Investigations of Mechanisms of Drug-induced Changes in Gene Expression: N-Methylformamide-induced Changes in Synthesis of the Mr 72,000 Constitutive Heat Shock Protein during Commitment of HL-60 Cells to Granulocyte Differentiation

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ABSTRACT

HL-60 cells were treated with the differentiating agent N-methylformamide and early changes in gene expression and protein content were investigated. Analysis of protein synthesis had previously shown an early (<12 h) fall in the synthesis of Mr 70,000 heat shock proteins (F. M. Richards, A. Watson, and J. A. Hickman. Cancer Res., 48: 6715–6720, 1988). The changes have now been characterized in detail and their kinetics compared to those of the expression of the c-myc protein. Immunofluorescence analysis, using antibodies to either the stress-inducible heat shock protein hsp70 (4G4) or a pan-Mr 70,000 heat shock protein antibody (3A3), showed that there was a striking reduction in the levels of the constitutive heat shock protein hsc70 when cells were incubated continuously with 170 mM N-methylformamide. A reduction in the level of hsc70 RNA was observed within 3 h and continued thereafter. In contrast, transcription of the hsc70 gene was induced within 1–2 h, after which the rate returned to basal level. There were no significant changes in the rate of transcription of the stress-inducible heat shock proteins hsp70 or hsp90. When N-methylformamide was removed from the cells, prior to commitment to differentiation, the levels of hsc70 were reestablished, whereas after 3 h of treatment there was no recovery. Western blotting with an antibody to the c-myc protein showed this to fall to virtually undetectable levels by 3 h under the same conditions. The results suggest that the loss of hsc70, which may perform a protein chaperoning role, was mediated at both transcriptional and posttranscriptional levels of regulation and was an early event closely associated with the commitment of HL-60 cells to differentiation. The fall in hsc70 was not associated with alterations in the cell cycle, nor were the kinetics of the change suggestive of a relationship with the decrease in content of c-myc protein.

INTRODUCTION

Structurally and mechanistically disparate pharmacological agents are able to induce the terminal maturation of HL-60 promyelocytic leukemia cells along the granulocyte-neutrophil pathway (1). An interesting question regarding this response to such disparate agents concerns the nature of the mechanisms whereby interactions of drugs, at presumably different loci, are able to initiate the molecular events that lead to the differentiated state (2). Of particular interest are the conserved changes in gene expression of the "immediate-early" response genes, such as c-myc (3, 4). In previous studies, observations were made that the induction of differentiation of HL-60 cells took place when concentrations of agents were used which were marginally below those which induced cytotoxicity (5). We went on to suggest that the induction of terminal differentiation may involve a stress response but paradoxically we observed that one of the earliest changes in protein observed in HL-60 cells treated with NMFM was a fall, rather than an increase, in the synthesis of the Mr 70,000 heat shock proteins (6). We were unable to identify which specific members of the Mr 70,000 heat shock proteins were being modulated.

Changes in the synthesis of heat shock proteins early during differentiation have been observed in Friend erythroleukemia cells treated with dimethyl sulfoxide (7), mouse embryonal cells treated with retinoic acid (8), HL-60 cells treated with phorbol esters (9, 10), THP-1 monocyte leukemia cells treated with γ-interferon or retinoic acid (10), K562 erythroleukemia cells treated with hemin (11), and in a variety of developmental processes (12, 13). The significance of these changes is not clear, and the qualitative, quantitative, and temporal aspects of them differ markedly according to cell type and conditions. Some of the changes appear to be associated with alterations in the proliferative status of the cells. The expression of the stress-inducible hsp70 has been shown to be cell cycle regulated (14).

The expression of the protooncogene c-myc is also associated with the proliferative capacity of a variety of cells (15) and a fall in expression has been considered to herald a withdrawal from the cell cycle (16). A fall in the expression of c-myc has also been observed to be a prelude to the terminal differentiation of HL-60 cells (3, 4). The kinetics of the change in c-myc expression was found to depend upon the nature of the inducer used and upon the lineage to which the cells become committed (17, 18). Because of a colocalization between c-myc protein and the stress-inducible hsp70 (19), we were interested in determining the precise characterization of the heat shock protein which changed as HL-60 cells were committed to differentiate, and whether there might be a relationship between the kinetics of this change and that of the expression of c-myc.

Detailed descriptions of the regulation of the expression of some of the genes which encode the heat shock proteins have emerged recently, most particularly those of the Mr 70,000 family (20). Their role in protecting the cell from the effects of elevated temperatures (21) and their constitutive role as protein chaperones (22, 23) is the subject of intense investigation. Because of the emerging knowledge of their molecular and cellular biology we consider study of changes in expression of the Mr 70,000 heat shock proteins may provide clues as to how a drug might bring about changes in gene transcription as well as perhaps clarifying what role these proteins may play in the process of differentiation. The solvent NMF was chosen as an archetypical differentiating agent (24) since it had been the subject of previous studies by us (5, 6, 25, 26).

MATERIALS AND METHODS

Materials. All materials were obtained from Sigma Chemical Co. (Poole, Dorset, United Kingdom) unless otherwise stated.

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1 Supported by grants from the Cancer Research Campaign (SP1518), NATO (890957), and the NIH (ES3).
2 To whom requests for reprints should be addressed.
3 The abbreviations used are: NMF, N-methylformamide; BSA, bovine serum albumin.
Culture and Treatment of Cells. HL-60 human promyelocytic leukemia cells were maintained in RPMI-1640 medium (Imperial Laboratories, Andover, Hampshire, United Kingdom) containing 10% fetal calf serum (TechGen, London, United Kingdom) supplemented with 2 μg/mL -glutamine, at 37°C, 5% CO2, 100% humidity. Cells were routinely maintained in logarithmic phase growth between 1 x 10^4 and 1 x 10^6 cells/mL by biweekly subculture and had an approximate doubling time of 24 h. Cells were counted by using a hemacytometer and short term viability was determined by the exclusion of a 0.1% solution of trypan blue. HeLa cells were grown under identical culture conditions and subcultured by trypsin-EDTA removal from tissue culture flasks (Costar, High Wycombe, Buckinghamshire, United Kingdom). HeLa cells were heat shocked for 1 h at 42°C, left for 2 h, and harvested and frozen before protein analysis as described below. NMf (Aldrich Chemical Co., Gillingham, United Kingdom) was added directly to the cell cultures to give a concentration of 170 mM, and cells were removed at predetermined time points for the assessment of differentiation and protein or RNA analyses.

Assessment of Differentiation. The percentage of differentiated HL-60 cells was assessed based on the ability to produce superoxide anion in response to TPA stimulation and reduce the dye nitroblue tetrazolium, as described in detail by us previously (5).

Cell Cycle Kinetics. Following drug treatment for up to 48 h, cells were resuspended in fixative (0.1% paraformaldehyde, 0.1% Triton) at a density of 1 x 10^6/mL for a minimum of 4 h at room temperature. Twenty μl propidium iodide solution (2.5 mg/mL) were added sequentially to each 500-μl sample, incubated for 5 min at room temperature, before cell cycle analysis using a Coulter EPICS Counter. The flow cytometer was set to excite at 488 nm with red fluorescence being collected through a 630 nm long pass filter. Cell cycle analysis was performed by using in-house software.

Measurement of Gene Transcription Rates. Isolation of nuclei and transcription run-on analysis were carried out according to previously published methods (11, 27). Briefly, isolated nuclei were labeled with [32P]UTP (Amer sham International, Aylesbury, Buckinghamshire, United Kingdom) and the transcription reaction was arrested by the addition of 600 μL of a stop buffer and a further 2-h incubation at 45°C. After chloroacetic acid precipitation of nucleic acids, the labeled nascent transcripts were hybridized to plasmid DNA immobilized onto nitrocellulose Hybond-C (Amer sham International, Aylesbury, Buckinghamshire, United Kingdom) and the mRNA levels were quantified using a phosphorimager (Storm, Molecular Dynamics, Sunnyvale, CA). Analysis of transcription was carried out along with the Northern blotting of the same RNA (32).

Isolation of RNA and Northern Blot Analysis. Total cellular RNA was isolated by using a modified version of the guanidinium isothiocyanate method as described previously (32). Briefly, 5 x 10^5-1 x 10^6 cells were lysed in 4 μL guanidinium isothiocyanate solution, layered onto 5.7 μL CsCl0.1 μL EDTA, and centrifuged overnight (35,000 x g, 4°C, 17 h). Following phenolic extraction and ethanol precipitation, 20 μg purified RNA were fractionated on a denaturing 1.2% agarose-formamide gel. Ethidium bromide (40 μg/mL) was included with the RNA to allow visualization of the loading of each gel. After overnight capillary transfer to Hybond-C, RNA was immobilized by UV irradiation and prehybridized in formamide containing buffer (50% formamide, 6 x SSC, 5 x Denhardt's, 0.1% SDS, 50 μg/mL tRNA), at 42°C for approximately 6 h. Filters were hybridized overnight to the 32P-labeled probe (Boehringer Mannheim, Lewes, East Sussex, United Kingdom) base-pair EcoRI fragment encoding the human 5′ sequence of hsc70 (ph 7.6). The filters were then washed in a series of buffers, as described above for analysis of transcription.

Immunoblot Analysis. Cells (2 x 10^5) were washed in PBS, denatured by boiling in reducing sample buffer (2% SDS, 10% glycerol, 0.002% bromophenol blue, 40 mM Tris, pH 6.8), and total cellular protein was separated by SDS-polyacrylamide gel electrophoresis by using 10% polyacrylamide gels. Following the electrophoretic transfer of proteins to nitrocellulose Hybond-C Extra, the membranes were blocked overnight in 5 mg/mL BSA in PBS, and washed in 0.1% Tween in PBS. The gels were stained for residual protein with Coomassie blue to ensure equal loading and transfer. Membranes were then incubated with either anti-hsp70 specific (4G4), anti-hsp70/hsc70 (3A3), mouse monoclonal antibodies (gifts from Dr. Shawn Murphy, Northwestern University, Evanston, IL), or rabbit antiserum pan c-myc antibody (a gift from Dr. Gerard Evan, Imperial Cancer Research Fund, London) for 1-2 h (4G4 and 3A3 antibodies were diluted 1:100 and c-myc 1:1000 in 5 mg/mL BSA in PBS before use) and washed with 0.1% Tween in PBS. This was followed by an additional 1-h incubation with affinity purified goat anti-mouse IgG or goat anti-rabbit IgG conjugated to horse radish peroxidase (diluted 1:60000 in 5 mg/mL BSA in PBS before use) and repeated washings with 0.3% and 0.1% Tween 20 in PBS. The antibody-specific proteins were visualised by using the enhanced chemiluminescence detection system according to the recommended procedure (Amer sham International). Filters that were to be probed with a different antibody were first incubated in stripping buffer (1x Tris, pH 6.8, 2% SDS, and 5% β-mercaptoethanol) for 20 min at 50°C. The immunoblotting procedure was then repeated as above.

RESULTS

Characterization of NMF-induced Changes in Levels of the Mf, 70,000 Heat Shock Proteins. Immunoblot analysis showed that continuous treatment of HL-60 cells with 170 mM NMF induced a time-dependent decrease in the cellular amount of a heat shock protein with a molecular weight of approximately 70,000 (Fig. 1). Analysis by SDS-polyacrylamide gel electrophoresis of changes in protein synthesis, after labeling with [35S]methionine confirmed our previous findings (6) of a fall in the synthesis of a Mf, 70,000 protein after approximately 6 h (data not shown). In order to identify which member of the hsp70 family changed, we compared the Western blot results by using two monoclonal antibodies, 3A3, which recognizes both the constitutive hsc70 and the inducible hsp70, and 4G4, which recognizes only hsp70. Protein samples from HL-60 cells exposed to NMF, and from heat-shocked HeLa cells, were analyzed by using the inducible hsp70-specific antibody, 4G4. The HeLa cells were used to provide a strongly inducible heat shock response, and therefore an increase in the level of the stress inducible hsp70 protein (Fig. 2A); Western blotting of HL-60 cells did not show a very significant change in the amount of this protein after heat shock, despite our previous finding of some change in synthesis (Fig. 1). The blot was then reprobed by using the antibody 3A3, which detects both the inducible and constitutive forms of hsp70. Both HL-60 and HeLa heat shock 70 proteins were immunologically reactive with 3A3 (Fig. 2B). Because of the slightly inducible nature of the constitutive heat shock protein, hsc70, and the severalfold induction of the stress inducible hsp70 protein following a heat shock, these two proteins from HeLa cells are not fully resolved. The detection of an additional upper band following incubation with 3A3 is, however, evident and is labeled accordingly (Fig. 2, Lane 6). This result suggested that there was a high basal expression of the constitutively expressed hsc70 protein which was reduced following NMF treatment. Although 1-dimensional analysis failed to detect the heat/stress-inducible protein hsp70 in HL-60 cells, small amounts of this protein were detected by using 2-dimensional
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Fig. 2. Detection of hsc70 protein expression in HL-60 cells using a combination of two mouse monoclonal antibodies specific for more than one member of the heat shock protein 70 family. Western blot analysis of total cellular protein from 2 × 10^5 cells/lane was first carried out by using (A) the monoclonal antibody, 4G4, specific for the stress-inducible form of the Mr 70,000 heat shock protein, hsp70. A protein sample from heat-shocked (42°C, 1 h) HeLa cells was included as a positive control. The filter was stripped and reprobed (B) with the use of the monoclonal antibody 3A3 to detect both the constitutive (hsc) and inducible (hsp) forms of hsp70.

Fig. 3. In vitro transcription in nuclei of HL-60 cells isolated during NMF treatment, and after a heat shock of 42°C for 1 h. Following isolation of nuclei and in vitro transcription reactions, [35S]UTP-labeled transcripts were hybridized to filter bound DNA: pH 3.2 (human hsp70); pUC 801 (human hsp90a); pUC 811 (human hsp90b); pHG2 (human grp78); pH7A.6 (human hsc70); pHPA-1 (human α-actin).

Western analysis, and the levels remained essentially unchanged throughout NMF treatment (data not shown). The onset of the selective decrease in the constitutive protein hsc70 was evident after 12 h of NMF exposure. The level of hsc70 fell to almost undetectable levels by 36 h, concomitant with the irreversible commitment of the cells to terminal differentiation (see below).

Changes in Heat Shock Gene Expression. Exposure to 170 μM NMF consistently induced the transient and selective increase in the transcription rate of the hsc70 gene within 1–2 h (Fig. 3). Significantly, the transcription of its stress-inducible counterpart, hsp70, and another heat shock gene hsp90, were unaffected. Following the transient induction of hsc70 transcription, there was a return to control levels by 4–8 h. Fig. 3 shows that heat and NMF treatment of HL-60 cells induced transcriptional changes of the α-actin gene, so that the basal and steady state levels of transcription of the other heat shock genes were taken to represent steady state levels of transcription. The expression of the hsc70 gene was also examined by Northern blot analysis. It was observed that hsc70 message levels were significantly reduced by 3 h (Fig. 4), despite the maintenance of basal levels of transcription (Fig. 3). Ethidium bromide staining of the gel prior to transfer (shown in Fig. 4, bottom) ensured equal loading of RNA. Significant levels of protein were detectable (Fig. 1) up until after 12 h, suggesting either that the protein had a significantly longer half-life than its message or that its half-life was extended in the presence of NMF.

Relationship between Changes in hsc70, c-myc Levels, Growth Arrest, and Commitment to Differentiation. The conditions for optimal HL-60 differentiation have been described previously (5); a 96-h continuous incubation with 170 μM NMF induced maximum differentiation to granulocyte-like cells. These parameters were re-investigated for the purpose of current experiments, and furthered to determine the time after which the cells became irreversibly committed to a terminally differentiated phenotype, the so-called commitment time. It was found that a minimum exposure time of between 36 and 48 h to NMF was required to commit cells, as assessed by nitroblue tetrazolium reduction (Fig. 5A). Flow cytometric analysis, during the first 48 h of NMF exposure, showed that there was no significant change in the distribution of cells in the cell cycle, at which time the cells were committed. Analysis of cell numbers confirmed this (Table 1). Interestingly, cell cycle progression of HL-60 cells occurred independently of much reduced levels of c-myc protein. We observed a rapid decline in the levels of c-myc protein well before proliferation ceased (Fig. 5B). Although appreciable levels of c-myc were undetectable by approximately 3 h, cells were still capable of initiating another full round of cell division (Table 1).

Immunoblot analysis of proteins isolated from cells which had been incubated for increasing times with 170 μM NMF, then washed before a 24-h drug-free recovery period, showed that the effects of NMF to suppress hsc levels were reversible up to, but not beyond the point of

Fig. 4. Northern blot analysis of hsc70 gene expression during NMF treatment (0-6 h). Total cellular RNA was isolated at the indicated times using the guanidinium isothiocyanate/CsCl method. Twenty μg RNA were separated on a denaturing formaldehyde gel, transferred to nitrocellulose, and hybridized to a 32P-labeled 600-base pair DNA fragment of hsc70 (pHA7.6).
commitment to granulocytic differentiation (Fig. 6). The reduction in treatment. Total cellular protein from 2 X 10s cells/lane was analyzed throughout the presence or absence of NMF for 96 h. Viable cell numbers were also assessed at each time to NMF (0—18 h) followed by drug-free incubation up to % h allowed determination of changes in cellular levels were defined. This is important since each member of the Mr 70,000 hsps, suggested, by elimination, that the heat shock protein which was reduced in amount was the constitutive hsc70. Analysis of the proteins by two-dimensional electrophoresis showed that there was no change in the hsc70 protein content after a 36-h exposure to NMF was not restored to control level after a 24-h recovery period.

DISCUSSION

HL-60 cells treated with NMF underwent an early fall in cellular levels of one of the members of the M, 70,000 heat shock family. This corresponded to our previous observations regarding the fall in synthesis of heat shock proteins (6). Immunoblot analysis using two antibodies, which discriminated in the pattern of recognition of the members of the M, 70,000 hsps, suggested, by elimination, that the heat shock protein which was reduced in amount was the constitutive hsc70. Analysis of the proteins by two-dimensional electrophoresis followed by immunoblotting, showed that there was no change in the stress/heat-inducible hsp70 (data not shown). Although a number of previous studies have identified changes in the synthesis of M, 70,000 family of heat shock proteins during differentiation, neither the precise identity of the protein whose expression was being modulated nor changes in cellular levels were defined. This is important since each member of this family of proteins may serve a different role in the cell (13). The heat shock protein M, 70,000 family is the most highly conserved of the heat shock proteins, containing between 8 and 10 members, some of which are expressed constitutively, while others are induced in response to a variety of stresses. Although the precise function of hsc70 remains unclear, reports have identified a range of hsc70-specific characteristics, including clathrin-uncoating ATPase activity (33, 34). Suggested functional roles for hsc70 also include involvement in the lysosomal degradation of intracellular proteins (35), the chaperoning of proteins from the cytoplasm to the nucleus (36), and as a binding protein for peroxisome proliferators (37). In comparison to the stress-inducible heat shock protein 70, regulation of the expression of hsc70 has not been investigated.

The change in the cellular level of hsc70 was accompanied by selective changes in the transcriptional rate of the hsc70 gene, in comparison to the stress-inducible hsp70 (Fig. 4). Nuclear run-on analysis, interestingly, showed a transient but significant change in transcriptional rate after 1–2 h of NMF incubation. Thereafter, the transcriptional rate appeared to fall back to control levels. The significance of this transient increase in transcription is unclear. Two recent reports observed transient increases in the expression of hsp27 (9) in HL-60 cells treated with the phorbol ester TPA, and in c-myc during interleukin 6-induced differentiation of M1 myeloid leukemia cells (38, 39). The transient increase in c-myc RNA preceded a subsequent reduction to undetectable levels and, although this was noted by the authors, no mechanistic explanation of the changes was offered. A transient increase in hsc70 protein synthesis or amount (by immunoblotting), corresponding to the rise in transcription rate, was not observed (data not shown). The disparity observed between transcription rate and the levels of RNA and protein suggest that the transcripts detected by nuclear run-on analysis may be incomplete and therefore not able to be fully processed to produce mature mRNA to be used for translation. This does not eliminate the possibility of the involvement of changes in the rate of transcription in some regulatory mechanism. The transcription rate of hsc70 was low (Fig. 3), but the constitutive levels of mRNA appeared to be considerable (Fig. 4), which is suggestive of the production of a stable message. The reduction in the amount of hsc70 mRNA to a level below that of control, under conditions of continued basal transcription, therefore suggests that NMF may affect message stability. Previous reports have shown that heat-inducible hsp70 expression is regulated at both transcriptional and posttranscriptional levels (40). Although down-regulation of hsc70 protein levels were also observed, these occurred at a time after the changes in RNA levels (compare Figs. 1 and 4). This suggests that either the stability of the protein is greater, or that RNA stability was changed after NMF treatment. This is under investigation.

The temporal change in hsc70 level induced by NMF was strongly suggestive of it playing a role in the maintenance of the block in differentiation which characterizes leukemic HL-60 cells. A comparison of the kinetics of the reduction in hsc70 protein levels with those of commitment (Fig. 5A) showed that as levels fell below those detectable by immunoblotting, cells were becoming committed to


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differentiate. Moreover, the fall in hsc70 levels was reversible only until 36 h of NMF treatment, a time which corresponded to the irreversible commitment to terminal differentiation (Fig. 6).

Early changes in transcription and in mRNA were followed by a fall in hsc70 protein levels by about 12 h. We were concerned that these falls in hsc70 levels might simply reflect accumulating numbers of cells moving out the cell cycle and to growth arrest, since the stress-inducible hsp70 had been shown to be cell cycle regulated (41). Analysis of the cell cycle and measurement of cell numbers did not support this hypothesis (Table 1). Additional experiments, not reported here, have shown that hsc70 protein levels remained unchanged when HL-60 cells were treated with 300 ms NMF, which induced a rapid cytoysis. Recently, it was reported that TPA induced an increase in the stress-inducible hsp70 mRNA in HL-60 cells committed to monocyte differentiation (9). These cells were rapidly growth arrested and it is possible that these changes may reflect changes in cell cycle rather than differentiation.

The loss of proliferative capacity of NMF-treated HL-60 cells coincided with their commitment to differentiate, which has been related to changes in the expression of the protooncogene c-myc (3, 4). We were interested in the temporal relationship, if any, between changes in the nuclear c-myc protein and hsc70, because hsc70 has been implicated in the translocation of proteins from the cytoplasm to the nucleus (36). Additionally, there has been an association reported between a heat shock protein and c-myc (19, 42). This raised the possibility that the fall in hsc70 might be associated with changes in c-myc expression in differentiating HL-60 cells. Immunoblotting showed that the fall in cellular levels of c-myc after NMF treatment occurred within 2 h (Fig. 5B), whereas changes in hsc70 levels did not occur until after 12 h (Fig. 1). This disparity of kinetic change does not support the idea that hsc70 may be involved with changes in c-myc levels.

Interestingly, the rapid fall in c-myc protein levels occurred well before any changes were observed in growth or differentiation of the HL-60 cells. Furthermore, the cells initiated another full round of division in the absence of detectable levels of c-myc protein. Similar observations have been reported by other workers who have suggested that the kinetics of the decline in c-myc varies both with the nature of the inducing agent and the lineage to which the cells become committed (16, 17, 43).

The loss of a constitutively expressed protein, which has a role as a molecular chaperone, from cells committed to differentiate by NMF raises a number of important questions. How NMF brings about this change and how general it is for agents which induce granulocyte-neutrophil differentiation remains to be answered. Our immediate aim is to discover which protein(s) is normally associated with hsc70 under basal conditions and whether the loss of their protein chaperone may play a critical role in the block in differentiation, indicative of the malignant phenotype. Additionally, we are interested in how a simple molecule like NMF can initiate subtle and selective changes in gene expression and posttranscriptional control.

REFERENCES


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