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Induction of a Ca^{2+},Mg^{2+}-dependent endonuclease activity during the early stages of murine erythroleukemic cell differentiation

(Friend cells/dimethyl sulfoxide/DNA fragmentation)

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ABSTRACT A Ca^{2+},Mg^{2+}-dependent endonuclease activity was detected in erythroleukemic cells undergoing differentiation *in vitro* in response to induction by dimethyl sulfoxide (Me_2SO) or hexamethylene-bis-acetamide (HMBA). The endonuclease activity was demonstrated in isolated nuclei within 6 hr after the addition of inducer, reached maximum levels between 24 and 48 hr, and returned to control levels within 72 hr. The activity caused single strand breaks in high molecular weight native DNA, which could be labeled at exposed 3′-OH termini with *Escherichia coli* DNA polymerase I and radiolabeled nucleotides. Alkaline elution studies revealed DNA fragmentation that appeared coincident with the presence of the endonuclease activity. The detection and levels of single strand DNA breakage correlated with induction of terminal differentiation by Me_2SO or HMBA. Induction of the endonuclease activity was reversible: depletion of Me_2SO from the growth medium after treatment for 6 and 18 hr led to a rapid decrease in the level of activity. Removal of the inducer prevented terminal differentiation, a finding that strongly suggests the endonuclease activity is present during the precommitment phase of differentiation. DNA fragmentation was not observed in cells incubated with hemin, which has been shown previously to increase the cytoplasmic level of globin mRNA without causing commitment to terminal maturation. Me_2SO did not induce the endonuclease activity or DNA fragmentation in an uninducible Friend cell line.

The differentiation response of Friend cells to a variety of inducers has proven to be an important system for studying erythroid maturation (for review, see refs. 1 and 2). In this regard, the commitment of a cell to terminal erythroid differentiation appears to require transitory metabolic changes. Specifically, when chemical inducers are used with Friend cells, the precommitment period is characterized by a prolongation of the G_1 phase of the cell cycle (3, 4) and an increase in the intracellular level of calcium ions (5). It has been suggested that the calcium influx proceeds through a Na^+/Ca^{2+} antiport mechanism, because the treatment of the cells with inhibitors of Na^+,K^+-ATPase activity led to stimulation of Ca^{2+} uptake and abolition of the 10-hr lag period normally associated with commitment of these cells to terminal differentiation (6).

In this communication, we demonstrate that chemical induction of Friend cells leads to the presence of a nuclear Ca^{2+},Mg^{2+}-dependent endonuclease activity that is capable of generating single strand DNA breaks in high molecular weight chromosomal DNA. Previous studies of Friend cells reported single strand breaks in the DNA subsequent to induction of differentiation by butyrate, dimethylacetamide, butyrylcholine, and dimethyl sulfoxide (Me_2SO) (7–9). In addition, DNA fragmentation has been shown to accompany the differentiation process in several other systems (10–12). We detect this Ca^{2+},Mg^{2+}-dependent endonuclease activity in the nucleus of Friend cells early in the induction process prior to the commitment of the cells to terminal differentiation.

MATERIALS AND METHODS

Growth of Cells and Tissue Sources. Friend tumor cell lines C7D and A6, previously cloned from subcutaneous tumors (13), were grown in Eagle's basal medium supplemented with 10% fetal calf serum and gentamicin sulfate at 50 μg/ml. Cell line 745-PC-4 (a derivative of cell line 745 from C. Friend, Mt. Sinai School of Medicine, New York) was obtained from V. Volloch and D. Housman (Massachusetts Institute of Technology, Boston). Logarithmic-phase cells at 10^5 cells per ml were supplemented with 192 mM Me_2SO, 3 mM hexamethylene-bis-acetamide (HMBA) (Sigma), or 100 μM hemin where indicated. Cell viability was estimated by trypan blue exclusion and the proportion of hemoglobin-containing cells was estimated by wet staining with benzidine hydrochloride (14).

Nuclei Isolation and Digestion. Approximately 10^8 cells were washed with 50 ml of buffer A (50 mM Tris·HCl, pH 7.6/25 mM KCl/10 mM $MgCl_2$/5 mM 2-mercaptoethanol/0.2 mM phenylmethylsulfonyl fluoride) containing 10 mM EGTA and 0.34 M sucrose. The cells were lysed with buffer A containing 0.75% (wt/vol) Triton X-100 in 0.25 M sucrose and layered over a 0.5 M sucrose buffer A cushion. Nuclei were sedimented (500 × *g*, 10 min, 4°C) and resuspended in 2.4 M sucrose/buffer A. The nuclei were centrifuged (10,000 × *g*, 20 min, 4°C), and the resultant nuclear pellet was washed twice with 0.25 M sucrose/buffer A.

Autodigestion of nuclei was for 3–24 hr at 37°C at a concentration of 10 A_{260} units/ml in 0.25 M sucrose/buffer A supplemented with 1 mM $CaCl_2$. Reactions were terminated with 15 mM EDTA (pH 7.5) and the nuclei were then sedimented (500 × *g*, 5 min, 4°C). Nuclei were stored in 40% (vol/vol) glycerol/buffer A at −70°C until analysis.

Where indicated, nuclei were lysed in 10 mM Tris·HCl, pH 8.0/1 mM EDTA. Insoluble chromatin was removed by centrifugation (10,000 × *g*, 10 min, 4°C). Supernatants were collected and A_{260}-absorbing material, which was rendered buffer soluble, was measured (15).

Preparation and Analysis of Chromatin Extracts. Chromatin derived from lysed nuclei was extracted in a buffer containing 0.35 M KCl/20 mM Tris·HCl, pH 7.6. After centrifugation (2000 × *g*, 20 min, 4°C), the supernatant was dialyzed overnight against 10 mM KCl/10 mM Tris·HCl, pH 7.6/5 mM 2-mercaptoethanol. Endonuclease activity in extracts was assayed by two methods: (*i*) Up to 50 μg of chromatin extract was incubated at 37°C with 2.5 μg of [^{3}H]thymidine-

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Abbreviations: Me_2SO, dimethyl sulfoxide; HMBA, hexamethylene-bis-acetamide; kb, kilobase(s).

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labeled *Escherichia coli* DNA [average length, 0.5 kilobases (kb)]/20 mM Tris·HCl, pH 7.6/10 mM $MgCl_2$/1 mM $CaCl_2$/5 mM 2-mercaptoethanol. Reactions were stopped by adding 10% perchloric acid. Activity was expressed as the amount of starting DNA that was rendered perchloric acid-soluble after a 90- to 240-min incubation.

(*ii*) Chromatin extract (10–100 ng) was added to 1 μg of closed circular pBR322 plasmid DNA and incubated for 60 min at 37°C in the presence of 10 mM $MgCl_2$/1 mM $CaCl_2$. Plasmid DNAs were analyzed by electrophoresis in a 1% agarose gel (16). Endonuclease activity was detected by the conversion of closed circular plasmid DNA to open circular and linear forms.

Analysis of Cellular DNA by Alkaline DNA Elution. Cultures were initiated at different cell densities ($5–25 \times 10^4$ cells per ml) to ensure harvesting them at the same cell density. DNA was labeled to the same specific activity by growing cells for the same amount of time (24 or 48 hr depending on the set of experiments) in the presence of 1.0 μCi of [2-^{14}C]thymidine per ml (56 mCi/mmol;1 Ci = 37 GBq; ICN). The labeling procedure did not affect the growth rate or the response to inducer. Cells of each sample (5×10^5) were slowly sedimented by gravity onto a 25-mm polyvinyl chloride filter membrane (2.0-μm pore size; Millipore) after dilution in 20 ml of ice-cold phosphate-buffered saline. The cells were washed twice with 5 ml of phosphate-buffered saline and lysed with 5 ml of a solution containing 2 M NaCl/40 mM EDTA, pH 10.0/0.2% *N*-lauroylsarcosine (Sigma)/0.5 mg of proteinase K per ml (Merck). The filters were washed once with 3 ml of 20 mM EDTA (pH 10.0) and eluted with 15 ml of a tetrapropylammonium hydroxide/EDTA solution (17) containing 0.1% $NaDodSO_4$. Filters were eluted into 10 equal fractions at ≈0.025 ml/min. Fractions were analyzed by liquid scintillation counting after the addition of 10 ml of Hydrofluor (National Diagnostics) supplemented with 0.7% acetic acid. DNA remaining on the filter was counted after acid depurination by the treatment of Kohn *et al.* (17). DNA fragmentation was measured as an increase in elution rate relative to the elution rate of cellular DNA from untreated cells.

Endonuclease Activity Measurement in Isolated Nuclei. Nuclei were isolated from cells (labeled with [2-^{14}C]thymidine) at different times after the addition of inducer. After incubation for 3 hr with 1 mM $CaCl_2$, these nuclei, containing 3.5 μg of DNA ($1–6 \times 10^4$ cpm/μg), were then applied to polyvinyl chloride filter membranes in 20 ml of buffer A containing 0.25 M sucrose/1 mM EGTA and were subjected to alkaline elution procedures as described above. Unincubated nuclei as well as nuclei incubated for 3 hr in the presence of 0.1 mM EGTA were examined as controls in a similar manner. Endonuclease activity was expressed as an increase in elution rate of DNA isolated from nuclei incubated with Ca^{2+} as compared to DNA from control nuclei.

Preparation of DNA. Nuclei were resuspended in a buffer containing 0.1% (wt/vol) $NaDodSO_4$/50 mM NaCl/10 mM EDTA/ 50 mM Tris·HCl, pH 8.0, and digested with 200 μg of proteinase K per ml and 50 μg of RNase A per ml overnight at 60°C. DNA was extracted twice with a 1:1 mixture of redistilled phenol/chloroform and extracted up to three times with a 24:1 mixture of chloroform/isoamyl alcohol. The aqueous phase was made 0.3 M with sodium acetate (pH 5.2) and precipitated overnight at −20°C with 2.5 vol of 95% ethanol. DNA was centrifuged (10,000 × *g*, 10 min, 4°C) and resuspended in buffer B (10 mM Tris·HCl, pH 8.0/1 mM EDTA).

DNA Polymerase I Assay. Purified cellular DNA (1 μg) was analyzed by a modification of the methods of Hagen (18) and incubated for 1–2 hr at 15°C in a reaction containing 20 mM Tris·HCl, pH 7.6/10 mM $MgCl_2$/1 mM dithiothreitol/20 μM dATP, dGTP, and TTP/1 μM [α-^{32}P]dCTP (600 Ci/mmol; New England Nuclear)/10 units of *E. coli* DNA polymerase I. The reaction was stopped with 15 mM EDTA (pH 7.5). Labeled DNA was isolated free of unincorporated triphosphates by centrifugation through a Sephadex G-50 column equilibrated with buffer B. Samples were precipitated with 10% perchloric acid, filtered, and radioactivity was determined by liquid scintillation counting in Ultrafluor (National Diagnostics).

Alkaline Gel Electrophoresis. Radiolabeled DNA samples were dissolved in 50 mM NaOH and loaded on a 2% alkaline agarose gel containing 50 mM NaCl/1 mM EDTA/30 mM NaOH. Samples were subjected to electrophoresis for 5 hr at 40 V with recirculating buffer. The gel was neutralized with 7% acetic acid, dried, and autoradiographed using a Cronex intensifier screen at −70°C.

RESULTS

We have noted a Ca^{2+},Mg^{2+}-dependent endonuclease activity in nuclei of spleens derived from mice infected with Friend virus (ref. 15; unpublished observations). Since the erythroleukemic spleen is largely composed of differentiating erythroid cells, we investigated whether chemical induction of precursor erythroleukemic tumor cells in tissue culture elicited a similar endonucleolytic activity.

An inducible Friend cell line, C7D, was grown in the presence of 192 mM Me_2SO or 3 mM HMBA. Staining of the cells with benzidine and microscopic examination revealed that >95% of the cells were undergoing terminal differentiation within 5 days of treatment with either inducer. At 48 hr after addition of Me_2SO, the globin mRNA level was increased 3- to 5-fold (G. Brawerman, personal communication). Nuclei were isolated from cells during induction by Me_2SO and incubated in the presence of Ca^{2+} and Mg^{2+} ions. Endonuclease activity was assayed by the release of DNA into a low ionic strength buffer fraction upon lysis of incubated nuclei (15). This method would measure enzymatic activity that led to internucleosomal double strand DNA breaks. However, this kind of activity was not observed in nuclei from chemically induced or uninduced C7D cells. We next examined 0.35 M chromatin extracts derived from differentiating C7D cultures for the presence of an endonuclease activity similar to that detected in extracts from erythroid spleen cell chromatin. No detectable levels of endonucleolytic activity were observed as measured by the release of ^{3}H-labeled DNA substrate into an acid-soluble fraction or by the nicking of closed-circular plasmid DNA.

Detection of Increased DNA Fragmentation During Induction of Friend Cells. As an alternative approach, we used a more sensitive assay to determine whether an endogenous enzyme activity leads to single strand breaks in the cellular DNA. Examination of these DNA lesions was noted by changes in the rate of DNA elution under denaturing conditions (19). [2-^{14}C]Thymidine was added to logarithmic-phase C7D cultures 24–48 hr prior to analysis. At different times after addition of Me_2SO, labeled cells were gently lysed onto polyvinyl chloride filter membranes and the DNA was eluted. By 24 hr after the addition of Me_2SO, DNA fragmentation was noted as an approximate 2-fold increase in the rate of DNA elution (Fig. 1*A*). This level of fragmentation was maintained up to 48 hr, decreased to control rates of DNA elution by 72 hr, and was maintained at low rates up to 120 hr. No such fragmentation was seen in control cells incubated and labeled under the same conditions in the absence of inducer.

In a separate set of experiments, DNA from cells at different times after the addition of Me_2SO was isolated and labeled at available 3′-OH termini by incubation in a DNA polymerase I assay. An increase of 15% in the specific activity of the DNA was found in cells induced for 24 or 48 hr as compared to control cells. Examination of this radiolabeled

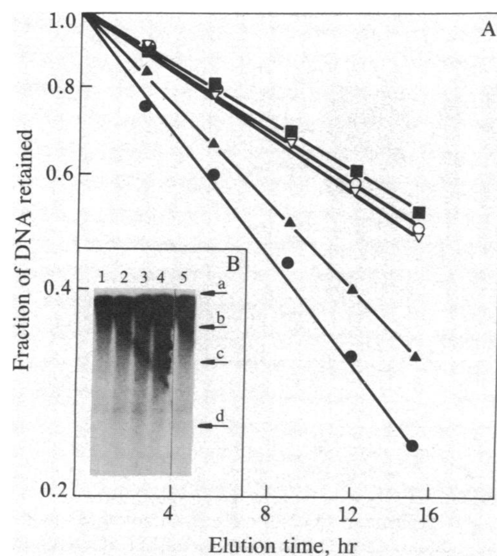

FIG. 1. Analysis of DNA fragmentation during Me_2SO induction. Cell DNA was analyzed by elution of ^{14}C-labeled DNA through polyvinyl chloride filter membranes under denaturing conditions. DNA was analyzed at 0 (○), 24 (▲), 48 (●), 72 (▽), and 96 (■) hr after the addition of 192 mM Me_2SO (*A*). In a separate experiment, DNAs were purified from whole cells at different times after addition of inducer and were radiolabeled by incubation with *E. coli* DNA polymerase I and [α-^{32}P]dCTP. These DNAs were analyzed by electrophoresis in a 2% agarose gel under alkaline conditions (*B*) and were examined by autoradiography (numbers refer to increasing times of induction as seen in *A*). Arrows denote a DNA standard representing 23.5 (a), 9.7 (b), 6.6 (c), and 4.3 (d) kb.

DNA in agarose gels by electrophoresis under alkaline conditions showed a detectable decrease in DNA strand length (Fig. 1*B*). Examination of the same DNAs under native DNA conditions showed no apparent decrease in DNA size at any time during induction (data not shown). Uninduced cells showed no evidence of DNA fragmentation by this assay.

Addition of 3 mM HMBA to an exponentially growing C7D culture was also accompanied by DNA fragmentation, as revealed by a change in the rate of DNA elution. Fragmentation was noted 6–12 hr after the addition of inducer and rapidly returned to control rates of elution by 24 hr after initial exposure to HMBA (Fig. 2*A*). HMBA also elicited an earlier and more synchronous appearance of benzidine-positive cells when compared to treatment of cultures with Me_2SO (Fig. 2*B*). In contrast to these two inducers, hemin when added at 100 μM to this cell line did not cause an increase in single strand DNA breaks under conditions that led to an approximate 5- to 7-fold increase in globin mRNA levels after 48 hr (G. Brawerman, personal communication). Under these conditions, however, histologic staining of the cells showed that none was undergoing morphologic changes indicative of terminal erythroid differentiation. This finding has been observed with other hemin-treated Friend cell lines (20–22). Single strand DNA breaks were also evaluated in an uninducible Friend cell line, A6, after addition of Me_2SO. No evidence of an increase in single strand DNA fragmentation by alkaline elution techniques or by the DNA polymerase I assay was found (Fig. 2*A*).

Ca^{2+},Mg^{2+}-Dependent Endonuclease Activity During Induction. To investigate a possible enzymatic generation of these DNA breaks, we examined the DNA from nuclei isolated from cells at different times after Me_2SO addition. Incubation of nuclei in the presence or absence of Ca^{2+}, Mg^{2+}, and Mn^{2+} ions revealed an endonuclease activity that required Ca^{2+} and either Mg^{2+} or Mn^{2+} ions. The activatable endonuclease activity slightly preceded the detection of DNA fragmentation. As evidenced by a relative increase in the rate of DNA elution under denaturing conditions (Fig. 3*A*), the endonuclease activity reached maximum levels in nuclei from cells derived between 24 and 48 hr after the addition of Me_2SO and decreased to a control level by 72 hr. Incubation of these nuclei for 3 hr showed a 3- to 4-fold increase in elution rate compared to nuclei incubated in the presence of Mg^{2+} alone or in nuclei derived from cell cultures 72 or 96 hr after addition of Me_2SO (Fig. 3*A*). By the same method, we detected an endonuclease activity in C7D cells 48 hr after addition of another inducer, 1 mM butyric acid (data not shown).

Examination of nuclei from another inducible Friend cell line, 745-PC-4, showed a similar Ca^{2+},Mg^{2+}-dependent endonuclease activity within 48 hr after addition of Me_2SO, which amounted to 80% of the activity observed in nuclei of the C7D cell line at 48 hr. Analysis of DNA from a Me_2SO-treated yet uninducible cell line (A6) did not reveal any endonuclease activity (Fig. 3*D*). No such endonuclease activity was detected in control cells at any time during 5 days of culture.

In a related set of experiments, the endonucleolytic activity was detected within 6 hr after the addition of Me_2SO and quantitated as a relative increase in incorporation of deoxyribonucleotides after incubation of the purified cellular DNA with *E. coli* DNA polymerase I and [α-^{32}P]dCTP (Fig. 3*B*). Analysis of the DNA on native gels did not reveal any double strand DNA breaks even after extensive digestion of the nuclei for 24–48 hr at 37°C (data not shown). Size analysis of the radiolabeled DNA in alkaline gels substantiated the presence of the Ca^{2+},Mg^{2+}-dependent endonuclease activity, because the DNA fragment size decreased appreciably after incubation of nuclei in the presence of both Ca^{2+} and Mg^{2+} ions (Fig. 3 *B* and *C*).

Endonuclease Activity in the Precommitment Phase of Induction. To associate the presence of the endonuclease ac-

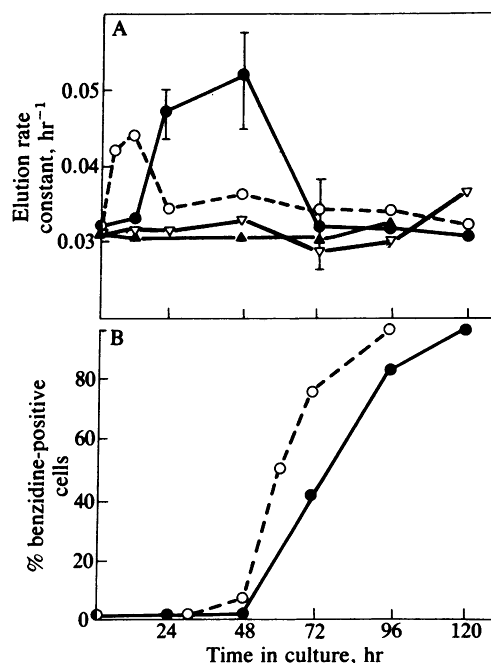

FIG. 2. Association of DNA fragmentation with terminal differentiation. The rate of alkaline elution of prelabeled DNA was monitored during induction of Friend cell line C7D with 192 mM Me_2SO (●), 3 mM HMBA (○), or 100 μM hemin (▲). DNA from an uninducible Friend cell line A6 treated with Me_2SO was also examined (▽) (*A*). The appearance of intracellular hemoglobin was estimated by staining with benzidine and is expressed as percentage of total cells in culture (*B*).

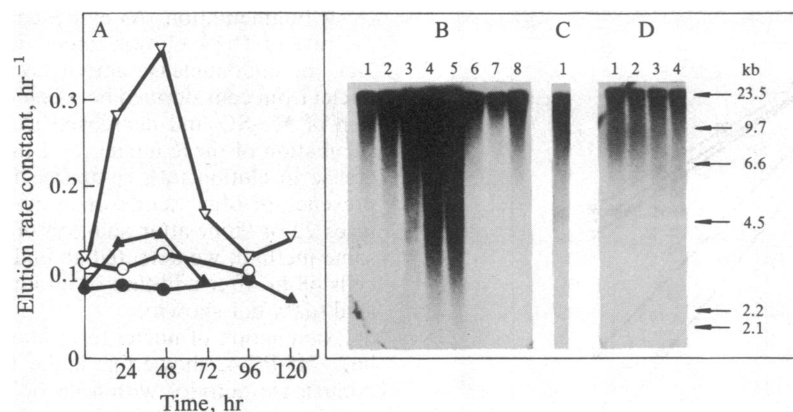

FIG. 3. Appearance of a Ca^{2+},Mg^{2+}-dependent endonuclease activity during Me_2SO induction. Nuclei containing labeled DNA were isolated at various times from control C7D cells (○, ●) and cells treated with Me_2SO (▽, ▲). Nuclei were incubated for 3 hr (open symbols) or not incubated (closed symbols) in the presence of 1 mM $CaCl_2$/10 mM $MgCl_2$. Endonuclease activity was observed as a relative increase in the rate of elution of DNA from incubated as compared to unincubated nuclei (*A*). In a similar experiment, nuclei were isolated from unlabeled C7D cells at 0, 6, 12, 24, 48, 72, 96, and 120 hr after the addition of Me_2SO (lanes 1–8) and incubated for 24 hr at 37°C in 1 mM $CaCl_2$/10 mM $MgCl_2$. The DNAs were purified, incubated with [α-^{32}P]dCTP in a polymerase assay, and subjected to analysis on a 2% alkaline agarose gel (*B*). DNA was similarly examined from nuclei derived from a C7D culture 48 hr after the addition of Me_2SO and incubated in the presence of 0.1 mM EGTA/10 mM $MgCl_2$ (*C*). DNAs were also examined from Ca^{2+},Mg^{2+}-incubated nuclei derived from uninducible Friend cells 0, 6, 12, and 24 hr after treatment with Me_2SO (lanes 1–4) (*D*).

tivity with a particular phase of the induced differentiation process (23), Me_2SO was removed from the growth medium at 6 and 18 hr after initial exposure. The presence of Me_2SO for only 6 hr activated a low level of endonuclease activity that was detectable by DNA fragmentation (Fig. 3*B*, lane 2) but was not, however, easily seen in the DNA polymerase I assay. At 18 hr after addition of Me_2SO, endonuclease levels characteristic of maximal activity at 24–48 hr were noted. Upon removal of inducer from the medium, the activity rapidly returned to control levels (Fig. 4). The depletion of inducer from the media at either 6 or 18 hr resulted in only 0–5% of the cells staining benzidine-positive 4 days after the initial exposure of cells to Me_2SO. These results strongly suggested that the activation of the endonuclease occurred during a precommitment phase of the induced differentiation process in the vast majority of these cells.

DISCUSSION

We have detected an activatable Ca^{2+},Mg^{2+}-dependent DNA nicking activity in nuclei of chemically induced differentiating murine erythroleukemia cells. The activity resulted in single strand DNA breaks, a finding previously reported

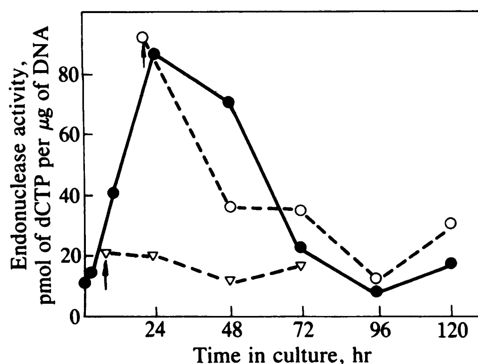

FIG. 4. Endonuclease activity after depletion of inducer. Me_2SO was depleted from a C7D Friend cell culture 6 (▽) and 18 (○) hr after an initial exposure to Me_2SO. Endonuclease activity was monitored as a Ca^{2+},Mg^{2+}-dependent increase in pmol of [α-^{32}P]dCTP incorporated per μg of DNA. Background incorporation derived from incubation of nuclei in the presence of Mg^{2+} ions alone was subtracted. A culture continuously exposed to Me_2SO is shown (●).

as a consequence of chemical induction (7–9). In this study, we have presented evidence to suggest that the breaks are generated enzymatically, because the presence of the endonuclease activity coincides with an increase in the steady-state level of DNA fragmentation. In addition, endonuclease activity and DNA strand breakage are transient and occur during the same time period (6–48 hr) after the addition of inducer (Figs. 2 and 3). In a prior study, Scher *et al.* (24) showed a 2-fold increase in endonuclease activity in cell-free extracts derived from Friend cells induced for 3–4 days in the presence of Me_2SO. In contrast to our nuclear activity, this endonuclease activity was released by cell lysis, required only Mg^{2+} ions for activity, and was stimulated later in the induction process. Our results contrast with those of Pantazis *et al.* (25), who were unable to detect DNA breaks during Friend cell differentiation using alkaline elution procedures. The 2-fold increase in DNA fragmentation we have observed may have been missed, however, by the use of a fluorimetric rather than a radiolabeled DNA assay.

Our evidence suggests that the fragmentation of DNA is only associated with Friend cells that terminally differentiate in response to chemical induction. In support of this, no fragmentation or endonuclease activity was found in hemin-treated cells that contained increased levels of globin transcripts and did not show morphologic evidence of terminal differentiation. While our results have not ruled out that Me_2SO or HMBA are not directly acting on the DNA, it is noted that hemin, which is known to cause DNA strand breaks *in vitro* (26), did not lead to appreciable strand breakage or activation of endonuclease activity. Moreover, neither Me_2SO nor HMBA elicited DNA breaks in the uninducible cell line. Our data would suggest that the increased synthesis of globin mRNA in response to hemin occurs independently of the other early events that commit cells to terminally differentiate. Recently, it has been reported that hemin-induced iron transport and heme biosynthesis are effects of hemin that are independent of commitment to terminal maturation (27).

The kinetics of appearance of hemoglobin-containing cells is not identical for all inducing agents (28, 29). In agreement with these reports, we note the appearance of benzidine-reactive cells treated with HMBA 12 hr earlier than cultures treated similarly with Me_2SO. If the rate of appearance of benzidine-reactive cells is a reflection of the rate of commit-

ment, it is of interest that the appearance of DNA fragmentation in HMBA-treated cultures is also earlier than that seen with Me_2SO (Fig. 2). While both HMBA and Me_2SO committed >95% of the cells to differentiate, the magnitude of HMBA-induced DNA strand breakage observed was less than that seen with Me_2SO. Since the steady-state level of DNA breakage is related to the relative endonucleolytic and repair capacity of the cell, the apparent reduced level of HMBA-induced breaks may be related to increased DNA repair. This is especially intriguing, since it has been recently shown that differentiating Friend cells are more radiosensitive in the first 2 days of the induction process (30). The increased sensitivity of these cells to x-ray damage may be related to the level of DNA breakage and/or endonucleolytic repair capacity of the cell. In addition, the transient appearance of endonuclease activity in the cultured Friend cell may reflect a tightly regulated activity of the endonuclease in relation to a particular aspect of the cell cycle, because the time of appearance of endonuclease activity correlates with a period of G_1 arrest (23, 31). This is important, because cells have been shown to accumulate DNA strand breaks during G_1 arrest (32).

As our own study indicates, an activated endonuclease activity appears during an early phase of induced erythroid maturation. Of interest, we have noted an enrichment of Ca^{2+},Mg^{2+}-dependent endonuclease activity in a proerythroid cell subpopulation of low buoyant density cells derived from differentiating erythroleukemic spleen cells and subcutaneous precursor erythroid tumor cells (unpublished observations). However, this endonuclease activity led to double strand DNA breaks and release of chromatin fragments, whereas only single strand DNA breaks were noted in induced Friend cells. Whether this difference is a reflection of the cell type, a different enzyme, actual enzyme levels, or the stage of differentiation is unknown.

Recently, it has been shown that the commitment of Friend cells to differentiate may be associated with changes in Ca^{2+} ion influx (3) and Na^+,K^+-ATPase activity (5). As in these studies, we find endonuclease activity to be present at a defined time early in the differentiation process. It appears to precede the commitment of these cells to differentiate. Whether the magnitude of change in intracellular Ca^{2+} ions is sufficient to affect the endonuclease activity we have observed remains to be determined. While estimates of free cytoplasmic Ca^{2+} ions have been made in Friend cells (0.14 μM) (6) and other tissues (0.11–0.97 μM) (33), the level of free intranuclear Ca^{2+} is not known. Purified Ca^{2+},Mg^{2+}-dependent endonucleases from calf thymus (34) and bull semen (35) require 0.5–1 mM for optimal activity, but this finding is difficult to assess in terms of what concentration might be required for activity in the cell. Still, the finding that Ca^{2+} ion influx and endonuclease activity occur during a similar early period of erythroid differentiation suggests that the two events may be related. Moreover, the presence of an activatable endonuclease activity and transiently formed single strand DNA breaks early in the induction process may signify a role of the enzyme in steps leading to terminal differentiation.

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