Deregulated messenger RNA expression during T cell apoptosis

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ABSTRACT

The IL-2 dependent murine cytotoxic T cell line CTLL-2 undergoes programmed cell death when deprived of its specific cytokine. We analyzed the expression of cell cycle related genes after IL-2 deprivation. Here we show that a generalized decrease and re elevation of the levels of mRNA takes place as part of the apoptotic program. The levels of several mRNAs encoding cell cycle functions, including cyclin D2, cyclin D3, cyclin B1, *c-myc* and *max* all declined at 1.5–3 h following IL-2 deprivation. Notably, the *max* mRNA, which was shown to be expressed in proliferating, growth arrested and differentiated cells, is down regulated with the same kinetics as the other mRNAs. Surprisingly, the mRNAs whose levels declined at 1.5–3 h rose again at 10–14 h, a time which closely followed the time of the first detection of apoptotic DNA degradation, at 8 h, but which precedes actual loss of viability, at 14 h, as measured by trypan blue exclusion. Of all analyzed genes only the expression of the S-phase specific histone H4 gene resists the initial decrease and declines gradually over the course of cell death. Measurement of c-Myc protein synthesis at a late stage of the apoptotic program revealed that the accumulated reinduced mRNA is not translated into protein. Because transcriptional regulation has been shown to be dependent on the chromatin structure, the reinduction may be triggered by relaxation of the chromatin caused by alterations in the chromatin structure of apoptotic cells.

INTRODUCTION

Programmed cell death is an important mechanism in the development and regulation of growth of cell populations in multicellular organisms. Cells dying by apoptosis undergo profound structural changes. In contrast to necrosis, where the cells swell and burst, apoptotic cells shrink (1,2). The shrinking cells undergo an apparent modification of their cytoskeleton, so that the plasma membrane becomes untethered and undergoes a process called 'zeiosis', or rapid blebbing (2,3). During the process of apoptosis, profound changes take place in the chromatin structure. The chromatin becomes very condensed and tends to collect in crescents around the nuclear envelope (4,5). Analyses of the chromosomal DNA revealed an initial cleavage of the DNA to

300 and/or 50 kb fragments prior to or in the absence of internucleosomal fragmentation (6). This fragmentation is considered to arise from the activation of one or more endonucleases. The relationship between the appearance of condensed chromatin and the endonucleolytic cleavage of DNA is uncertain. It has been implied that in thymocytes chromatin condensation is a result of internucleosomal cleavage of DNA (7).

The precise nature of the signal cascades which induce the cellular suicide program is not yet known. Recent results indicate that conflicts between cell growth promoting and repressing signals may provide one general mechanism for triggering the apoptotic pathway (8,9). The c-Myc and adenovirus E1A oncoproteins, which were shown to activate cell proliferation in the presence of other growth stimulating factors, induce rapid cell death when expressed in a growth arrested cell (10–12). Further evidence supporting the conflicting signal model was obtained from results on transgenic mice homozygous for a deletion of the retinoblastoma gene product, Rb (13–15). The pRb protein is proposed to be a negative regulator of cell proliferation. Extensive abnormal cell death occurs in the brain of these mice at embryonic day 11.5 and beyond. This cell death was seen in neuronal and hematopoietic lineages at about the time that first evidence of terminal differentiation is normally seen. This suggests that the inability of these cells to cease proliferation and induce growth-repressing differentiation signals triggers their entry into programmed cell death.

In order to study the molecular mechanism of programmed cell death we have chosen the IL-2 dependent murine T cell line CTLL-2 (16). These cells are tumor specific activated cytotoxic T cells, which undergo apoptosis when deprived of their specific cytokine (17). During immune responses, high numbers of antigen specific helper and cytotoxic T cells are present in an organism. After exhaustion of the antigen, the majority of these specific T cells are depleted by programmed cell death (2). Apoptosis of CTLL-2 cells induced by IL-2 deprivation may therefore resemble the depletion of cytotoxic T lymphocytes when serum levels of IL-2 decrease following an immune response.

MATERIALS AND METHODS

Cell culture

CTLL-2 cells were cultured at 37°C, 90% humidity and 5% CO₂ in a medium containing 90% RPMI 1640 (Cellgro), 10% heat inactivated fetal calf serum (Hyclone), 2 µM 2-mercaptoethanol (Sigma) and 10 U/ml human recombinant interleukin 2 (Gibco).

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For IL-2 deprivation the cells were washed twice with IL-2 free medium and resuspended in IL-2 free medium. We have defined the time after the second washing step as the time of start of IL-2 deprivation. For analyzing the effects of the protein synthesis inhibitor cycloheximide on programmed cell death, a solution of 100 µg/ml cycloheximide in PBS was added to the cell suspension to a final concentration of 5 µg/ml.

Trypan blue staining

For analyzing cell viability, 50 µl cell suspension was mixed with 50 µl of a solution of 0.4% trypan blue stain in 0.85% saline (Gibco). Cells excluding (viable cells) or cells stained by trypan blue (dead cells) were analyzed with the help of a microscope.

DNA fragmentation analysis

DNA fragmentation was analyzed by preparing chromosomal DNA from CTLL-2 cells as described (42). Chromosomal DNA (3 µg) was loaded on a 1% agarose gel and DNA bands were visualized by staining with ethidium bromide.

Flow cytometry

Light scattering and cell cycle distribution experiments were performed with a FACScan flow cytometer (Becton & Dickinson). After different time periods of IL-2 deprivation, 5×10^5 cells were pelleted by centrifugation and resuspended in 0.5 ml PBS buffer supplemented with 1% fetal calf serum. While vortexing, 5 ml of ice cold ethanol was added. The cell suspension was incubated overnight at 4°C. The fixed cells were pelleted by centrifugation and resuspended in 0.75 ml PBS buffer supplemented with 1% fetal calf serum. Ribonuclease A was added to a final concentration of 0.1 mg/ml. After the addition of propidium iodide to a final concentration of 0.05 mg/ml, the suspension was incubated for 2 h at 37°C. The ethanol fixed and propidium iodide stained cells were analyzed by flow cytometry for light scattering properties and DNA content. The percentages of cells in different stages of the cell cycle were calculated with the help of the computer program ModFit, supplied by Verity Software, Topsham, Maryland.

Northern blotting

Total RNA was isolated by the guanidinium-thiocyanate-phenol-chloroform single step extraction method (43). Fifteen micrograms of total RNA per lane was separated on a formaldehyde-agarose gel and transferred to a DURALON-UV membrane (Stratagene) by capillary elution. cDNA fragments were purified by gel elution and labeled with [α - 32 P]dCTP by random primed DNA labeling (44). The following DNA fragments were used: cyclin D2, 1.2 kb *EcoRI* fragment of pBluescript-cyl2 (45); cyclin D3, 1.7 kb *EcoRI* fragment of pBluescript-cyl3 (45); cyclin B1, 1.3 kb *XhoI-EcoRI* fragment of p18.2 (46); c-myc, 1.0 kb *XbaI-SacI* fragment of pSV2-myc (47); max, 0.5 kb

HindIII-BamHI fragment of pcDNA-I-max2 (Hopewell, R. and Ziff, E.B., unpublished); histone H4, 1.1 kb *EcoRI-HindIII* fragment of pHu4A (19). For hybridization, the labeled DNA probes were heat denatured and transferred to the DURALON membrane which was prehybridized for 30 min at 63.5°C in 15 ml QUIKHYB solution (Stratagene). The hybridization was performed for 4 h at 63.5°C. The membrane was washed for 30 min at room temperature using a solution containing 300 mM sodium chloride, 30 mM sodium citrate and 0.1% SDS. A second washing step was performed for 30 min at 59.5°C using a solution containing 15 mM sodium chloride, 1.5 mM sodium citrate and 0.1% SDS. The blots were autoradiographed. For reuse of the membrane, the hybridized labeled DNA probes were stripped off by pouring a boiling solution containing 15 mM sodium chloride, 1.5 mM sodium citrate and 0.1% SDS over the membrane. The membrane was incubated in the solution for 15 min on a shaker. The procedure was repeated two or three times.

Radioimmune assay

For metabolic labeling of CTLL-2 cells the cells were washed once with RPMI medium without L-methionine (Gibco) and supplemented with 10% dialyzed fetal calf serum (Hyclone) and 2 µM 2-mercaptoethanol (and 10 U/ml IL-2, the proliferating cells). Proliferating CTLL-2 cells and CTLL-2 cells, deprived of IL-2 for 12 h, were suspended in 2 ml of the above medium containing 0.5 mCi 35 S-methionine and the cells were incubated for 2 h at 5% CO₂, 37°C and 90% humidity. The medium was removed and the cells were washed once with PBS buffer. One millilitre of lysis buffer (250 mM NaCl, 50 mM Tris-HCl pH 7.4, 0.1% NP-40, 2 µg/ml leupeptin, 2 µg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride, 1 µg/ml pepstatin, 10 mM NaF, 0.2 mM sodium orthovanadate) was added and the cells were incubated for 20 min at 4°C for lysis. The lysate was centrifuged for 20 min at 4°C and 14 000 r.p.m. The protein concentration of the supernatant was determined by a Bio-Rad detergent compatible protein assay. The protein concentration was adjusted to 0.4 mg/ml by dilution with lysis buffer. One hundred microlitres 10% protein A Sepharose (in lysis buffer) and 3 µl of 10× diluted normal rabbit serum were added to 0.9 ml of the cell extract and the suspension was rocked for 1 h at 4°C. After removal of the protein A Sepharose by centrifugation, 3 µg of an immunopurified polyclonal rabbit serum raised against a recombinant Myc protein (26) or 3 µg of purified mouse monoclonal antibodies recognizing the C-terminal region of the Myc protein (C33, Santa Cruz Biot.) was added and the solution was incubated for 1 h on ice. When using a mouse monoclonal antibody 3 µg of rabbit anti mouse IgG1 (Pharmingen) was added and the solution was incubated for 20 min on ice. One hundred microlitres of 10% protein A Sepharose solution was added and the suspension was rocked for 1 h at 4°C. Afterwards the protein A Sepharose was pelleted and washed four times with lysis buffer. SDS protein sample buffer (20 µl) was added and the sample was boiled. The proteins were separated on 7.5% SDS polyacrylamide gel.

Figure 1. Viability of CTLL-2 cells deprived of IL-2 in the presence and absence of cycloheximide. (A) CTLL-2 cells were incubated for 0, 3, 6, 9, 12, 14 and 22 h in IL-2 free medium or in IL-2 free medium containing 5 µg/ml cycloheximide. The cell viability was determined by trypan blue staining. The percentage of trypan blue excluding cells is shown for each time point. (B) DNA fragmentation in CTLL-2 cells deprived of IL-2 for 0, 5, 8, 10, 12 and 14 h. A significant decrease of unfragmented and increase of fragmented DNA is observed between 8 and 10 h of deprivation. The size of the molecular weight marker M (*HaeIII* digest of double stranded phiX174 DNA) are 1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118 and 72 bp. (C) Changes in light scattering properties of CTLL-2 cells deprived of IL-2 for 0, 4, 8 and 12 h. The decrease in forward light scattering of the apoptotic cell population is emphasized by outlining the population of viable and apoptotic cells.

Figure 2. Flow cytometry analysis of the cell cycle distribution in CTLL-2 cells deprived of IL-2 for different time periods. CTLL-2 cells were deprived of IL-2, fixed with ethanol and stained with propidium iodide. The number of cells with a 2N (G1 phase), >2N <4N (S phase) and 4N (G2/M phase) DNA content was measured by flow cytometry analyzes. (A) Plots of DNA content versus cell number of CTLL-2 cells deprived of IL-2 for 0, 4, 8 and 12 h. The area of S phase cells is hatched and the area of G1, G2/M and apoptotic cells is solid. (B) Plot of the number CTLL-2 cells in different stages of the cell cycle (G1, S, G2/M) versus the time of IL-2 deprivation. The percentages of cells in different phases of the cell cycle were calculated with the help of the computer program ModFit (Verity Software).

RESULTS

In IL-2 deprived CTLL-2 cells, cell death is first detectable after 9 h of deprivation (Fig. 1A). The maximum loss of cell viability occurs between 11 and 15 h of cytokine deprivation as determined by trypan blue staining (Fig. 1A). After 22 h, 90% of the cells are dead. When cells undergo apoptosis they change their morphology resulting in a change in their light scattering behaviour (18). When analysing the light scattering properties of cytokine deprived CTLL-2 cells, first evidence of a population of apoptotic cells with a highly reduced forward light scatter value was detectable as early as 8 h after IL-2 removal (Fig. 1C). Morphological changes therefore precede the staining of apoptotic cells by trypan blue by ~1 h. Another very often observed characteristic of cells undergoing apoptosis is the degradation of their chromosomal DNA into single and multiple nucleosomes (2). When analysing the chromosomal DNA of CTLL-2 cells induced to undergo apoptosis we observed increasing evidence of DNA fragmentation at 8 h followed by extensive DNA fragmentation between 10 and 14 h (Fig. 1B). As determined by trypan blue staining, light scattering and chromosomal DNA analyses, we see first evidence of cell death at 8 h after IL-2 deprivation followed by a extensive loss of viability between 10 and 14 h. Programmed cell death of CTLL-2 can be partially prevented by the protein synthesis inhibitor cycloheximide (17). In the presence of cycloheximide, cell death is delayed by 5–7 h (Fig. 1A).

By analysing the cell cycle distribution of CTLL-2 cells after IL-2 deprivation we discovered that the G1/S phase transition is blocked within 2–4 h after the removal of IL-2. The flow cytometry analysis of proliferating CTLL-2 cells shows a negative slope of the curve of the distribution of their S phase population (Fig. 2A). However after 4 h of IL-2 deprivation, the number of early S

phase cells with a DNA content slightly higher than 2N is markedly decreased, leading to a change in the slope of the distribution of the S phase population from minus to plus (Fig. 2A). Since no cells are depleted by cell death at this time, the decreased number of early S phase cells indicates that the transition of cells from G1 phase to S phase is blocked. Progression through S phase however is still possible leading to a greatly diminished S phase population and an accumulation of cells in G1 phase at later deprivation times (8 h) (Fig. 2A and B). IL-2 deprivation therefore causes a block of the G1/S phase transition of the cell cycle, but still allows cells that have already entered S phase to progress through the cell cycle, leading to an accumulation of CTLL-2 cells in G1 phase before the onset of cell death.

To establish a possible basis for the growth arrest and subsequent induction of apoptosis, we analyzed gene expression in proliferating and IL-2 deprived CTLL-2 cells by Northern blotting. Our results show that the expression of mRNA by several cell cycle regulatory genes including *c-myc*, *max*, cyclin *D2*, cyclin *D3* and cyclin *B1* is repressed within the first 3 h of IL-2 deprivation (Fig. 3), indicating that repression of genes necessary for cell cycle progression precedes the block in the G1/S phase transition which we observe at 2–4 h following deprivation. These genes remain down regulated until the onset of massive cell death and chromatin degradation. The expression of the histone *H4* gene is normally restricted to cells which are replicating their DNA (19) and its expression after IL-2 removal correlates with the decreasing number of cells in S phase (Figs 2B and 3). All genes analyzed, with the exception of histone *H4*, show an accumulation of mRNA at a very late stage of IL-2 deprivation (14 h) (Fig. 3), when the cells were already committed to cell death (Fig. 4). Since the mRNA, ribosomal RNA (Fig. 3) and chromosomal DNA (Fig. 1B) prepared from these cells are

Figure 3. Gene expression in proliferating and IL-2 deprived CTLL-2 cells. Total RNA from CTLL-2 cells deprived of IL-2 for 0, 1.5, 3, 4, 6, 8, 10, 12 and 14 h was analyzed by Northern blot analysis. The blotted RNA was hybridized with the following [α - 32 P]dCTP labelled cDNA probes: mouse cyclin D2, cyclin D3, cyclin B1, *c-myc* and *max*, and human histone H4. The same blot was hybridized separately with different probes. For quantitation of the amount of total RNA the ethidium bromide stained bands of the 28S ribosomal RNA are shown. Complete RNA transfer was verified.

partially degraded, it seems likely that these highly expressed mRNAs arise from dying or dead cells rather than of a subpopulation of cells unable to undergo apoptosis. In contrast to all initially down regulated genes, which remain down regulated until the onset of programmed cell death, the expression of cyclin D2 mRNA is upregulated between 3 and 8 h of IL-2 deprivation (Fig. 3). Western blot analyses of the protein levels however did not show a reinduction of cyclin D2 protein during this time period (data not shown).

The Myc protein was shown to play a key role in the induction of apoptosis in different cells types (10,11,20). We have analyzed if the *c-myc* mRNA induced in dying CTLL-2 cells is translated to give an increased expression of c-Myc protein. We have metabolically labelled proliferating and dying CTLL-2 cells, which were deprived of IL-2 for 12–14 h, with 35 S-methionine. The rate of c-Myc expression was measured by immunoprecipitating the c-Myc proteins from the labelled cells. The proteins of the precipitates were resolved by SDS polyacrylamide gel electrophoresis. Autoradiography revealed a very high expression of c-Myc protein in proliferating cells, but nearly no c-Myc protein expression in the dying lymphocytes (Fig. 4). This shows that the *c-myc* mRNA which accumulates at high levels in dying

Figure 4. Expression of the c-Myc protein in proliferating and dying CTLL-2 cells. Proliferating (+IL-2) and CTLL-2 cells deprived of IL-2 for 12 h (–IL-2) were metabolically labelled with 35 S-methionine for 2 h. The cells were lysed and the Myc proteins were immunoprecipitated using an immunopurified polyclonal rabbit serum (1) or mouse monoclonal antibodies (2) raised against the c-Myc protein. The precipitated proteins were separated by 7.5% SDS polyacrylamide gel electrophoresis. The protein bands were visualized by autoradiography.

CTLL-2 cells is not translated into c-Myc protein. The reinduction of mRNAs can therefore be seen as a consequence of the apoptotic process rather than an inducer of the program. This is consistent with the fact that the reinduction of the mRNAs occurs at a very late time (12–14 h) within the apoptotic program, when protein synthesis no longer has an influence on the rate of apoptosis (Fig. 5). The addition of the protein synthesis inhibitor cycloheximide to IL-2 deprived T cells delays the apoptotic program (Fig. 1A). We have added the protein synthesis inhibitor at different time periods after IL-2 deprivation and measured the cell viability after 22 h of cytokine deprivation (Fig. 5). Addition of cycloheximide after 4 h of IL-2 deprivation resulted in the same percentage of surviving cells after 22 h as in cells where cycloheximide was added directly at the time of IL-2 deprivation (Fig. 5). However, addition of cycloheximide after ≥ 6 h of cytokine deprivation lead to a marked decreased in the percentage of viable cells after 22 h. These results indicate that CTLL-2 cells become committed to cell death between 6 and 8 h of IL-2 deprivation.

DISCUSSION

Our results show that induction of apoptosis by IL-2 deprivation in CTLL-2 cells causes a very early block in the G1/S transition of the cell cycle. Cells which are in S or G2/M phase are still able to progress through the cell cycle leading to an accumulation of cells in G1 phase before the onset of massive cell death.

We have shown that the *c-myc* gene, which is necessary for G1–S phase progression (21–23), is strongly down regulated after IL-2 deprivation. This down regulation may contribute to the early cell cycle block. c-Myc protein is necessary for T cell receptor induced cell death of a T cell hybridoma cell line (20). In CTLL-2 cells the pattern of expression of *c-myc* indicates that the c-Myc protein, which is very unstable, is not involved in the activation of the apoptotic program. The Max protein is proposed to act as a repressor of transcription in growth arrested cells and in cooperation with the c-Myc protein as an activator of transcription in proliferating cells (24–26). The *max* gene was previously shown to be expressed in growth arrested, proliferating and differentiated cells (27,28). Under other conditions of

Figure 5. Prevention of apoptosis by the protein synthesis inhibitor cycloheximide in IL-2 deprived CTLL-2 cells. CTLL-2 cells were deprived of IL-2 and cycloheximide was added to different cell samples to a final concentration of 5 μ g/ml after 0, 2, 4, 6, 8, 10, 12 and 14 h of IL-2 deprivation. Cell viability was measured after 22 h of IL-2 deprivation by trypan blue staining. A plot of the time of cycloheximide versus the cell viability after 22 h is shown.

growth regulation, the levels of *max* mRNA remain constant (27,28). However, after IL-2 deprivation, *max* mRNA is down regulated with the same kinetics as the *c-myc* mRNA or the mRNA for the G2/M phase specific cyclin B1. Since the Max protein has, in contrast to the c-Myc and cyclin B1 proteins, a very long half life, it may still have a function in the apoptotic program.

The absence of IL-2 receptor signaling does not cause a general shutdown of cell cycle related genes in IL-2 deprived CTLL-2 as indicated by continued expression of the histone *H4* gene. The expression of histone H4 is restricted to cells which are replicating DNA. Histone H4 expression after IL-2 removal correlates with the decreasing number of S phase cells and is consistent with the observation that cells which have already entered S phase are able to progress through the cell cycle after IL-2 removal.

Our analysis of gene expression in cytokine deprived CTLL-2 cells reveals a difference between expression of the cyclin *D2* gene and other cell cycle related genes studied here. The cyclin *D2* gene is selectively induced during the period from 3 to 8 h following IL-2 deprivation, a time when the cells become committed to cell death. Western blot analyses, however, did not confirm the induction of cyclin D2 protein and showed instead a constant decline of protein over the time period from 0 to 11 h of IL-2 deprivation (data not shown). The cyclin D2 protein may still play a role in the induction cascade of the apoptotic program. The elevated expression of cyclin *D2* mRNA between 3 and 8 h could be necessary to ensure the presence of the very rapidly metabolised cyclin D2 protein during the committing step. It is noteworthy that cyclin D1 is induced during the committing step of programmed cell death of NGF deprived postmitotic neurons (29) and that constitutive expression of D type cyclins in rodent fibroblasts is toxic in a cyclin protein concentration dependent manner (30).

Our studies show a coordinate decrease of cyclin *D2*, cyclin *D3*, cyclin *B1*, *c-myc* and *max* mRNAs at 1.5–3 h following IL-2 deprivation followed by a re-induction at 10–14 h, a time following onset of DNA degradation. The mechanisms of repression and of reinduction are not known. Because they affect multiple genes, they may reflect common features of the regulation of gene expression such as assembly of general transcription factors into a complex on DNA at a promoter and recruitment of RNA polymerase to this site (31,32) by transcription factors which regulate the general transcriptional machinery (33).

The packaging of DNA into chromatin (34,35) compacts and organizes the DNA and may also regulate transcription. Changes in chromatin structure may either precede or accompany the transcriptional activation of genes and modification of chromatin structure *in vivo* can affect gene expression (35,36). Unfolding of the highly condensed chromatin structure seems to be an essential step in gene activation.

A broad range of genes is promiscuously transcribed in an unregulated manner by the basal transcriptional machinery with naked DNA templates free of histone (35). Packaging of DNA into chromatin causes a general repression of transcription that can be relieved by enhancer element associated transcription factors and/or general transcription factors (36,37). Binding of transcription factors to enhancer elements can derepress a chromatin template, but not a histone free template (38). Thus, the compact chromatin may represent a ground state in which gene activity is repressed by DNA folding and histone binding. Binding of enhancer proteins may partially unfold the condensed chromatin, relieving inhibition and allowing the general transcription factors to bind to the promoter and initiate RNA synthesis (35).

By analyzing gene expression in cytokine deprived lymphocytes we show that IL-2 deprivation of CTLL-2 causes a repression of

the mRNA expression of all genes we have analyzed within 3 h, with the exception of the histone *H4* gene. This raises the possibility that the chromatin structure of these repressed genes has changed from a relaxed activated state to a higher folded repressed state. IL-2 deprivation of these cells induces an apoptotic program which would cause profound changes in the chromatin structure of the cells. Between 8 and 10 h of IL-2 deprivation we detect the degradation of the chromosomal DNA into nucleosomal fragments. Parallel with the degradation of DNA we observe an induction of all genes which were initially down regulated. The structural changes of the chromatin caused by degradation processes in apoptotic cells may therefore result in de-repressed unfolded template DNA fragments with a high rate of basal transcription.

Unlike other cell cycle related mRNAs studied here, the histone *H4* mRNA does not accumulate at the time when the chromatin becomes degraded. Experiments altering the chromatin structure by mutating histone genes in yeast have shown a promoter specificity for deregulation of gene expression (35,39). Changes in the chromatin structure caused by missing histones resulted in the selective hyperactivation of a subset, but not all, analyzed promoters. Since the regulation of the histone *H4* gene differs from the other genes in that it resists the initial decrease after IL-2 deprivation, it may also be differently regulated by changes in chromatin structure. The histone *H4* mRNA also differs in that it lacks a stabilizing poly A tail and has instead a 3' stem and loop structure which regulates its degradation (40). In the absence of DNA replication, histone mRNAs are degraded within minutes (40,41). This extreme instability could prevent an accumulation of histone mRNA even if the gene is induced by basal transcription from degraded DNA in apoptotic cells.

We have found a deregulated expression of mRNA from several cell cycle related genes in apoptotic cytotoxic T lymphocytes. The fact that these mRNAs are not translated into protein, as shown for the c-Myc protein, and the accumulation of the mRNA at a time when the cells are already committed to cell death, lead us to the conclusion that the observed phenomenon is a consequence rather than an inducer of the apoptotic program. The very tight correlation of the timing and extent of the mRNA accumulation and the degradation of chromosomal DNA suggests that the increased mRNA expression is triggered by the relief of chromatin mediated inhibition of transcription in apoptotic cells.

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