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Transcriptional and Posttranscriptional Regulation of H1 Histone Gene Expression by 1-β-D-Arabinofuranosylcytosine

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SUMMARY

Recent studies have demonstrated that 1-β-D-arabinofuranosylcytosine (ara-C) activates the transcription of the *jun/fos* early response genes in human myeloid leukemia cells. The basis for ara-C-induced control of gene expression remains unclear. However, down-regulation of H1 histone mRNA levels has been reported as one of the earliest changes in specific gene expression associated with ara-C treatment. In this report, we describe the mechanisms responsible for H1 histone expression by this agent. Treatment of HL-60 cells with ara-C resulted in a decrease in H1 histone mRNA levels that was detectable by 15 min. In contrast, this down-regulation by ara-C was completely blocked by treatment of the cells with cycloheximide. Nuclear run-on analyses demonstrated that ara-C treatment is associated with inhibition of H1 histone gene transcription. The results also demonstrate that cycloheximide abrogates the transcriptional down-regulation by ara-C but alone has no detectable effect. We also show that ara-C treatment is associated with a decrease in stability of the H1 histone transcript and that this effect is also reversed by inhibition of protein synthesis. Taken together, these findings demonstrate that ara-C regulates H1 histone expression at both the transcriptional and posttranscriptional levels. The results also indicate that control of this gene by ara-C involves the activation of at least two signaling events that require *de novo* protein synthesis.

Ara-C is an effective agent in the treatment of human acute myelogenous leukemia (1). Certain insights are available regarding the effects of ara-C on DNA replication; however, the precise mechanisms responsible for the lethal effects of this agent are still unclear. Ara-C incorporates into DNA of leukemic cells, and the extent of ara-C-DNA formation correlates with inhibition of DNA synthesis (2). The incorporation of ara-C into DNA also correlates with loss of clonogenic survival (3, 4). Other studies have extended these results by demonstrating that the effects of this agent are related to both incorporation into DNA and the sequence of the DNA template (5, 6). Taken together, these findings are consistent with the hypothesis that the incorporated ara-C residue alters reactivity of the 3′ terminus and slows elongation by functioning as a relative chain terminator.

Recent studies have demonstrated that the effects of ara-C on myeloid leukemia cells include the activation of specific gene expression. In particular, the c-*jun* gene, which has been implicated in the regulation of cell growth, is induced at the transcriptional level by ara-C (7, 8). Similar findings have been obtained for the related *jun*-B and c-*fos* genes (9–11). The products of these genes function as components of the AP-1 transcription factor, which binds to a heptameric DNA consensus sequence (12). Because AP-1 binding sites are known to be involved in the regulation of various genes responsive to phorbol esters and growth factors, these findings indicated that ara-C may activate a cascade of gene expression.

The signaling mechanisms responsible for the regulation of gene expression by ara-C are unknown. However, one of the earliest reported changes in gene expression produced by inhibitors of DNA synthesis involves down-regulation of H1 and core histone transcripts (13, 14). H1 histone is involved in the higher order structure of nucleosomes and has been considered to be functional in mechanisms responsible for repression of gene expression (15). However, little is known about the role of H1 histone in the cellular response to DNA-damaging agents, such as ara-C. Whereas H1 histone mRNA levels are regulated by both transcriptional and posttranscriptional mechanisms (13–17), the effects of ara-C on expression of this gene have been attributed to destabilization of the H1 histone transcripts (13, 14). The present results demonstrate that H1 histone gene transcription is inhibited in ara-C-treated cells and that this effect is mediated by a labile protein. This mechanism, as well as a decrease in stability of the H1 histone transcript, contributes to the rapid down-regulation of H1 histone gene expression in ara-C-treated cells.

Materials and Methods

Cell culture. KG-1 human myeloid leukemia cells (American Type Culture Collection, Bethesda, MD) were grown in Iscove's modified

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ABBREVIATIONS: ara-C, 1-β-D-arabinofuranosylcytosine; CHX, cycloheximide; kb, kilobase(s).

Dulbecco's medium supplemented with 10% heat-inactivated fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine. HL-60 cells were grown as previously described (18). The cells were treated with 10^{-5} M ara-C (Sigma Chemical Co., St. Louis, MO), 10 μg/ml CHX (Sigma), 5 μg/ml actinomycin D (Sigma), and 1 mM hydroxyurea (Sigma).

RNA isolation and Northern blot analysis. Total cellular RNA was isolated using a modification of the guanidine-isothiocyanate technique (18). The RNA (20 μg) was analyzed by electrophoresis in 1% agarose-formaldehyde gels, transferred to nitrocellulose filters, and hybridized to the following ^{32}P-labeled DNA probes: 1) the 1.44-kb *Eco*RI/*Pvu*II fragment of the human H1 histone gene in the pFNC16A plasmid (19) and 2) the 2.0-kb *Pst*I fragment of a chicken β-actin DNA purified from the pA1 plasmid (20). Hybridizations were performed as described (18). Autoradiograms were scanned using an LKB Ultrascan XL laser densitometer, and relative signal intensity was determined using the Gelscan HL software package.

Nuclear run-on assays. Nuclei were isolated and newly elongated transcripts were labeled with [α-^{32}P]UTP (800 Ci/mmol; DuPont-New England Nuclear) at 26° for 30 min (18). The labeled RNA was hybridized to the following DNAs: 1) the 2.0-kb *Pst*I fragment of the chicken β-actin pA1 plasmid (20), 2) the 1.1-kb *Bam*H1 insert of the human β-globin gene (21), and 3) the 1.44-kb *Eco*RI/*Pvu*II fragment of the human H1 histone gene pFNC16A plasmid (19). The digested DNAs were heated to 65° for 15 min, separated in 1% agarose gels, and transferred to nitrocellulose filters. Conditions for prehybridization, hybridization, and washing have been described (18).

Results

The effects of ara-C on expression of the H1 histone gene were initially studied in KG-1 myeloid leukemia cells. The H1 histone probe hybridized to a major transcript of approximately 0.8 kb, which is consistent with the mature mRNA. Hybridization was also detectable to a minor transcript that may represent a polyadenylated species (22) (Fig. 1). Treatment with 10^{-5} M ara-C was associated with a decrease in H1 histone mRNA levels that was detectable at 1 hr (Fig. 1). Similar findings were obtained when HL-60 cells were treated with this agent (Fig. 1). The down-regulation of H1 histone gene expression at 1 hr was 78% for KG-1 cells and 90% for HL-60 cells (Fig. 1). No further decreases in H1 mRNA levels were detected despite continuous ara-C exposure (Fig. 1). These findings indicated a basal level of H1 histone expression that is insensitive to the effects of this agent. A detailed time-course study was performed to define more precisely the rapid decline in H1 histone mRNA levels. Decreases in H1 histone transcripts were detectable at 15 min of ara-C exposure and continued until 1 hr, when ara-C-insensitive H1 mRNA levels were apparent (Fig. 2). Densitometric scanning demonstrated a decrease in ara-C-sensitive H1 histone expression with a half-life of 30 min (Fig. 2). In contrast, there was little if any effect of ara-C on actin mRNA levels (Fig. 2). These findings indicated that the effects of ara-C were specific for the H1 histone gene.

Previous studies in HeLa cells have demonstrated that H1 histone mRNA levels are stabilized by inhibition of protein synthesis (13, 16, 23). Treatment of KG-1 cells with CHX for 1 hr had little effect on H1 histone mRNA levels, whereas inhibition of protein synthesis with this agent for 3 hr was associated with a detectable increase in these transcripts. The exposure of cells to both ara-C and CHX resulted in a block in the down-regulation of H1 histone expression observed with ara-C alone (Fig. 3). The absence of an effect of ara-C and CHX on actin gene expression indicated that protein synthesis is required for down-regulation of H1 histone expression by ara-C.

The down-regulation of H1 histone expression could be controlled by transcriptional and/or posttranscriptional mechanisms. In order to address this issue, nuclear run-on assays were performed to determine the effects of ara-C on relative rates of H1 histone gene expression. The actin gene (positive control) was constitutively transcribed in KG-1 cells, whereas there was no detectable transcription of the β-globin gene (negative control) (Fig. 4). The H1 histone gene was also constitutively transcribed in untreated KG-1 cells, and the transcription rate of this gene was decreased after treatment with ara-C for 1 hr (Fig. 4). Treatment with CHX alone had little effect on relative rates of gene transcription. However, CHX inhibited the ara-C-induced down-regulation of H1 histone gene transcription (Fig. 4). Densitometric scanning of three experiments demonstrated that ara-C decreased the rate of H1 histone gene transcription by 84 ± 7% (mean ± standard

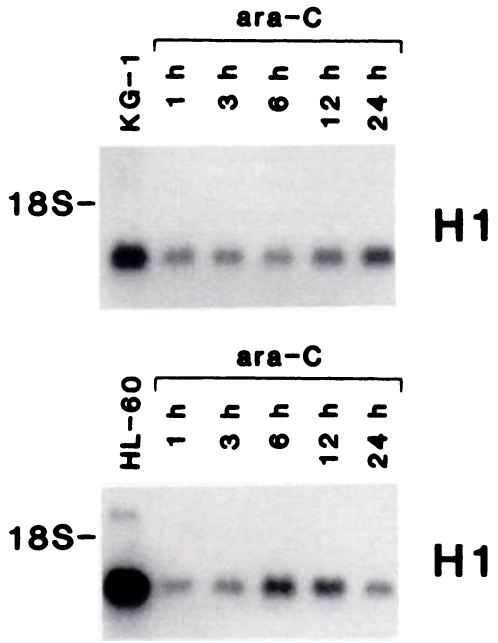

Fig. 1. Down-regulation of H1 histone mRNA levels in KG-1 and HL-60 cells treated with 10^{-5} M ara-C. Total cellular RNA (20 μg) was isolated at the indicated times and hybridized to a ^{32}P-labeled H1 histone probe.

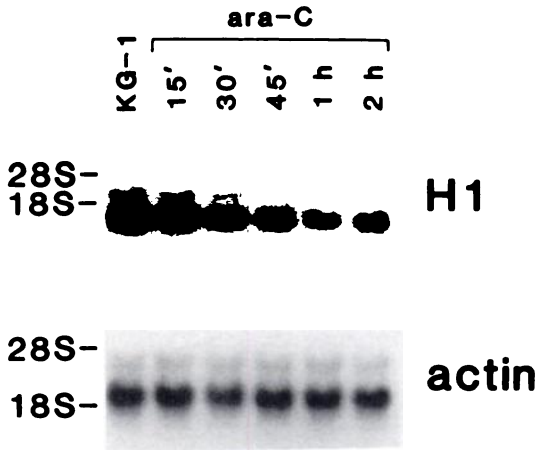

Fig. 2. Kinetics of ara-C-induced decreases in H1 histone transcripts. KG-1 cells were treated with 10^{-5} M ara-C for the indicated times. Total cellular RNA (20 μg) was hybridized to the ^{32}P-labeled H1 histone and actin probes.

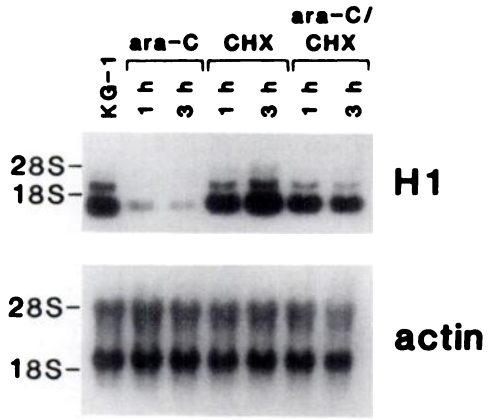

Fig. 3. Effects of ara-C and CHX on H1 histone mRNA levels. KG-1 cells were treated with 10^{-5} M ara-C, 10 μg/ml CHX, or both agents. At the indicated times, total cellular RNA (20 μg) was isolated and hybridized to the ^{32}P-labeled H1 histone and actin probes.

Fig. 4. Effects of ara-C and CHX on the rate of H1 histone gene transcription. KG-1 cells were treated with 10^{-5} M ara-C, 10 μg/ml CHX, or both agents for 1 hr. Nuclei were isolated, and newly elongated ^{32}P-labeled RNA transcripts were hybridized to 2 μg of actin, β-globin, and H1 histone DNA inserts after restriction enzyme digestion and Southern blotting. The *solid lines* in the schematic diagram represent the relative positions of the DNA inserts.

deviation) and that this effect was completely reversed by CHX. These results indicated that a newly synthesized protein is necessary for the inhibition of H1 histone transcription by ara-C.

In order to study the posttranscriptional regulation of H1 histone mRNA levels, we exposed KG-1 cells to actinomycin D for various intervals, to inhibit transcription. The half-life of H1 histone transcripts, as determined by densitometric scanning, was 120 min (Fig. 5). Similar studies were performed on cells treated with ara-C and actinomycin D. Under these experimental conditions, the half-life of the H1 histone mRNA was decreased to 32 min (Fig. 5). In contrast, there was no detectable effect of actinomycin D or the combination of ara-C and actinomycin D on actin mRNA levels (data not shown). These results indicated that ara-C treatment is associated with a decrease in stability of these transcripts.

We also compared the effects of CHX on both the transcriptional and the posttranscriptional control of H1 gene expression by ara-C. Cells were treated with CHX for 1 hr and used for run-on analysis or exposed to actinomycin D to determine transcript stability. In this experiment, treatment with ara-C for 1 hr decreased the rate of H1 histone gene transcription by 64%, and this effect was completely reversed by CHX (data not shown). Moreover, the half-life of the H1 histone transcript was prolonged by CHX to >3 hr and 145 min in control and ara-C-treated cells, respectively (Fig. 6). Taken together, these findings supported the requirement of *de novo* protein synthesis in both the down-regulation by ara-C of H1 histone gene transcription and the increased turnover of these transcripts.

In order to determine whether CHX-sensitive down-regulation of H1 histone transcription is limited to ara-C, similar

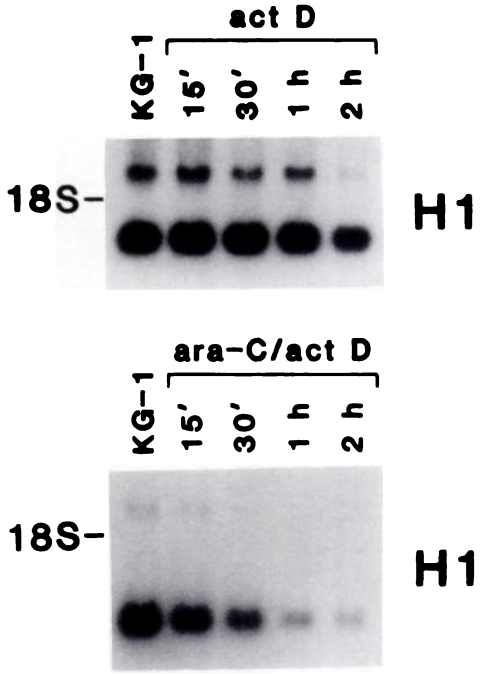

Fig. 5. Effects of ara-C on stability of H1 histone transcripts. KG-1 cells were treated with 5 μg/ml actinomycin D (*act D*) or with 10^{-5} M ara-C and actinomycin D. At the indicated times, total cellular RNA (20 μg) was isolated and hybridized to the ^{32}P-labeled H1 histone probe. Hybridization to the actin probe demonstrated equal loading of the lanes.

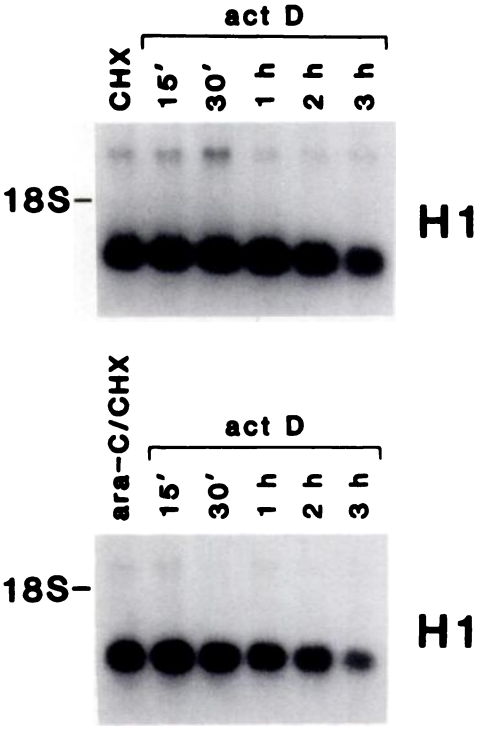

Fig. 6. CHX regulates H1 histone expression at the posttranscriptional level in ara-C-treated cells. KG-1 cells were treated with 10 μg/ml CHX or 10^{-5} M ara-C and CHX for 1 hr. The cells were then incubated in the presence of 5 μg/ml actinomycin D (*act D*). At the indicated times, total cellular RNA (20 μg) was isolated and hybridized to the labeled H1 histone probe. Hybridization to the actin probe demonstrated equal loading of the lanes.

studies were performed with hydroxyurea as another inhibitor of DNA replication. Treatment of KG-1 cells with hydroxyurea for 1 hr was associated with a 93% decrease in H1 histone mRNA levels (Fig. 7A). Moreover, this effect was completely reversed by CHX (Fig. 7A). Nuclear run-on assays were performed to determine whether this effect involves a transcriptional mechanism that requires protein synthesis. Hydroxyurea decreased the rate of H1 histone gene transcription by 85%. In contrast, this agent had little effect on transcription of the actin and β-globin genes (Fig. 7B). Whereas treatment with CHX alone had no detectable effect on H1 histone transcription, this agent inhibited the down-regulation of this gene by hydroxyurea (Fig. 7B). These results indicated that inhibition of DNA synthesis is coupled to decreases in H1 histone transcription by a CHX-sensitive signaling event.

Discussion

The recent demonstration that ara-C induces expression of the *jun/fos* family of early response genes (7–10) has suggested that this agent may activate a program involving the transcriptional activation of specific genes. The timing and magnitude of the response of this early response gene family to ara-C are concentration dependent. Ara-C incorporates into DNA and inhibits DNA replication in a concentration-dependent manner (2–4). These effects of ara-C are also associated with induction of DNA strand breaks (24, 25). Thus, the activation of early response gene expression by this agent may be related to inhibition of DNA synthesis or DNA fragmentation. The signaling pathways that contribute to ara-C-induced cellular responses are unclear and may include a cascade of events. In this context, the activation of *jun/fos* gene transcription is first detectable at 6 hr after addition of this agent (7).

Previous studies have demonstrated that the down-regulation of H1 histone mRNA levels by inhibitors of DNA synthesis is detectable within 1 hr (13, 14) and, thus, represents a more rapid change in gene expression, compared with induction of the *jun/fos* family. Similar findings were obtained in the present studies, with down-regulation of H1 histone mRNA levels being detectable after 15 min of ara-C exposure. Although this decrease continued for up to 1 hr, a fraction (~10–20%) of the H1 transcripts remained insensitive to these effects of ara-C. Previous studies have demonstrated a basal level of core histone synthesis that is insensitive to inhibitors of DNA synthesis (26). Similar mechanisms may regulate H1 histone expression and contribute to the basal level of these transcripts in ara-C-treated cells. Histone genes are transcribed throughout the cell cycle, and the rate of transcription is increased during entry into S phase (17, 27). Posttranscriptional control of histone mRNA stability also contributes to the increase in expression during S phase (28, 29). Although previous work has demonstrated that treatment of cells with inhibitors of DNA synthesis is associated with down-regulation of core histone gene transcription (16, 17, 30), another study has indicated that changes in H1 histone transcription are not involved in the down-regulation of mRNA levels observed during treatment with these agents (13). In contrast, the present studies demonstrate that treatment of KG-1 cells with ara-C or other DNA synthesis inhibitors, such as hydroxyurea, is associated with a decrease in the rate of H1 histone gene transcription. The finding that ara-C treatment results in nearly 85% inhibition of H1 histone transcription at 1 hr suggests that this agent rapidly activates a signal transduction pathway involved in the negative control of this gene. This regulation could involve inhibition of the synthesis of a protein required for *trans*-activation or the induction of a negative regulatory factor.

The studies with CHX were performed to define further the mechanisms responsible for ara-C-induced down-regulation of H1 histone transcription. The finding that inhibition of protein synthesis has little effect on the transcription rate of this gene in control cells suggests that the effects of ara-C are unrelated to decreased synthesis of a protein required for *trans*-activation of the H1 histone promoter. Nonetheless, ara-C treatment could be associated with inactivation of such a transcription factor. Another potential explanation for the findings is that inhibition of protein synthesis disrupts the signaling pathway that couples inhibition of DNA synthesis and decreases in H1 gene transcription. The finding that CHX treatment abrogates the effects of ara-C also raises the possibility that this inhibition of DNA synthesis activates the synthesis of a factor involved in the negative regulation of this gene. The isolation of a putative promoter region for the H1 histone gene has resulted in the identification of two protein-binding domains (31). However, little is known about the functional elements that control transcription of this gene. Moreover, there is presently no evidence for a factor that is involved in the negative regulation of the H1 histone promoter. In any event, previous work has demonstrated that CHX blocks the down-regulation of H3 histone gene transcription (30) and, thus, similar signaling mechanisms may contribute to the negative control of both H1 and core histone genes.

The present results further indicate that the ara-C-induced down-regulation of H1 histone mRNA levels involves control at both the transcriptional and posttranscriptional levels. Both of these levels of regulation also appear to require protein synthesis. In this regard, whereas the half-life of H1 histone transcripts was decreased in ara-C-treated cells, this effect was abrogated by CHX. A 3′ stem loop structure is involved in the rapid degradation of histone mRNAs, and this mechanism requires active histone protein synthesis (32–34). Previous studies have suggested that histone expression is autoregulated by binding of these proteins to their mRNA, thereby destabilizing the transcript (35). Furthermore, the ribonuclease in-

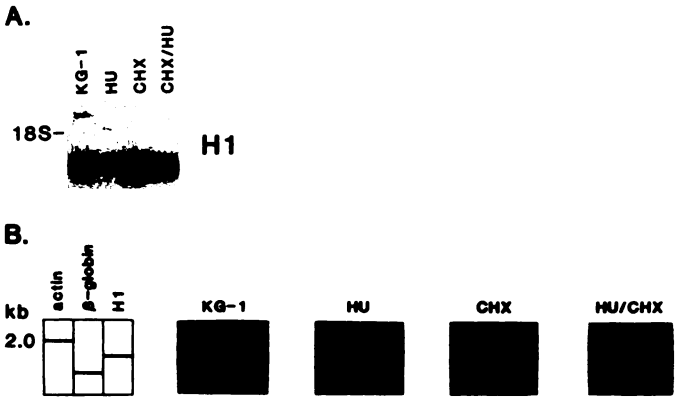

Fig. 7. Requirement of protein synthesis for the regulation of H1 histone transcription by hydroxyurea. KG-1 cells were treated with 1 mM hydroxyurea (*HU*), 10 μg/ml CHX, or both agents for 1 hr. A, Total cellular RNA (20 μg) was isolated and hybridized to the labeled H1 histone probe. B, Nuclei were isolated and newly elongated ^{32}P-labeled RNA transcripts were hybridized to actin, β-globin, and H1 histone DNA inserts after restriction enzyme digestion and Southern blotting. The *solid lines* in the schematic diagram represent the relative positions of the DNA inserts.

volved in this process recognizes the 3′ stem loop after the termination of translation (33). Thus, inhibition of protein synthesis can increase H1 mRNA stability by blocking translation of this transcript. Taken together with the demonstration that expression of this gene is regulated at the transcriptional level, these findings indicate that ara-C activates at least two distinct signaling pathways that control H1 histone mRNA levels. The identification of a CHX-sensitive pathway that functions in the down-regulation of H1 histone gene transcription should define at least one of these early signaling events that are activated by ara-C and contribute to the control of specific gene expression by this agent.

Finally, although one of the effects of ara-C on gene expression includes down-regulation of H1 histone mRNA levels, the role of this event in the cellular response to this agent remains unclear. H1 histone interacts with specific DNA sequences (37–39) and could function in control of individual gene transcription by displacing bound *trans*-acting factors (40). However, there is presently no evidence to suggest that down-regulation of H1 histone expression contributes to the activation of *jun/fos* gene expression. Moreover, other studies have demonstrated that H1 histone serves as a substrate for the $p34^{cdc2}$ kinase and, thus, may play a role in the uncoiling of chromatin in preparation for replication or entry into mitosis (40). Down-regulation of H1 histone expression could, therefore, inhibit progression through S phase in the presence of agents that distort chromatin structure or damage DNA.

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