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Maximal Binding Levels of an H1 Histone Gene-Specific Factor in S-Phase Correlate with Maximal H1 Gene Transcription

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Levels of *trans*-acting factor (H1-SF) binding to the histone H1 gene-specific motif (5′-AAACACA-3′ [L. S. Coles and J. R. E. Wells, Nucleic Acids Res. 13:585–594, 1985]) increase 12-fold from G1 to S-phase in synchronized cells and decrease again in G2 phase of the cell cycle. Since the H1 element is required for S-phase expression of H1 genes (S. Dalton and J. R. E. Wells, EMBO J. 7:49–56, 1988), it is likely that the increased levels of H1-SF binding component play an important role in S-phase regulation of H1 gene transcription.

We have shown previously that an H1 histone gene upstream element (5′-AAACACA-3′) is required for S-phase regulation of H1 gene transcription (6). In these experiments, a 5-kilobase fragment of chicken DNA containing an H1 gene and a divergently transcribed H2A-H2B gene pair was transfected into HeLa cells together with the *neo* gene as a dominant selectable marker and cell lines were isolated. Analysis of transcripts from these chicken histone genes showed that deletion or base substitution in the H1 element caused loss of S-phase expression of the H1 gene while not affecting the normal burst of H2A-H2B gene transcription during S-phase. It was also shown that a *trans*-acting factor (H1-SF) binds to the H1 core element region and that clustered point mutations in this region not only prevented the normal S-phase burst of H1 gene transcription within cells but also eliminated factor binding to this region as judged by gel retardation assays in vitro.

In this communication, we show that levels of H1 *trans*-acting factor binding activity correlate with the profile of H1 gene transcription at different stages of the cell cycle. This contrasts with the situation of a histone H2B octamer-binding factor which does not show such variation in binding activity during the cell cycle (7).

Enrichment of H1-SF binding activity by column chromatography. Nuclei were isolated from cells of the transformed chicken erythroid cell line AEV *ts34* A6L1 (LSCC HD2, kindly provided by T. Graf, Institute of Virus Research, Heidelberg, Federal Republic of Germany). Salt-extracted proteins recovered from AEV *ts34* nuclei were precipitated with 0.33 mg of ammonium sulfate per ml and centrifuged at 50,000 × *g* for 30 min. This crude nuclear extract (~90 mg of protein) was loaded on a Sephacryl S-300 gel filtration column (bed volume, 200 ml), and 5-ml fractions were collected. Columns (at 4°C) were run at 0.5 ml/min in TM buffer (50 mM Tris hydrochloride [pH 7.9], 1 mM EDTA, 1 mM dithiothreitol, 12.5 mM $MgCl_2$, 20% glycerol) plus 0.1 M KCl and 0.5 mM phenylmethylsulfonyl fluoride. Fractions were tested for specific binding to the H1 element (Fig. 1A) by using a gel mobility shift assay (6). Pooled samples containing H1-SF (Fig. 1B) were dialyzed against 0.1× TM buffer and passed over DEAE-Sepharose in this buffer to remove nucleic acid and other contaminants (3). Figure 1B shows two classes of proteins eluting at different peak volumes. Upper retarded complexes (NS) have previously been shown to be nonspecific by competition assay (6), while peak binding activity specific for the H1 AAACACA motif eluted primarily in fractions 15 and 16 (Fig. 1B). By calibrating the column with molecular weight standards, it is estimated that H1-SF elutes at a molecular weight of approximately 90,000 under nondenaturing conditions (Fig. 1A and B). Fractions containing peak binding activity were pooled and DNA target specificity was tested by competition assay with excess amounts of unlabeled heterologous and homologous competitor DNA as described previously (6; S. Dalton, Ph.D. thesis, University of Adelaide, Adelaide, Australia, 1987).

It has proven useful to pass pooled fractions containing peak binding activity from the Sephacryl S-300 column over DEAE-Sepharose equilibrated with 0.1× TM buffer. The H1-SF material does not bind and is collected in the flow-through fraction (Dalton, Ph.D. thesis), but other contaminants such as nucleic acids and phosphatases are removed (3). Such preparations of H1-SF show only a single mobility shift band in gel retardation assays even in the absence of heterologous competitor DNA (Dalton, Ph.D. thesis). These preparations have been used to characterize H1-SF further. Biochemical analysis shows that the binding activity is sensitive both to heat treatment (70°C, 5 min) and to proteinase K digestion, but that preincubation with RNase has no effect on levels of H1-SF binding capacity (Dalton, Ph.D. thesis). Thus, H1-SF in nondenaturing conditions appears to be a protein of 90,000 daltons.

Maximal levels of H1-SF binding activity are found in S-phase nuclei. We have recently shown that the H1 gene-specific element (4) is required for the characteristic S-phase increase of H1 gene expression and that deletion of the motif or base substitutions within the 5′-AAACACA-3′ sequence prevent this burst of transcription. We also showed that the factor H1-SF, which binds to the H1 box, does not bind to an oligomer containing base substitutions in the H1 element. In the same series of experiments, it was shown that S-phase expression of closely linked chicken H2A-H2B genes was not influenced by alterations to the H1 gene motif (6). In the experiments described here, we investigated the relative levels of the H1 *trans*-acting factor, H1-SF, and of the previously characterized H2B octamer-binding factor, OTF-1 (7), in relation to the cell cycle. Because binding of the octamer factor is apparently independent of the cell cycle (7), we wished to investigate whether the same was true for H1-SF. In the experiments (described below), we used an oligomer (40-mer) containing the H2B octamer element as a control to monitor the levels of octamer-binding protein in

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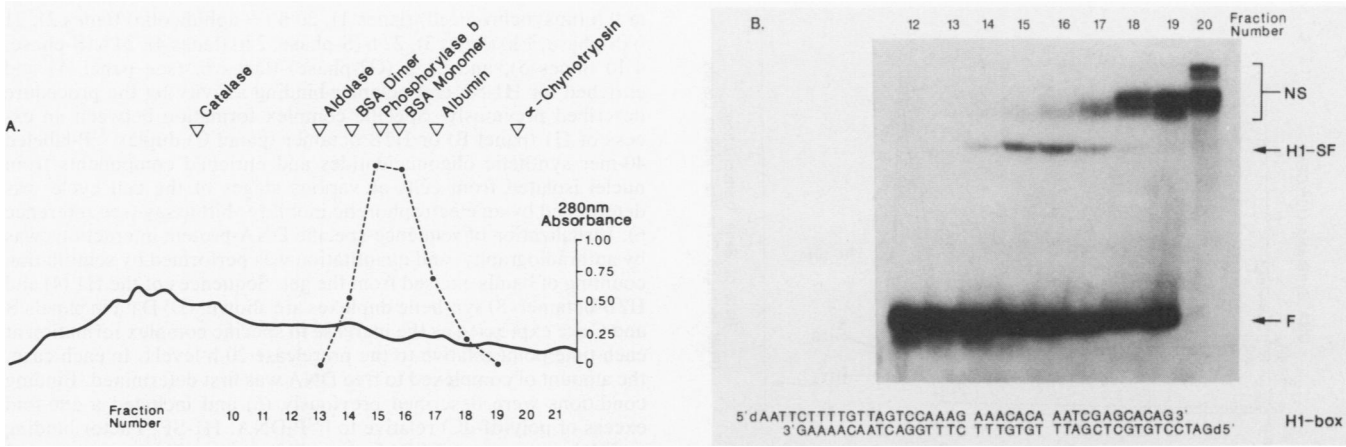

FIG. 1. Enrichment of sequence-specific binding activity by gel filtration. (A) Crude nuclear extract (~90 mg of protein) was loaded on a Sephacryl S-300 gel filtration column (bed volume, 200 ml), and 5-ml fractions were collected as described in the text. Typical A_{280} protein elution profile is shown (——) with respect to fraction number and standard molecular weight markers (Sigma Chemical Co.) which were used to calibrate the column. The markers were as follows (molecular weights in parentheses): α-chymotrypsin (25,000), albumin (45,000), bovine serum albumin monomer (66,000), phosphorylase *b* (97,000), bovine serum albumin dimer (130,000), aldolase (158,000), and catalase (232,000). Superimposed on the protein elution profile is the relative level of H1-SF (--●--) collected from the corresponding fractions, as determined by the gel mobility shift assay shown in panel B. Similar results were obtained when poly(dI-dC) or *Escherichia coli* DNA were used as binding competitors, except that nonspecific bands were eliminated. (B) Gel mobility shift assays were performed under conditions described previously (6) for the detection of H1-SF except that 0.5 μg of extract protein was included in the experiments shown here and heterologous competitor DNA was omitted. Lanes are numbered corresponding to the extract fraction used in the corresponding binding assays (see panel A). Positions of nonspecific (NS), free oligomer duplex (F), and H1-SF-DNA complex (H1-SF) are indicated. Similar quantitative results were obtained when excess poly(dI-dC) or *E. coli* DNA was included, resulting in loss of NS binding activity. Sequence of the synthetic H1 duplex is shown.

nuclear extracts from different stages of the cell cycle. Aliquots of the same extracts were used to monitor the levels of H1-SF by gel retardation assays. As shown below (Fig. 2), we verify that there is little fluctuation in the H2B-specific *trans*-acting factor (7), whereas the level of binding activity of H1-SF in nuclear extracts mirrors the S-phase peak in H1 transcriptional activity.

Cells synchronized with aphidicolin (5) were pulse-labeled with [^{3}H]thymidine to monitor DNA synthesis (Fig. 2A). In addition, nuclei were prepared from cells at different stages of the cell cycle and pulse-labeled with [^{32}P]UTP, and labeled RNA was extracted for dot-blot analysis to filter-bound chicken histone DNA (5; Dalton, Ph.D. thesis). As shown in Fig. 2A, the pattern of maximal H1 and H2B histone gene expression, as measured by radioactivity in nascent transcripts, is coincident with the initiation of DNA synthesis during S-phase. Populations of cells from these experiments were also used to prepare nuclear protein extracts enriched for H1-SF and the H2B octamer-binding protein which copurify through Sephacryl S-300 and passage through DEAE-Sepharose (Dalton, Ph.D. thesis). For gel retardation assays, equal amounts of total protein (2) containing the semipurified binding factors were incubated with an excess of each of two ^{32}P-labeled duplex oligonucleotides (40-mers; Fig. 2D). One consisted of the H1 histone gene element described above, and the other was a 40-mer containing the H2B-histone octamer element (5′-ATTTGCAT-3′). Point mutations in either of these elements eliminate sequence-specific binding activity (6; Dalton, Ph.D. thesis).

The data depicted in Fig. 2B compare levels of H1 and H2B complexes in extracts enriched for binding activity prepared from cells at different stages of the cell cycle. The pattern is clearly different in the two cases. Whereas levels of the H2B octamer-derived complex do not fluctuate substantially at different stages of the cell cycle (also see reference 7), the H1-SF complex increases about 12-fold in S-phase extracts compared with interphase-derived extracts. Essentially the same result is obtained with crude extracts of nuclear proteins (Dalton, Ph.D. thesis). We suggest that this temporal increase in the level of H1-motif binding factor is likely to play an important role in the burst of H1 gene transcription during S-phase in vivo. It also suggests that the cell cycle regulation of histone H2B genes is not principally achieved via different levels of *trans*-activator (as identified here) interacting directly with the H2B octamer motif.

Although the binding activity of H1-SF increases substantially in S-phase, it is not yet certain whether this is due to de novo synthesis of the factor or to modification of preexisting molecules to a binding mode. Preliminary evidence indicates the latter possibility may be involved (our unpublished observations). There are a number of variations on this theme. In *Saccharomyces cerevisiae*, the factor GCN4, which stimulates genes required for amino acid synthesis, is subject to translational control; low amino acid levels stimulate translation of GCN4 mRNA which is itself constitutively produced (12). The immunoglobulin light-chain gene enhancer-binding factor NF-κB is produced constitutively but is modified to an activating mode by physiological signals (11). Phorbol ester activation of a restricted set of genes also proceeds in part by posttranslational activation (possibly phosphorylation) of the *trans*-activating factor AP-1 (1, 9). Finally, the yeast mating-type switch system provides an example not only of cell cycle regulation of the HO gene-activator, SW15, but also of differential partitioning of this factor to mother cells following cell division, ensuring a mating type switch in the mother cell but not in the daughter cell (10).

Our further studies are directed towards characterization of the H1 *trans*-acting factor at the molecular level. Nevertheless, the clear increase in the binding mode of H1-SF in S-phase compared with interphase cells is strongly sugges-

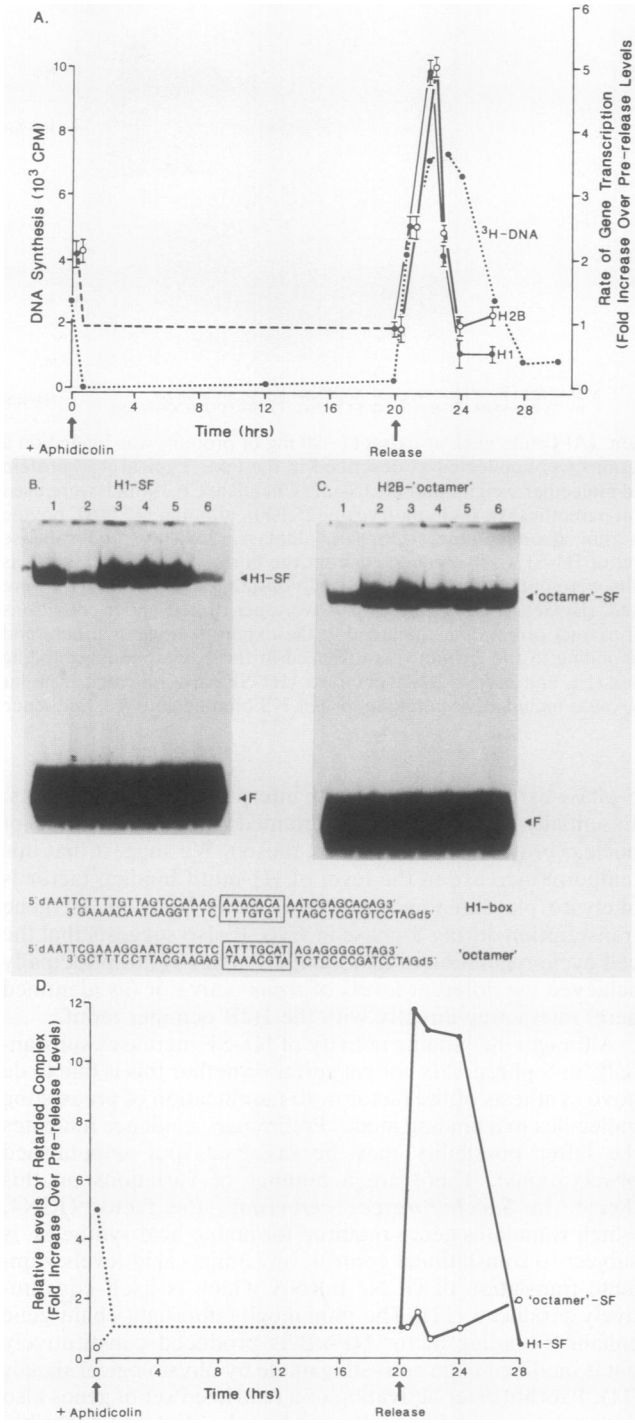

FIG. 2. Nuclear factor binding to the H1 box increases during early S-phase. (A) Where indicated, aphidicolin (5 μg/ml) was added to randomly growing cultures of AEV *ts34* cells in exponential growth phase, which were then washed in fresh media after 20 h as described previously (5). Cells were pulse-labeled with [^{3}H]thymidine (1 μCi/ml) for 15 min. [^{3}H]thymidine incorporation in trichloroacetic acid-insoluble material (---●---), indicating the rate of DNA synthesis, in addition to rates of H1 and H2B gene transcription (5) at corresponding times, is shown. Error bars represent the standard error of the mean from three experiments. In a typical experiment with 5×10^6 cpm of total labeled input RNA, hybridization to H1 and H2B gene probes was 0.6×10^3 and 0.4×10^3 cpm, respectively, at 20 h. (B and C) Crude extracts were prepared from AEV *ts34* cells at 0 h (unsynchronized) (lanes 1), 20 h (+ aphidicolin) (lanes 2), 21 h (S-phase, 1 h) (lanes 3), 22 h (S-phase, 2 h) (lanes 4), 24 h (S-phase, 4 h) (lanes 5), and 28 h (G2-phase) (lanes 6) (see panel A) and enriched for H1-SF and octamer-binding activity by the procedure described previously. Specific complex formation between an excess of H1 (panel B) or H2B octamer (panel C) duplex ^{32}P-labeled 40-mer synthetic oligonucleotides and enriched components from nuclei isolated from cells at various stages of the cell cycle was determined by an electrophoretic mobility shift assay (see reference 6). Visualization of sequence-specific DNA-protein interactions was by autoradiography, and quantitation was performed by scintillation counting of bands excised from the gel. Sequences of the H1 (4) and H2B-octamer (8) synthetic duplexes are shown. (D) Data in panels B and C are expressed as the increase in specific complex formation at each time point relative to the prerelease 20-h levels. In each case, the amount of complexed to free DNA was first determined. Binding conditions were described previously (6) and included a 100-fold excess of poly(dI-dC) relative to [^{32}P]DNA. H1-SF, Factor binding to H1 box; 'octamer'-SF, factor binding to octamer sequence of H2B gene. Complex formation is expressed as the fold change over prerelease (20 h + aphidicolin) levels.

tive of a quantitative role for this factor in regulating S-phase transcription of H1 histone genes in vivo.

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