect on transcription. (c) Single round transcription assays were carried out in the presence of 1% sarkosyl, which prevents reinitiation by RNA polymerase. Under these conditions, addition of SSB (0.5, 1 and 5 μ M) results in a strong stimulation of transcription. TBP was present in limiting amounts (4 ng per reaction).

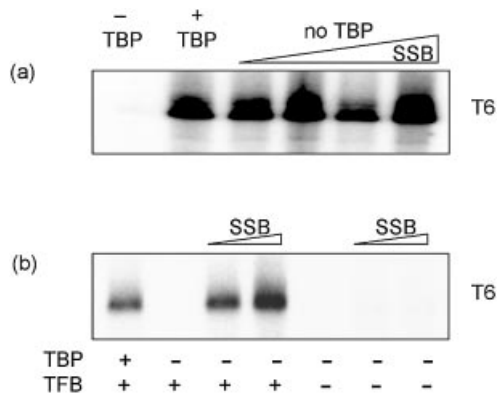


Figure 3. SSB can replace TBP, supporting transcription in the presence of TFB. (a) In the absence of TBP, there is no transcription from the T6 promoter in the presence of TFB and RNA polymerase (lane 1). Addition of SSB (0.1, 0.5, 1 and 5 μ M) restores transcriptional activity to levels equivalent to those observed when TBP is present. (b) The ability of SSB to replace TBP in transcription assays requires the presence of TFB. Removal of both TBP and TFB results in a complete loss of transcriptional activity that is not restored by addition of SSB (0.5, 1 μ M).

SSB relieves transcriptional repression by the chromatin protein Alba

Most studies of archaeal transcription have utilized naked DNA. However, the physiological substrate *in vivo* is archaeal chromatin. One of the major chromatin protein components in

S. solfataricus is the Alba (Sso10b) protein, which is present in all hyperthermophiles (34). The repressive effect of Alba on transcription *in vitro* has been demonstrated previously (21), and was confirmed here (Fig. 4b). However, when the same assays were then performed in the presence of SSB, we observed a dramatic increase in transcriptional activity from both the SSV T6 and 16S RNA promoters (Fig. 4a and b), suggesting an efficient relief of transcriptional repression. These conditions are likely to be much closer to the physiological conditions for archaeal transcription *in vivo*, as both Alba and SSB are known to be abundant proteins in *Sulfolobus* (21,22). The ability of SSB to rescue transcription from repression by Alba *in vitro* requires the presence of the SSB C-terminal tail, as an SSB lacking the C-terminal tail (SSBΔC) failed to stimulate transcription (Fig. 4b). As the truncated mutant retains the ability to bind to ssDNA (22), this suggests that the specific interaction of SSB with RNA polymerase is essential for stimulation of transcription under these conditions.

Chromatin remodelling by SSB allows assembly of TBP and TFB at promoters and relieves transcriptional repression by Alba

The high level of stimulation of transcription by SSB on Alba-coated templates suggests a role for SSB *in vivo* may be in overcoming Alba-mediated repression. One possibility is that SSB helps the TBP and TFB proteins overcome local chromatin structure to initiate transcription; in other words,

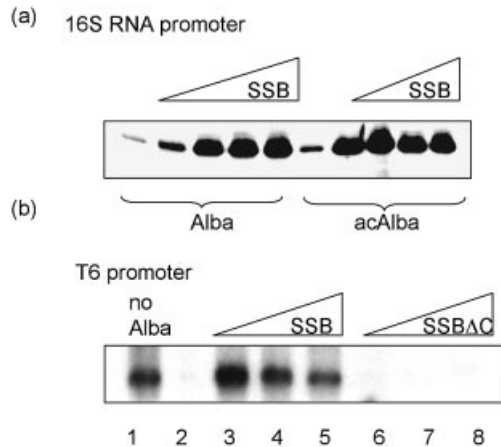


Figure 4. Repression of transcription by Alba can be reversed by full-length SSB. (a) The presence of the archaeal chromatin protein Alba (either recombinant or native acetylated protein at a concentration of 5 μ M) to *in vitro* transcription assays using the 16S rRNA promoter resulted in strong repression of transcriptional activity. This repression was reversed by addition of SSB (0.1, 0.5, 1 and 5 μ M), with a maximal effect between 0.5 and 1 μ M SSB. (b) Repression of transcription from the T6 promoter by recombinant Alba (lane 2) is reversed by addition of 0.5, 1 and 5 μ M full-length SSB (lanes 3–5) but not by SSBAC at the same concentrations (lanes 6–8).

SSB remodels chromatin at the promoter site. In support of this, an experiment to monitor TBP binding to the T6 promoter in the presence of Alba shows that this is dependent on the presence of SSB (Fig. 5). A biotinylated DNA duplex corresponding to the T6 promoter was used to monitor the ability of TBP to bind to the promoter in the presence of recombinant Alba. After pull-down and washing of the biotinylated DNA, the presence of the TBP protein was detected using a rabbit polyclonal antibody. In the presence of Alba, TBP was effectively excluded from binding to the promoter (lane 4). Even in the presence of all the components required for the formation of the pre-initiation complex (RNA polymerase, TBP and TFB), no TBP could be detected (lane 5). This is consistent with the observation that promoter DNA reconstituted with Alba is transcriptionally repressive. The interaction of TBP with the promoter could only be detected when SSB was included along with all the components required for the formation of the pre-initiation complex (lane 4). TBP binding to the promoter was not observed when TFB was absent and SSB present (lane 2), confirming previous observations that TBP has only weak DNA binding affinity in the absence of TFB (35). Thus, SSB appears to aid the formation of the pre-initiation complex at a promoter site when archaeal chromatin is reconstituted *in vitro*.

SSB melts promoter sequences in reconstituted chromatin

In eukaryotes, chromatin-remodelling proteins play a vital role in opening DNA regions to allow access for the transcription machinery. SSBs have the ability to destabilize DNA duplexes by trapping transient breathing intermediates in a single-stranded form, and thus have the potential to disrupt chromatin structure (36). Thus, it seemed plausible that the stimulation of transcription by SSB related to its ability to melt duplex DNA and disrupt chromatin structure transiently around promoters,

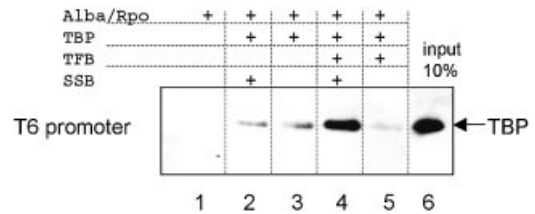


Figure 5. SSB facilitates binding of TBP to the T6 promoter in the presence of Alba. TBP does not bind a biotinylated T6 promoter sequence in the presence of Alba (lane 1), even in the presence of all the components required for the formation of the pre-initiation complex (RNA polymerase, TBP and TFB) (lane 5). Addition of SSB allows TBP binding to the promoter DNA duplex (lane 4). This effect is dependent on the presence of TFB (lane 2). Protein concentrations used in this assay were as follows: RNAP, 0.5 μ g; TBP, 2 μ g; TFB, 2 μ g; SSB, 0.5 μ M; Alba, 5 μ M.

giving access to the transcriptional apparatus. We investigated the ability of SSB to melt sequences around the promoters of the 16S rRNA promoter, and the SSV T6 promoter. These and many other *Sulfolobus* promoters have an AT-rich region at the transcription start site and a GC-rich sequence downstream of the initiation codon (Fig. 2a). Duplex DNA species corresponding to the upstream and downstream regions of these two promoters were constructed and purified, with one strand labelled with [32 P]ATP as described in Materials and Methods. All four duplexes had identical 4 bp GC clamps at either end to minimize differences due to duplex fraying at the termini. Following incubation for set times with SSB and/or Alba, the DNA duplex species were analysed by native polyacrylamide gel electrophoresis to separate double- and single-stranded species. At 40°C, SSB melted the upstream promoter regions but not the downstream regions of both promoters (Fig. 6a). This was probably due to the differential stability of the respective duplexes arising from their differing GC content. At the more physiological temperature of 70°C, all duplexes were melted efficiently by SSB. Melting could be inhibited by the presence of Alba, consistent with a role for Alba in the stabilization of double-stranded DNA at high temperatures (Fig. 6b). However, addition of SSB to DNA duplexes coated with Alba resulted in a loss of protection of the upstream AT-rich promoter duplex, which was melted under conditions where the downstream duplex remained in duplex form (Fig. 6b). As all archaeal promoters have at least some AT-rich character due to the ubiquitous TATA box, this may allow selective melting of promoter regions by SSB. DNA duplexes containing TATA box sequences have been shown to have conformational flexibility and local bending (37)

DISCUSSION

Archaeal transcription can be reconstituted *in vitro* using promoter DNA, purified RNA polymerase and recombinant basal transcription factors TBP and TFB. There is thus no absolute requirement for the large number of transcription accessory factors required in eukaryal transcription (29). Reconstitution of archaeal chromatin by addition of the DNA-binding protein Alba, however, results in the efficient repression of transcriptional activity *in vitro* (21), suggesting that a requirement for mechanisms to overcome chromatin-

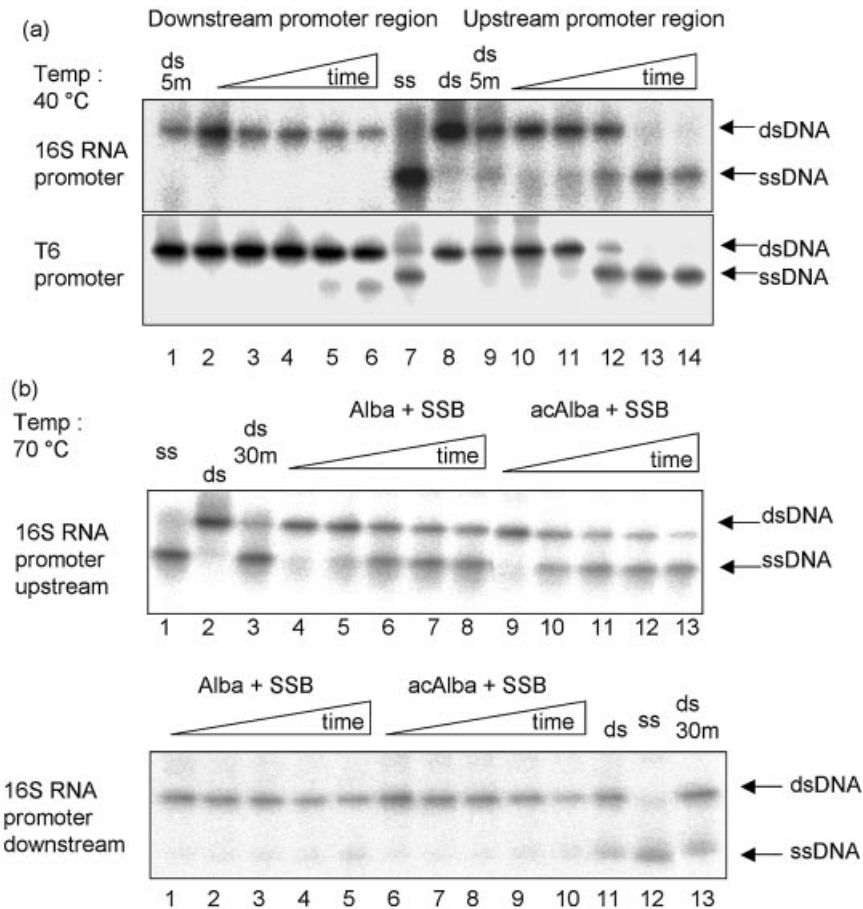


Figure 6. SSB melts DNA promoter sequences in the absence and presence of Alba. **(a)** For each duplex DNA species, SSB (2.5 μ M) was added and incubated at 40°C. Samples were analysed for DNA melting by non-denaturing gel electrophoresis at 0, 15 and 30 s, and 1 and 5 min time points (lanes 2–6 and 10–14). SSB melted both the 16S rRNA and T6 upstream promoter regions (lanes 10–14) but had little effect on the downstream promoter regions of either promoter (lanes 2–6). Control lanes for all gels are as follows: ss, single-stranded DNA marker; ds: duplex DNA marker; ds 5m, duplex DNA incubated for 5 min at the indicated temperature without proteins; ds 30m, duplex DNA incubated for 30 min at the indicated temperature without proteins. **(b)** DNA melting assays repeated as in (a) after reconstitution of archaeal chromatin by addition of recombinant or acetylated Alba (5 μ M) to the 16S rRNA promoter duplexes. After incubation with 2.5 μ M SSB at 70°C, samples were taken at 0 and 30 s, and 1, 5 and 30 min time points and analysed as before. Alba protected the downstream promoter region from SSB-induced melting even after 30 min at 70°C (lower panel). However, the upstream promoter region, containing the TATA box sequence, was efficiently melted by SSB (top panel).

mediated transcriptional repression may be relevant *in vivo*. We have demonstrated a specific physical interaction between *Sulfolobus* SSB and RNA polymerase that is independent of DNA and requires the acidic C-terminal tail of SSB. One of the functional consequences of this interaction is to relieve the repression of transcription from reconstituted chromatin.

The physical basis for stimulation of transcription by *Sulfolobus* SSB is likely to relate to SSB's ability to bind ssDNA and destabilize DNA duplexes. An interaction mediated by the C-terminal tail of SSB leaves the ssDNA-binding domain free to assume this function. We have shown that SSB can melt duplex DNA sequences around the TATA box of two *Sulfolobus* promoters and that this probably relates to the highly AT-rich composition of these sequences. Promoter DNA duplexes coated by Alba are amenable to selective melting by *Sso*SSB at temperatures approaching physiological relevance for *Sulfolobus*, which grows at 80°C.

Localized disruption of chromatin structure at promoters by SSB allows efficient initiation of transcription by facilitating the binding of the TBP and TFB proteins. However, this is not

sufficient in itself, as a C-terminally truncated form of SSB, which retains the ability to bind ssDNA (22), does not stimulate transcription. Thus the role of SSB in transcription appears to be 2-fold: disruption of chromatin structure at the promoter and recruitment of RNA polymerase to form the pre-initiation complex. Figure 7 shows a schematic representation of the potential roles of SSB in archaeal transcription *in vivo*. Previous work has established that the archaeal basal transcription machinery could be distilled down to the TBP, TFB and RNA polymerase proteins (Fig. 7a), and that reconstitution of this machinery catalysed transcription from naked DNA promoters *in vivo* [reviewed in Bell and Jackson (15) and Reeve (38)]. We have shown that RNA polymerase forms a stable and specific complex with SSB in solution, and that SSB can substitute for TBP, supporting transcription *in vitro* in the presence of TFB and RNA polymerase. Importantly, transcription using SSB is dependent on TFB, and appears to have the same start site in model promoters as transcription with TBP. This suggests that the pre-initiation complex assembles in the same way, with conservation of the

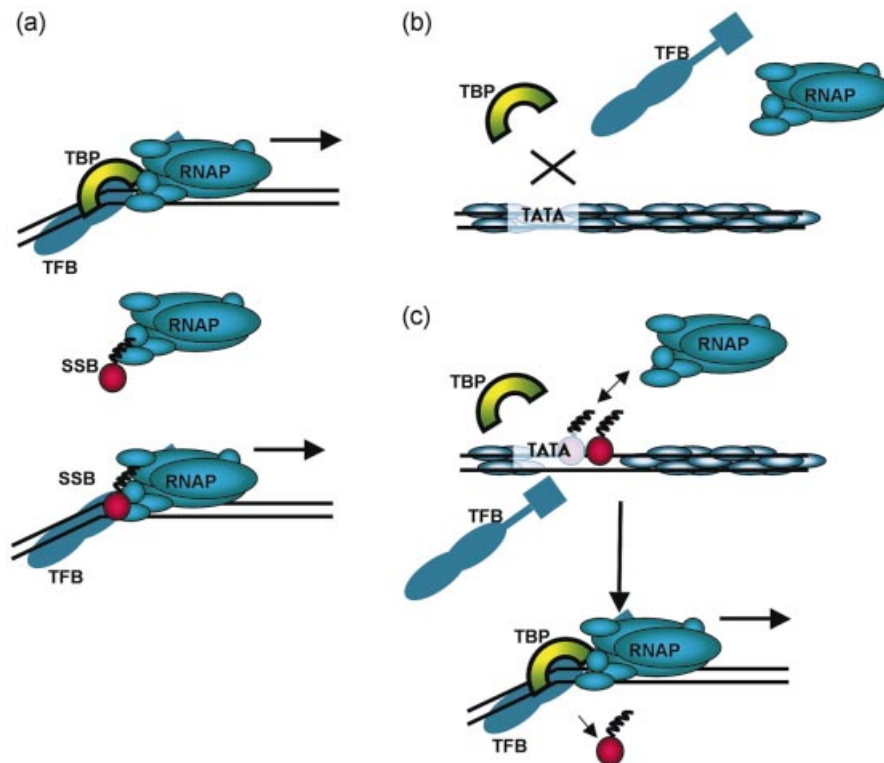


Figure 7. The role of SSB in archaeal transcription. (a) The accepted model for basal transcription requires assembly of TBP and TFB at the promoter site, followed by binding of RNA polymerase to form the pre-initiation complex that is competent for transcription. This model does not take account of the observed inhibitory effect of archaeal chromatin components such as Alba (21). We have demonstrated that *Sulfolobus* SSB interacts with RNA polymerase in solution via its C-terminal acidic tail, and that full-length SSB can substitute for TBP, supporting transcription from two model promoters in the presence of TFB and RNA polymerase. (b) An archaeal promoter reconstituted with the chromatin protein Alba does not support transcription by the basal transcription apparatus RNA polymerase, TFB and TBP [this work and Bell *et al.* (21)]. (c) We have shown that the *Sulfolobus* SSB protein can selectively melt (or remodel) reconstituted archaeal chromatin containing an AT-rich promoter sequence. SSB is required for binding of the TBP protein and formation of the pre-initiation complex at promoters in reconstituted chromatin. SSB reverses the repression of transcription observed when promoters are coated by the chromatin protein Alba, and this activity requires the C-terminal tail of the protein, demonstrating that an interaction with RNA polymerase is required rather than just the general ssDNA-binding affinity of SSB. SSB is likely to dissociate from RNA polymerase upon establishment of the pre-initiation complex.

interactions between TFB, bound at the BRE upstream of the TATA box, and RNA polymerase. The ability of SSB to specifically and efficiently initiate transcription in the absence of TBP is startling. Prior to this finding, TBP was believed to be an essential core transcription component. The requirement for TFB in SSB-driven transcription suggests strongly that we are observing a true substitution of SSB for TBP, rather than a non-specific effect. Furthermore, it is well established that TFB requires substantial deformation of the promoter to bind [e.g. Kosa *et al.* (18)]. Typically this is brought about by the induction of an $\sim 90^\circ$ bend by TBP; this bend, in conjunction with protein-protein interactions between TBP and TFB, defines the geometry of the ternary complex. We hypothesize that localized DNA melting in the vicinity of the TATA box, mediated by SSB, may overcome this requirement for TBP. Whilst most canonical promoters are likely to utilize TBP in preference to SSB, our results suggest a re-assessment of the situations in which transcription might occur independent of TBP, e.g. non-canonical promoters lacking a clear TATA box, or when TBP becomes depleted in the cell as a result of DNA damage or other stresses.

We have also shown that transcription *in vitro* is repressed once promoter DNA has been reconstituted with the abundant

chromatin protein Alba (Fig. 7b), confirming previous findings (21). We have demonstrated a dramatic reversal of this repression by addition of the SSB, probably a result of the ability of SSB to melt promoter sequences transiently, and shown that this rescue requires a specific interaction between SSB and RNA polymerase through the C-terminal tail of SSB (Fig. 7c). We predict that SSB dissociates from RNA polymerase upon establishment of the pre-initiation complex with TFB and TBP (preliminary data not shown).

These data should prompt a re-assessment of the basal transcription machinery of archaea. As transcription *in vivo* takes place in the context of chromatin rather than naked DNA, the SSB may be an important component of the transcriptional machinery *in vivo*. It should be noted that not all archaeal SSBs have an acidic C-terminal tail. The specific mode of interaction of SSB with RNA polymerase in other species is therefore likely to vary. The inhibition of transcriptional activity by chromatin proteins has also been demonstrated in the euryarchaeon *Pyrococcus furiosus* (39). It would be fascinating to discover whether the *Pyrococcus* SSB can rescue transcription under those conditions.

The data reported here might be relevant to our understanding of transcription initiation in eukarya. Recent work

with murine cells in which TBP has been knocked out demonstrated that, whilst RNA polymerase I and III transcription was shut down in the absence of TBP, RNA polymerase II transcription continued at a high level (40). The factor or factors substituting for TBP in mammalian cells have not yet been identified. It is possible that SSB in *Sulfolobus* represents a forerunner of the TBP-associated factors [TAF(II)s] present in TFIID in eukaryotes. The TFTC [TBP-free, TAF(II)-containing complex] has been shown to support TBP-independent transcription *in vitro* (41). One component of TFIID, hTAF(II)68, has been shown to have the ability to bind ssDNA/RNA (42), and could be a functional analogue of *Sulfolobus* SSB. It is possible, for example, that other TAF(II)s could harbour undetected OB-folds capable of ssDNA binding.

Our findings may also be pertinent to the evolution of chromatin-remodelling proteins. *Sulfolobus* SSB, with no enzymatic activity and a simple ssDNA-binding function, represents one of the simplest proteins with this activity studied to date. This paradigm may extend to the much more complex remodelling factors in eukarya with the inclusion of OB-fold domains for ssDNA binding. Such domains are very hard to identify by sequence analysis, and usually make their presence known after structural studies; recent examples include OB-folds uncovered in the structures of BRCA2 (43) and RecG (44). We have detected orthologues of *Sulfolobus* SSB in the genomes of metazoa, and are currently assessing potential roles for these proteins in eukaryal transcription.

ACKNOWLEDGEMENTS

We thank Catherine Botting and Robin Antrobus for expert mass spectrometry services, Liza Cubeddu, Dorothee Götz, Clare Jelinska and Ross Wadsworth for helpful discussions, and Clare Jelinska and Joanne Parker for recombinant and native Alba protein. This work was funded by the BBSRC. M.F.W. is a Royal Society University Research Fellow.

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