The Chromatin Remodeling Protein, SRCAP, Is Critical for Deposition of the Histone Variant H2A.Z at Promoters*

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Studies in Saccharomyces cerevisiae indicate that the histone variant H2A.Z is deposited at promoters by the chromatin remodeling protein Swr1 and plays a critical role in the regulation of transcription. In higher eukaryotes, however, little is known about the distribution, method of deposition, and function of H2A.Z at promoters. Using biochemical studies, we demonstrated previously that SRCAP (SNF-2-related CREB-binding protein activator protein), the human ortholog of Swr1, could catalyze deposition of H2A.Z into nucleosomes. To address whether SRCAP directs H2A.Z deposition in vivo, promoters targeted by SRCAP were identified by a chromatin immunoprecipitation (ChIP)-on-chip assay. ChIP assays on a subset of these promoters confirmed the presence of SRCAP on inactive and active promoters. The highest levels of SRCAP were observed on the active SP-1, G3BP, and FAD synthetase promoters. Detailed analyses of these promoters indicate sites of SRCAP binding overlap or occur adjacent to the sites of H2A.Z deposition. Knockdown of SRCAP levels using siRNA resulted in loss of overlap or occur adjacent to the sites of H2A.Z deposition. The highest levels of SRCAP were observed on inactive and active promoters where it has been postulated to provide the correct promoter architecture to facilitate activation of transcription (3–5). Activation of transcription results in decreased levels of H2A.Z and an increase in acetylated H2A.Z, which has been proposed to facilitate disassembly/reassembly of nucleosomes (6–8).

In higher eukaryotes, the genomic distribution and the biological function(s) of H2A.Z are poorly defined. In mammals, H2A.Z is essential for embryonic development and chromosome segregation, and increased H2A.Z expression is implicated in cardiac hypertrophy (9–11). Studies done in chicken cells suggest that deposition of both H2A.Z and acetylated H2A.Z is required at active promoters but not at inactive promoters (12, 13). The specific role that H2A.Z plays at active promoters in higher eukaryotes has not been established.

The exchange of H2A.Z into nucleosomes in S. cerevisiae has been demonstrated by genetic and biochemical approaches to be carried out by the catalytic subunit of the SWR-C complex, termed Swr1 (14, 15). A SRCAP complex, which is the human ortholog of the SWR-C complex, has recently been purified and found to catalyze in vitro incorporation of H2A.Z into chromatin (16). However, the ability of the SRCAP complex to direct H2A.Z deposition in vivo has not been demonstrated. A role of SRCAP in activation of transcription has been indicated by its ability to serve as a coactivator for several transcription factors including CREB, the glucocorticoid receptor, and androgen receptor (17).

The mechanism(s) by which SRCAP is targeted to specific promoters and regulates their transcription has not been elucidated. SRCAP recruitment might occur through interaction with coactivators such as the histone acetyltransferase CBP (17, 18), the p160 coactivator (GRIP1), and the methyltransferase CARM-1 (17). Alternatively, SRCAP may be recruited to nucleosomes containing specific histone modifications. Histone modifications found in promoters of active genes, e.g. triMetH3K4, acetylated H2B, and acetylated H4 have been reported to be enriched in H2A.Z-containing nucleosomes purified from chicken and HeLa cells (12, 13, 19). The loss of specific histone acetyltransferases GCN5 and Sas3 has also been reported to reduce H2A.Z deposition (5).
In this study, chromatin immunoprecipitation (ChIP) assays were used to analyze recruitment of SRCAP to promoters in human A549 cells. These studies demonstrate that although SRCAP is recruited to both active and inactive promoters, the highest amounts are present on active promoters. Using the SP-1, G3BP, and FAD synthetase promoters as model systems, we report that the sites of SRCAP recruitment overlap or occur adjacent to the sites of deposition of H2A.Z and acetylated H2A.Z. Knockdown of SRCAP expression results in decreased deposition of H2A.Z and acetylated H2A.Z and decreased levels of SP-1, G3BP, and FAD synthetase mRNA. These studies provide the first evidence supporting the hypothesis that SRCAP mediates in vivo deposition of H2A.Z.

MATERIALS AND METHODS

Antibodies—Anti-SRCAP affinity-purified rabbit polyclonal antibody was generated against SRCAP as described (16). Other commercial antibodies were RNA polymerase II 8WG16 monoclonal (Covance Research Products), tri-methylated lysine 4 of histone H3 polyclonal, Histone H2A.Z polyclonal, and Lys-4, Lys-7, and Lys-11 acetylated on histone H2A.Z polyclonal (Abcam) and mouse monoclonal anti-β-actin antibody (Sigma).

Cell Culture—The human A549 lung adenocarcinoma cell line (ATCC) was cultured in the Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% fetal bovine serum (Cellgro) and penicillin/streptomycin (Invitrogen). Media (Invitrogen) was supplemented with 1% formaldehyde and was stopped by the addition of glycine to a final concentration of 125 mM. The cells were harvested with cold phosphate-buffered saline, pelleted in 0.1% Nonidet P-40, 1 mM dithiothreitol, and protease inhibitors (Roche Applied Science). The cells were homogenized 15 times with the tight pestle of a Dounce homogenizer. The resulting nuclei were collected by centrifugation at 500 × g for 5 min at 4 °C, and resuspended in 25 mM HEPES (pH 7.8), 1.5 mM MgCl₂, 10 mM KCl, 0.1% Nonidet P-40, 1 mM dithiothreitol, and protease inhibitors (Roche Applied Science). The cells were homogenized 15 times with the tight pestle of a Dounce homogenizer. The resulting nuclei were collected by centrifugation at 500 × g for 5 min at 4 °C, and resuspended in 25 mM HEPES (pH 7.8), 10 mM EDTA, 0.5 mM EGTA, 140 mM NaCl, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS, and protease inhibitors (Roche Applied Science)). The nuclei were sonicated at 12 20-s bursts with 1 min of cooling on ice in between each burst (output 5, constant setting, Branson Sonifier 450) to shear chromatin to ~0.3–1-kb fragments. Samples were diluted 2-fold in sonication buffer and preclarified to reduce nonspecific background with protein A/G-agarose slurry (Santa Cruz Biotechnology) in Sonication buffer. The preclarified extract was incubated overnight with 5 μg of the antibody at 4 °C. A 50-μl sample was set aside as the input sample. The immune complex was recovered with protein A/G-agarose slurry by 2 h of incubation at 4 °C. The bead complexes were washed twice for 5 min each with the following buffers in succession ordered sequence: low salt wash buffer (sonication buffer), high salt wash buffer (50 mM HEPES (pH 7.8), 500 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, and 0.1% SDS), LiCl wash buffer (20 mM Tris-HCl (pH 8.0), 1 mM EDTA, 250 mM LiCl, 0.5% sodium deoxycholate, and 0.5% Nonidet P-40), and TE buffer (10 mM Tris-HCl and 1 mM EDTA (pH 8.0)). Precipitated chromatin complex was eluted from beads through two 1-h incubations at room temperature with elution buffer (1% SDS and 0.1 M NaHCO₃) with vortexing every 10 min. The cross-linking (IP samples and input) was reversed by overnight incubation at 65 °C in the presence of 250 mM NaCl and 25 ng of RNase A. The protein in the samples was digested with 50 ng of proteinase K (Invitrogen) for 1.5 h at 42 °C. The DNA was extracted by phenol chloroform (pH 7.49–7.79) (Invitrogen) once and by chloroform (Sigma) once and precipitated with ethanol and sodium acetate in the presence of 20 μg of glycogen (Roche Applied Science). Immunoprecipitated samples were resuspended in 50 μl of deionized H₂O, and input samples were resuspended in 200 μl deionized H₂O.

PCR Analysis—The DNA from ChIP assays was analyzed by real-time PCR (DNA Engine Opticon 2 System, Bio-Rad) with 2X FastStart SYBR Green Master Mix (Roche Applied Science), 500 nM forward primer, and 500 nM reverse primer that spanned the region of interest. The real-time PCR protocol was: 95 °C for 10 min for activation of FastStart Taq DNA polymerase followed by a three-step amplification (denaturation: 95 °C for 30 s, annealing: 60 °C for 30 s, and extension: 72 °C for 40 s) and a quantification program of 35 cycles. Fold changes in copy number were assessed by comparison with a linear standard curve. Primers sequences are listed in supplemental Table 1.

Gene Expression—Total cellular RNAs were extracted with TRIzol® reagent (Invitrogen) according to the manufacturer’s protocol. Reverse transcription reactions were done on 4 μg of total RNAs, oligo-dT (Promega), and were carried out by using SuperScript™Ill reverse transcriptase (Invitrogen) according to the manufacturer’s protocol. Specific primers for the respective genes were used in the real-time PCR reaction as a measurement of transcriptional activity. Primers sequences are listed in supplemental Table 1.

Knockdown of SRCAP—The Dicer substrate siRNA targeting control and SRCAP used in this study were chemically synthesized and high pressure liquid chromatography (HPLC)-purified by Integrated DNA Technologies. The sequences are listed in supplemental Table 1. Transfections were carried out with 40 nM by DharmaFECT® 1 siRNA transfection reagent (Dharmacon) according to the manufacturer’s protocol into A549 cells. The cells were harvested 72 h later for protein Western blot analysis for SRCAP as described (16), and the β-actin was detected using anti-β-actin antibody (Sigma).

RESULTS

SRCAP Is Present on Both Inactive and Active Promoters—To identify human promoters that recruit SRCAP, the A549 lung carcinoma cell line, which expresses high levels of SRCAP, was used as a model system. With these cells an anti-SRCAP antibody developed in our laboratory (16) was used to perform a ChIP assay to isolate DNA that associates with SRCAP. This DNA was amplified by ligation-mediated PCR and used to screen a human promoter array (data not shown). Several of the promoters identified in this preliminary study were reexamined by standard ChIP assay for the ability to bind SRCAP. Binding
of SRCAP to the human β-globin (BG) promoter, which is inactivated during development in non-erythrocyte cells, was also examined. The results of these studies indicate that the highest levels of SRCAP are present on SP-1, FAD synthetase, and G3BP promoters. Intermediate levels of SRCAP were present on the PPM1B (protein phosphatase 1B isomorph 2) promoter, and lower but detectable levels of SRCAP were present on the SLAM-7, SLC19A2, C15, TOP2A, JAK1, and BG promoters (Fig. 1A).

To determine whether the amount of SRCAP present on individual promoters correlates with transcriptional activity, mRNA levels were measured for each gene (Fig. 1B). Eight of these genes (SP-1, G3BP, SLC19A2, JAK1, PPM1B, C15, TOP2A, and FAD synthetase) were considered active, as indicated by the expression of mRNA. The level of SLAM-7 and BG mRNAs was very low, indicating that these genes are transcriptionally inactive. This conclusion is supported by the results of additional ChIP assays indicating that very low levels of both RNAP II and the histone modification triMeH3K4 are present on the SLAM-7 and BG promoters (data not shown). Collectively, these studies indicate SRCAP is present on both active and inactive promoters. However, higher levels of SRCAP are recruited to a subset of active genes, e.g. SP-1, FAD synthetase, and G3BP.

Profiling SRCAP Location within the SP-1 Promoter—The SP-1 promoter was chosen for further characterization because of the presence of high levels of SRCAP and because the location of the transcription start site (TSS) has been characterized in several studies (23, 24). Binding sites for SRCAP and RNAP II were identified by ChIP assay using several primer sets that span the promoter and coding regions (Fig. 2A). A peak of SRCAP binding was detected within the upstream promoter region −601 to −270 (Fig. 2A, primer sets −601/−475 and −474/−270).

The anti-RNAP II antibody used in these studies detects the total pool of RNAP II including serine 5 phosphorylated RNAP II associated with initiation and serine 2 phosphorylated RNAP II associated with elongation of transcription (25). Not surprisingly, two peaks were observed for the binding of RNAP II. One peak, consistent with transcriptional initiation, was located in
the upstream promoter region −1109 to −750 (Fig. 2A, primer sets −1109/−983 and −859/−750). Previous studies have reported that these regions bind RNAPII and the TAF1 subunit of TFII.D (24). A second peak occurred within the coding region that is consistent with transcriptional elongation. To rule out the possibility that the decrease in RNAPII levels observed at −601 to −165 was due to loss of specific promoter fragments, following sonication and prior to immunoprecipitation, the input DNA was tested by PCR for the presence of intact promoter fragments. The results indicate that DNA within the promoter or coding regions was not degraded at any position by sonication (Fig. 2C).

To test whether a similar pattern of SRCAP and RNAPII binding occurred on other genes, the G3BP (Fig. 3) and FAD synthetase (Fig. 4) promoters were analyzed. Within the G3BP promoter, where the predicted binding site for TAF1 (24) and the identified RNAPII binding site (similar to the SP-I promoter) are located a considerable distance (−561) from the TSS, the SRCAP binding site was also located at a distance upstream at −294 (Fig. 3). In contrast, on the FAD synthetase promoter where the predicted TAF1 binding site (24) and the identified RNAPII binding sites are located close to the TSS, the SRCAP binding site also occurred close to the TSS (Fig. 4).

SRCAP Is Recruited Adjacent to the Sites of triMeH3K4 Deposition—Nucleosomes containing H2A.Z have been found to be enriched in the histone modification triMeH3K4 (19). This suggested that the location of triMeH3K4 might provide a signal for recruitment of SRCAP to the SP-I promoter. ChIP studies indicated that triMeH3K4 deposition extensively overlapped the upstream RNAPII binding site. It did not, however, overlap substantially with the SRCAP binding site, suggesting that it does not mark specific promoter locations that recruit SRCAP (Fig. 5).

The Peak of Acetylated H2A.Z Deposition Occurs Adjacent to the Peak of SRCAP Binding—Recent studies indicate the deposition of acetylated H2A.Z occurs at active promoters (7, 12, 13). This observation led us to ask whether SRCAP mediates deposition of acetylated H2A.Z at the SP-I promoter. To test this hypothesis, ChIP assays were carried out using an antibody that recognizes acetylated H2A.Z. The results indicate that acetylated H2A.Z levels within the SP-I promoter are uniform over a wide region, −3457 to −1109, with a slight peak at −1241 and two prominent peaks at −859 and +7 directly adjacent to but not overlapping the peak site of SRCAP binding. Consistent with reports of acetylated H2A.Z distribution on other genes, greatly reduced levels of acetylated H2A.Z were also observed in the coding region of the SP-I (12, 13).

ChIP experiments were also carried out using antibodies raised against an internal peptide of H2A.Z that recognizes acetylated and non-acetylated H2A.Z. The pattern of total H2A.Z deposition mirrored the pattern observed with one
Knockdown of SRCAP expression by siRNA decrease SRCAP binding to the SP-1, G3BP, and FAD synthetase promoters. To decrease the expression of SRCAP, A549 cells were transfected with SRCAP or control siRNAs (Con). Seventy-two hours post-transfection total SRCAP levels were measured by Western blot analysis (A), and the level of SRCAP specifically associated with the SP-1, G3BP, and FAD synthetase promoters was measured by ChIP assay (B). The amount of SRCAP observed on the SP-1 promoter (−601), the G3BP promoter (−294), and the FAD synthetase promoter (−87) is shown relative to the amount observed with the control siRNA. The graph represents the results and the S.E. of three or more independent ChIP experiments.

Knockdown of SRCAP Expression Results in Loss of Acetylated H2A.Z at the SP-1, G3BP, and FAD Synthetase Promoters—To test whether SRCAP plays a role in deposition of acetylated H2A.Z at promoters, SRCAP levels were knocked down using siRNA. Western blot analysis indicated that SRCAP levels in A549 cells transfected with SRCAP siRNA decreased to less than 10% of the level observed in cell transfected with nonspecific control siRNA. In contrast, the SRCAP siRNA did not affect expression of β-actin (Fig. 7A). In further support of the specificity of the SRCAP knockdown, transfection of a second nonspecific control siRNA did not result in a decrease of SRCAP levels (data not shown).

In parallel studies, the ability of the SRCAP siRNA to decrease SRCAP levels at the SP-1, G3BP, and FAD synthetase promoters was also assessed by ChIP assays. These studies indicate that an ~60% decrease in SRCAP levels associated with these promoters occurred following treatment with SRCAP siRNA compared with cells treated with control siRNA (Fig. 7B).

Treatment with SRCAP siRNA induced within the SP-1 promoter a 60% decrease in H2A.Z deposition at position −1241 and a 60% decrease in acetylated H2A.Z deposition at position −859 (Fig. 8). Deposition of acetylated H2A.Z at the second peak (SP-1 +7) was also reduced by 70% (supplemental sFig. 1). Within the G3BP promoter, treatment with SRCAP siRNA reduced at G3BP −561 deposition of H2A.Z by 80% and deposition of acetylated H2A.Z by 60%. Deposition of acetylated H2A.Z at the second peak (G3BP +79 in Fig. 6B) also was...
Knockdown of SRCAP expression decreases total and acetylated H2A.Z deposition at the SP-1, G3BP, and FAD synthetase promoters. To test whether SRCAP is essential for deposition of H2A.Z and acetylated H2A.Z, A549 cells were transfected with SRCAP siRNA and control (Con) siRNA, and ChIP assays were performed as described in the legend for Fig. 6. Levels of H2A.Z and acetylated H2A.Z at sites within the G3BP promoter (G3BP –561) and FAD synthetase promoter (FAD –87), which were observed previously (Fig. 6) to have high levels of these proteins, were assessed for changes induced by loss of SRCAP. Within the SP-1 promoter, changes in levels of H2A.Z deposited at –1241 and acetylated H2A.Z deposited at –859, sites previously observed to have high levels of these proteins, were also assessed. The graph represents the results and S.E. of four independent ChIP experiments.

Knockdown of SRCAP Expression Results in Decreased SP-1, G3BP, and FAD Synthetase mRNA Expression—To determine whether loss of SRCAP recruitment altered transcription, SP-1, G3BP, and FAD synthetase mRNA levels were measured in A549 cells transfected with control and SRCAP siRNAs. As shown in Fig. 9, treatment with SRCAP siRNA resulted in an ~50% decrease in the level of SP-1, G3BP, and FAD synthetase mRNA compared with cells treated with control siRNA. In contrast, treatment with the SRCAP siRNA did not effect either expression of β-actin protein (Fig. 7A) or β-actin mRNA levels (Fig. 9).

DISCUSSION

Previous studies done in our laboratory using transient transfection approaches indicated that SRCAP plays a role in activation of transcription. Consistent with this role, in a transgenic Drosophila melanogaster model, SRCAP co-localized with RNA II to several sites on polytene chromosomes (26). Unfortunately, the resolution of techniques used in these studies precluded determination of whether SRCAP bound to promoters, coding regions, or other chromosomal sites. To determine whether SRCAP was associated with promoters it was necessary to identify potential target genes. Studies in S. cerevisiae report the presence of H2A.Z on approximately two-thirds of the genes. Despite these results, Swr1 has been detected on only a small number of genes (5), suggesting that in human cells SRCAP might also be present on a small number of promoters. Therefore, rather than examining SRCAP levels on randomly selected genes, ChIP-on-chip analysis was performed to screen the entire genome for promoters targeted by SRCAP. It should be noted that a promoter array was screened, not a whole genome array, and as a consequence, non-promoter regions targeted by SRCAP were not detected. Data from these studies indicate that in A549 cells about 10% of promoters are targeted by SRCAP. From the promoters identified in this study, 10 were reexamined by standard ChIP analysis. This analysis indicates that SRCAP is associated with the promoters of both active and inactive genes. Further, the amount present on individual promoters does not appear to depend strictly on whether a gene is active, because the BG and SLAM-7 genes, which are inactive, have comparable levels of SRCAP as the JAK1 gene, which is active. Interestingly, the highest levels of SRCAP were found on promoters of three active genes (SP-1, G3BP, and FAD synthetase). The presence of highest levels of SRCAP on active promoters might reflect the rapid turnover of nucleosomes observed at a number of other promoters and the specific need to redeposit H2A.Z to maintain optimal promoter architecture for activation of transcription.

The SRCAP knockdown experiments indicated a correlation between loss of SRCAP at promoters and loss of deposition of H2A.Z and acetylated H2A.Z. Although these results do not directly demonstrate that SRCAP mediates deposition of H2A.Z, they indicate a critical role of SRCAP in this process. This is supported by the results of the ChIP assays that indicated the sites for H2A.Z deposition and SRCAP binding substantially overlap within the G3BP and FAD synthetase promoters. Because SRCAP mediates H2A.Z deposition in vitro (16), it is reasonable to postulate that SRCAP is involved in deposition in vivo, especially given the fact that no other proteins (except orthologs of SRCAP in other species) have been identified with this activity. This conclusion is further supported by the findings that the S. cerevisiae ortholog of SRCAP has also been demonstrated to deposit H2A.Z at promoters.

In contrast, H2A.Z deposition and the SRCAP binding sites did not substantially overlap in the SP-1 promoter. This result would suggest that at least in some cases SRCAP bound at the promoter is able to interact with adjacent nucleosomes and cause deposition of H2A.Z. Such interactions would be consistent with the documented ability of coactivators and transcription factors to interact with chromatin and general transcription factors at adjacent sites and indeed at sites located several thousand base pairs away. Alternatively, in the case of the SP-1 promoter, deposition of H2A.Z might occur at the SRCAP...
binding site with other remodeling events leading to dispersal of H2A.Z-containing nucleosomes.

SRCAP binding occurs in a relatively small region of the SP-1 promoter, nucleotides −601 to −270. This region is located between the upstream binding site for RNAP II/TAF1 positioned at −834 and the transcription start site. Several mechanisms might be used to direct SRCAP recruitment to this region, including histone modifications and binding of specific transcription factors. Although triMeH3K4 is found at active promoters and enriched in nucleosomes containing H2A.Z (19), the lack of overlap with the SRCAP binding site within the SP-1 promoter suggests that this modification does not represent a bona fide targeting site for recruitment of SRCAP. Other histone modifications are enriched in nucleosomes containing H2A.Z, including triMeH3K27 and triMeH3K9 (19). These modifications, however, have been associated with heterochromatin and are unlikely to play a role in targeting SRCAP to active promoters. The finding that recruitment of SRCAP also occurs adjacent to sites of recruitment of RNAP II/TAF1 in the G3BP and FAD synthetase promoters is intriguing. Although speculative, it is possible that components of these different complexes interact and provide a method for co-localization to sites within promoters.

Deposition of acetylated H2A.Z was also found to overlap or flank the sites of SRCAP binding. Studies using a SRCAP transgenic Drosophila model indicate that SRCAP recruits the histone acetyltransferase CBP (CREB-binding protein) to chromatin (26), raising the possibility that deposition and acetylation of H2A.Z occur by a SRCAP-CBP complex.

Studies in S. cerevisiae indicate that the highest levels of H2A.Z are deposited at inactive promoters. H2A.Z is lost following activation, suggesting a model in which the SWR-C complex is preferentially associated with active promoters (3–5). The recent finding that increased levels of acetylated H2A.Z occur at active promoters (7, 12, 13) suggests a model in which non-acetylated H2A.Z deposited at inactive promoters becomes acetylated upon activation. An alternative model is that the SWR-C complex is present at active promoters, where it catalyzes deposition of non-acetylated H2A.Z, which becomes acetylated, or that it catalyzes deposition of acetylated H2A.Z. Reports from other laboratories are conflicting in regard to whether H2A.Z is acetylated prior to deposition into chromatin (6, 8). Our finding that SRCAP is present at active promoters and that knockdown of SRCAP expression results in loss of deposition of acetylated H2A.Z supports the second model. From our data, however, it is not possible to determine whether non-acetylated H2A.Z is first deposited and then acetylated or whether acetylated H2A.Z is deposited directly.

Despite the ability to knock down the total cellular level of SRCAP by 90%, we were unable to decrease SRCAP levels at the SP-1, G3BP, and FAD synthetase promoters greater than 60%, which indicates that the SRCAP complex is stabilized at these promoters. This loss was paralleled by a corresponding 60–80% loss of H2A.Z deposition and a 40–50% decrease in mRNA levels. These findings are consistent with those obtained in S. cerevisiae, in which the loss of H2A.Z or deletion of Swr1 represses transcription (5). It is not clear, however, whether the decreased transcription observed results solely from the loss of H2A.Z incorporation or whether the loss of other SRCAP activities is a contributor. This latter possibility is suggested by our studies indicating that SRCAP contains several additional domains capable of activating transcription independent of the histone exchange reaction (17, 18).

In summary, previous work has shown that deposition of acetylated H2A.Z supports the second promoters and that knockdown of SRCAP expression results in loss of deposition of acetylated H2A.Z. The current work completes the picture as to how SRCAP may regulate transcription. It demonstrates for the first time that SRCAP is located at endogenous promoters and provides for several genes a detailed map of the location of SRCAP with respect to the RNAP II binding site and the transcription start site. These studies also provide the first evidence that SRCAP regulates transcription of endogenous chromatin bound promoters (this was not demonstrated in previous studies using transient transfection reporter genes). Finally, these studies provide the first link in vivo between SRCAP and deposition of H2A.Z.

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