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Modulation of a Human Dihydrofolate Reductase Minigene Following Release from Amino Acid Deprivation Involves Both 5′ and 3′ Nucleotide Sequences

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SUMMARY

A dihydrofolate reductase (DHFR) expression system composed of a DHFR minigene constructed from human DHFR genomic and cDNA sequences stably transfected into DHFR$^-$ Chinese hamster ovary cells was used to study the modulation of DHFR levels in response to release from amino acid deprivation. The addition of complete medium to cells grown for 48 hr in medium lacking isoleucine and glutamine caused the transfected cells to undergo a synchronous cycle of DNA replication. When DHFR protein levels assayed at the time of maximum DNA synthesis were compared to that present in the deprived state, levels rose 3.2- to 4.9-fold. By contrast, DHFR levels in cells transfected with a DHFR expression construct made from mouse DHFR cDNA fused to viral promoter, intervening, and polyadenylation sequences were not inducible under the identical conditions. Human DHFR minigene deletion or substitution constructs were used to determine which nucleotide sequences were responsible for amino acid-modulated expression. Although deletion of sequences upstream from 322 base pair 5′ to the start of transcription did not affect DHFR expression, removal of sequences between 322 and 113 base pairs reduced DHFR induction by approximately 50%. Deletion of nucleotide sequences within the 3′ nontranslated region of the gene also reduced the level of induction by approximately 50%. Reduction in the levels of DHFR RNA relative to total cellular RNA was also found. Thus, both 5′ and 3′ nucleotide sequences are involved in the modulation of DHFR levels following release from amino acid deprivation.

Dihydrofolate reductase (DHFR) is an essential enzyme necessary for the regeneration of reduced folate cofactors which are required for the production of thymidylate, purines, and glycine (1). Although expression of the DHFR gene is required in all cells, previous studies have shown that DHFR levels can be modulated by estrogen, methotrexate, cAMP, viral infection, contact inhibition, serum deprivation, and amino acid deprivation (2–11). DHFR levels are also regulated during the cell cycle with maximal mRNA and enzyme synthesis occurring in S phase (12–15). Since some modulators of DHFR levels alter the cellular growth rate, these modulators may have their effect solely by manipulating the cell cycle. Previous cellular studies on the regulation of DHFR expression have indicated that its regulation is apparently complex, with different modulators acting through different mechanisms.

Release from serum deprivation appears to affect DHFR gene expression primarily at the level of transcription (9). In contrast, studies by Collins *et al.* (2) on modulation of DHFR following the addition of amino acids to amino acid-deprived cells demonstrated no difference in the relative content and stability of DHFR mRNA or the relative transcription rate of the DHFR gene in amino acid-starved and exponentially growing cells. However, the rate of conversion of heterogenous nuclear RNA into cytoplasmic DHFR mRNA was slower in starved than in growing cells, resulting in a 3-fold excess of intranuclear DHFR mRNA in starved cells. Thus, there appear to be differences between the regulation of DHFR expression produced by amino acid compared to serum deprivation.

To better understand the DNA sequences responsible for, and the mechanisms involved in, the regulation of DHFR expression, a human DHFR minigene was constructed (16). When the minigene was stably transfected into DHFR gene-deleted Chinese hamster ovary (CHO) cells (17), it restored DHFR gene function. Moreover, release from serum deprivation, which causes transcriptional modulation of the endogenous DHFR gene in normal cells (9), also induced DHFR levels in minigene-transfected cells. Because cellular experiments suggest that there may be significant differences between the mechanisms of serum and amino acid modulation of DHFR levels, growth modulation by the addition of amino acids to cells starved for isoleucine and glutamine was studied using our DHFR minigene expression system. In this paper, we demonstrate that the human DHFR minigene-transfected cells can respond to amino acid stimulation. Analysis of a series of

ABBREVIATIONS: DHFR, dihydrofolate reductase; CHO, Chinese hamster ovary; IMEM, improved minimal essential medium; FCS, fetal calf serum; bp, base pair; kb, kilobase.

DHFR minigenes showed that DHFR sequences, both 5′ to the start of transcription and in the 3′ nontranslated portion of the gene, were involved in the responsiveness to amino acid deprivation.

Materials and Methods

Amino acid stimulation. The DHFR$^-$ CHO DG21 cell line (17), which can grow only in medium supplemented with glycine, hypoxanthine, and thymidine, was transfected with various human DHFR minigene constructs shown in Fig. 1 by calcium phosphate-mediated DNA transfer and clonal lines and/or pooled cultures of transfected cells isolated by selection in unsupplemented medium (16). For most transfected cell lines, the number of minigene copies and the level of DHFR protein were increased by passing the cells in stepwise increasing concentrations of methotrexate up to 10 μM, which caused a 10- to 20-fold increase in DHFR protein levels. Minigene-transfected cells were plated at 1–1.5 × 10^6/150-cm^2 flask in improved minimal essential medium (IMEM) supplemented with 2 mg/ml proline and 5% fetal calf serum (FCS). When the cells were 60% confluent, the medium was changed to IMEM without isoleucine and glutamine plus 5% dialyzed FCS. After 48 hr, two thirds of the cells were harvested and one third of the cells were stimulated by transferring them to IMEM with 5% FCS. After 16 hr, the stimulated cells were harvested. DHFR enzyme levels were determined using a [^{3}H]methotrexate (Moravek Biochemicals) binding assay (18). Total cellular protein levels were determined by a Bradford protein assay (19).

Results

Synchronization of transfected cells. Previous cellular studies investigating DHFR mRNA metabolism showed that cells starved for isoleucine and glutamine underwent a synchronous round of cell division following the addition of complete medium (2). Previously, we demonstrated that a synchronous round of cell division also followed the release of DHFR minigene-transfected cells from serum deprivation which was accompanied by a 3-fold rise in DHFR protein levels between the serum-deprived state and the time of maximum DNA synthesis (16). Therefore, the minigene-transfected cells were examined to determine if a cycle of DNA replication also followed release from amino acid deprivation.

Cell lines were selected which had been transfected with the human DHFR minigene shown in Fig. 1. These cells at 60% confluency were placed in medium lacking isoleucine and glutamine for 48 hr, then cell growth was stimulated by replacement with complete medium. The ability of these cells to be synchronized by release from amino acid-deprived conditions was analyzed by monitoring [^{3}H]thymidine incorporation into acid-precipitable DNA. Fig. 2 indicates that the minigene-transfected cells underwent a cycle of synchronous DNA replication with the maximum thymidine incorporation occurring 16 hr following the medium change.

Modulation of DHFR levels in minigene-transfected cells. Since previous studies on serum modulation of DHFR showed that DHFR levels and the DNA synthesis rate increased in parallel (10), DHFR protein levels were determined for several clonal lines of minigene-transfected cells in the amino acid-deprived state and at 16 hr after the replacement of amino acids, at which time the rate of DNA synthesis was maximal. Table 1 shows that several independently isolated clonal lines of minigene-transfected cells responded to the replacement of amino acids with a 3.2- to 4.9-fold increase in cellular DHFR enzyme levels between the two growth states. Several lines of minigene-transfected cells were analyzed in order to determine the clonal variability which may result from the integration of minigenes into different chromosomal locations.

The modulation of DHFR levels in cells transfected with another DHFR expression vector, pMTVdhfr, were also examined. This vector, which is shown in Fig. 1, was constructed by Lee *et al.* (20) from a mouse DHFR cDNA and a mouse

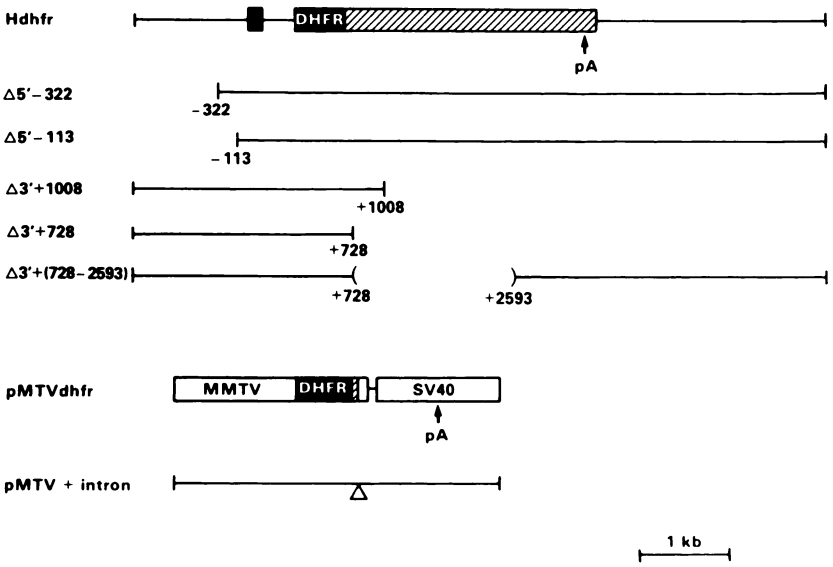

Fig. 1. Structure of DHFR expression constructs. The human DHFR minigene (Hdhfr) was constructed from genomic and cDNA DHFR DNA sequences (16). The minigene consists of 1.25 kb of DNA 5′ to the start of transcription, the entire protein coding region plus intron 1, the 3′ nontranslated region of the gene, and 2.7 kb of 3′ flanking sequence. ■, translated regions; ▨, the 3′ nontranslated region; ——, flanking and intervening sequences. The *cross-hatched nontranslated region* of the minigene contains the single polyadenylation site (*pA*) used in transfected CHO cells. The human DHFR minigene was used as the basis for other minigene constructs. HdhfrΔ5′ – 322 is a minigene which beings at a restriction site 322 bp 5′ to the start of transcription of the major human DHFR mRNA (21, 32). HdhfrΔ 3′ + 728 is a minigene which terminates at a site in the 3′ nontranslated region of the gene, 728 bp from the start of transcription of the mRNA (21, 32, 33). HdhfrΔ3′ + (728–2593) is a minigene with an internal 1.9-kb Bgl II-Bam HI fragment deleted from the 3′ nontranslated region. pMTVdhfr is a construct made by Lee *et al.* (20) from a mouse DHFR cDNA, an MMTV LTR promoter, and SV40 intervening and polyadenylation sequences. The homologous parts of Hdhfr and pMTVdhfr are the coding sequences and the beginning of the 3′ nontranslated region. The pMTV + intron construct was made by inserting a Bal I-Eco RI fragment containing the intron from the human DHFR minigene (intron 1) with Bam HI linkers into the Bgl II site at the junction between the mouse DHFR cDNA and the SV40 sequences in the 3′ nontranslated region of pMTVdhfr.

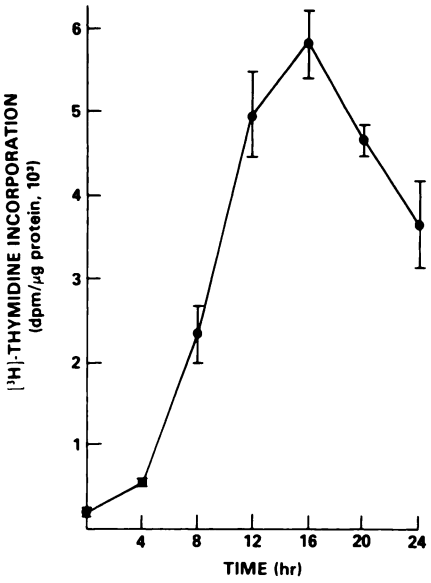

Fig. 2. [^{3}H]Thymidine incorporation in amino acid-stimulated minigene-transfected cells. Minigene-transfected cells (Hdhfr-6A) were plated in six-well Linbro plates at 40,000 cells/well and grown in amino acid-depleted medium as described under Materials and Methods. At $t = 0$ the medium was changed to complete medium and at various times cells were labeled for 1 hr with 5 μCi/well [^{3}H]thymidine (62 Ci/mmol [*methyl*-^{3}H]thymidine, ICN Pharmaceuticals). At the end of the incubation the cells were harvested and the acid-precipitable [^{3}H]thymidine incorporation and protein concentration were determined in duplicate for each sample. Each point represents the mean (±standard deviation) of triplicate samples.

TABLE 1

Amino acid induction of DHFR enzyme levels

Transfecting DNA with clone number	DHFR level[a]		Relative increase	
	Depleted	Induced	Increase	Mean ± sd
	pmol/mg protein			
Hdhfr-3A	1.06	3.24	3.1	
	0.79	2.67	3.4	3.2 ± 0.2
	0.87	2.80	3.1	
Hdhfr-5A	2.22	11.72	5.7	
	2.06	9.79	4.8	4.9 ± 0.8
	2.91	11.96	4.1	
Hdhfr-6A	4.66	21.10	4.5	
	4.24	17.24	4.1	4.2 ± 0.2
	3.85	15.91	4.1	
Hdhfr-7A	4.14	14.38	3.5	
	4.68	14.96	3.2	3.5 ± 0.3
	3.58	13.67	3.8	
pMTV-18A	6.33	6.05	1.0	
	7.35	6.84	1.0	0.9 ± 0.1
	6.01	4.84	0.8	
pMTV-12A	2.47	1.45	0.6	
	2.05	1.27	0.6	0.7 ± 0.1
	1.45	1.14	0.8	

[a] DHFR levels were determined in cells grown in amino acid-depleted medium and in amino acid-depleted cells 16 hr after the addition of medium containing isoleucine and glutamine. DHFR levels and total cellular protein concentrations were calculated in duplicate.

mammary tumor virus LTR promoter and with an SV40 intervening and polyadenylation sequences following the cDNA. We previously showed that pMTVdhfr could rescue the DHFR$^-$ CHO cells and that pMTVdhfr-transfected cells synthesized functional DHFR protein (16). A thymidine incorporation curve essentially identical to that in Fig. 2 was produced by DHFR$^-$ CHO cells transfected with pMTVdhfr following release from amino acid deprivation. However, as shown in Table 1, in contrast to the increase in DHFR levels in minigene-transfected cells following release from amino acid deprivation, no increase in DHFR levels was found in cells transfected with pMTVdhfr when treated under the identical conditions (0.7- to 0.9-fold). While it is possible that the MMTV promoter has a negative effect on the induction of DHFR levels, these results suggest to us that DHFR sequences in the human minigene, but not in pMTVdhfr, were responsible for amino acid modulation of DHFR expression.

Modulation of DHFR levels in cells transfected with minigene deletions. In order to examine which sequences were involved in the regulation of the human DHFR gene, minigenes which deleted or substituted sequences in the promoter, intron 1, most of the 3′ nontranslated, or the 3′ flanking regions were constructed. DHFR$^-$ CHO cells were transfected with these minigenes which are shown in Fig. 1, DHFR$^+$ cells were selected, and the change in DHFR protein levels was analyzed following release from amino acid deprivation.

Fig. 3 shows the amino acid-stimulated increase of DHFR protein levels in cells transfected with two minigenes with different amounts of 5′ nucleotide sequences deleted. Clonal cell lines transfected with HdhfrΔ5′ − 322 showed amino acid-stimulated induction of DHFR levels (3.7- to 5.6-fold) similar to that obtained with the entire minigene (3.2- to 4.9-fold). Thus, DHFR sequences upstream from −322 were not required for amino acid modualted DHFR expression. In contrast, analysis of clonal cell lines transfected with HdhfrΔ5′ − 113 show reduced induction of DHFR levels following amino acid stimulation (1.1- to 3.1-fold). Pooled cells transfected with this construct produced an average increase of only 2.2 ± 0.4-fold. This 50% reduction in DHFR inducibility suggested that nucleotide sequences between −322 and −113 were involved in the modulation of DHFR expression following release from amino acid deprivation.

To examine whether sequences in intron 1 could be respon-

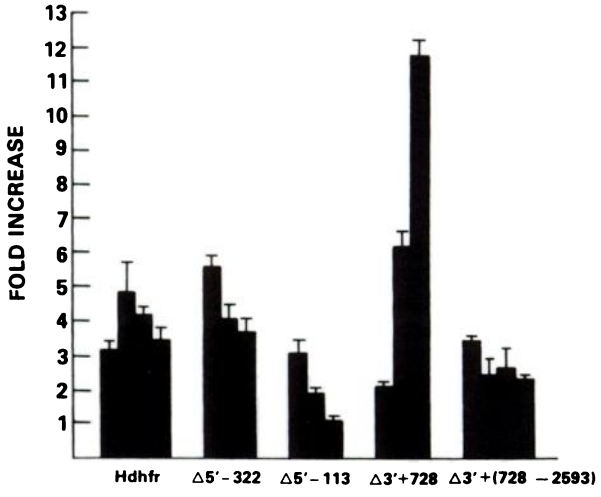

Fig. 3. Increase in DHFR protein levels in the amino acid-stimualted minigene-transfected cells. The -fold increase in DHFR protein levels as determined in Table 1 has been plotted as a bar graph. Each bar represents the results obtained from a clonal line of transfected cells and is the mean induction (±standard deviation) of, in general, triplicate samples. The DHFR minigenes are described in Fig. 1.

sible, a minigene was constructed in which the intervening sequence from Hdhfr was inserted into the 3′ nontranslated region of pMTVdhfr. DHFR protein levels in pooled cells transfected with this minigene (pMTV + intron), like those transfected with the pMTVdhfr parent construct, were not inducible following amino acid stimuation (1.1 ± 0.1-fold). This result suggested that sequences within the first intervening sequence by themselves were not responsible for amino acid-stimulated DHFR expression.

Induction of DHFR levels was examined in cells transfected with a minigene construct in which 3′ sequences were removed. Fig. 3 shows the results obtained with HdhfrΔ3′ + 728, a construct that ended 90 bp downstream from the termination of translation. This deletion removed most of the 3′ nontranslated and all of the 3′ flanking region of the minigene. Clonal cell lines transfected with HdhfrΔ3′ + 728 showed much more variability in DHFR induction following release from amino acid deprivation than was found with other constructs. Three clones of transfected cells were analyzed and showed inductions of 2.1(±0.1)-, 6.2(±0.4)-, and 11.7(±0.5)-fold.

This hypervariability may relate to the DHFR mRNA produced in these cells. The 3′ nontranslated region of the human DHFR gene contains multiple polyadenylation sites producing mRNAs of 0.72, 0.97, and 3.6 kb (21). Northern transfer analysis of DHFR mRNA indicated that there were multiple DHFR mRNA species in the DHFR gene-amplified human MCF-7 cell line (Fig. 4, lane *2*). In contrast, CHO cells transfected with the complete minigene produce a single 3.8-kb species (Fig. 4, lane *1*). This 3.8-kb mRNA is the predominent human DHFR mRNA found in most human cells (22–24). Cells transfected with HdhfrΔ3′ + 728 (Fig. 4, lane *4*) and HdhfrΔ3′ + 1008 (Fig. 4, lane *3*) which lack the polyadenylation site used in the transfected CHO cells, did not form mRNA that corresponded in size to any human DHFR mRNA. The sizes of the DHFR mRNA species produced in HdhfrΔ3′ + 728 and HdhfrΔ3′ + 1008 transfected cells are longer than the minigenes, suggesting that in these cells the DHFR mRNAs may be terminated in CHO DNA sequences outside these truncated minigenes. Thus, the DHFR mRNA in these cell lines most likely contains both hamster and human DNA. These CHO sequences, which would vary from cell line to cell line, might influence the response to amino acid induction by altering the RNA processing, transport, stability, or translational efficiency.

Another DHFR minigene was constructed which deleted part of the 3′ nontranslated region of the minigene but retained the polyadenylation site used in the transfected CHO cells. Clonal cell lines transfected with this minigene, HdhfrΔ3′ + (728–2593), showed a lower level of induction (2.3- to 3.3-fold) than the complete minigene (3.2- to 4.9-fold) and did not show the hypervariability found with HdhfrΔ3′ + 728. Pooled cells transfected with this minigene had only a 1.9 ± 0.1-fold increase in DHFR levels. The result, that cells transfected with HdhfrΔ3′ + (728–2593) had only 50% of the amino acid inducibility of the complete minigene, provided additional evidence which suggested that DHFR sequences in the 3′ nontranslated region of the gene influenced the modulation of DHFR expression following the release from amino acid deprivation.

Modulation of DHFR RNA levels. Fig. 5 shows DHFR RNA levels in total cellular RNA following release from amino acid deprivation. The relative DHFR RNA levels of two clonal cell lines transfected with Hdhfr were analyzed (Fig. 5, A and B) and showed similar patterns of RNA accumulation. Fig. 5 also shows DHFR RNA levels in two cell lines transfected with minigene deletion constructs HdhfrΔ5′ − 113 (Fig. 5C) and HdhfrΔ3′ + (728–2593) (Fig. 5D) which failed to induce DHFR protein levels in the same manner as cells transfected with the complete minigene.

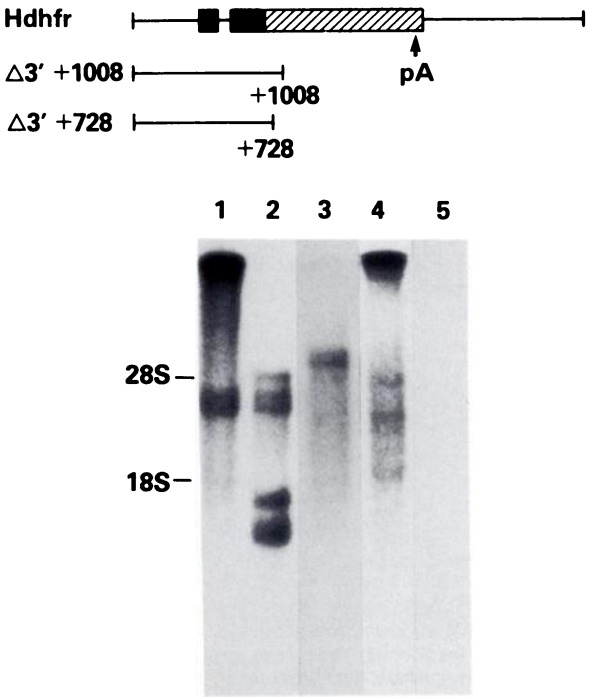

Fig. 4. Northern blot analysis of human DHFR mRNA in minigene-transfected cells. The methods for purifying the poly A$^+$ mRNA, fractionating the glyoxalated mRNA (10 mg) on 1.4% agarose gels, and transferring the mRNA to ABM paper were described previously (16). The blot was hybridized to ^{32}P-labeled human DHFR cDNA pHD84 (23). In normal human cells, the DHFR gene is polyadenylated at positions +719, +724, and +971 from the start of transcription of the DHFR mRNA in addition to the site used in CHO cells (21). Lane *1*, human DHFR minigene Hdhfr (clone 3A)-transfected CHO mRNA; lane *2*, human MCF-7 gene-amplified mRNA; lane *3*, HdhfrΔ3′ + 1008 (clone 50A)-transfected CHO mRNA; lane *4*, HdhfrΔ3′ + 728 (clone 1A)-transfected CHO mRNA; lane *5*, nontransfected CHO DG21 mRNA. Lanes *1*, *2*, and *5* have been shown previously (16).

These experiments indicated that the differences between the deprived and stimulated states seen at the level of DHFR protein were caused at least in part by changes in the level of DHFR RNA. However, since cells transfected with the complete minigene had only a 2-fold rise in RNA levels compared to the 3- to 5-fold rise in DHFR protein levels, there may also be post-transcriptional modulation of DHFR protein levels through changes in RNA transport or translational efficiency.

Discussion

Previous cellular studies analyzed growth modulation of DHFR levels by release from serum and amino acid deprivation. These cellular pulse-labeling studies suggested that, under the former condition, changes in DHFR are mediated at the level of transcription, whereas post-transcriptional mechanisms are involved under the latter condition (2, 9). What relationship, if any, exists between the regulation of DHFR by these two conditions and the normal cell cycle regulation of DHFR is unclear at this time (12–15). Previously, we developed a DHFR

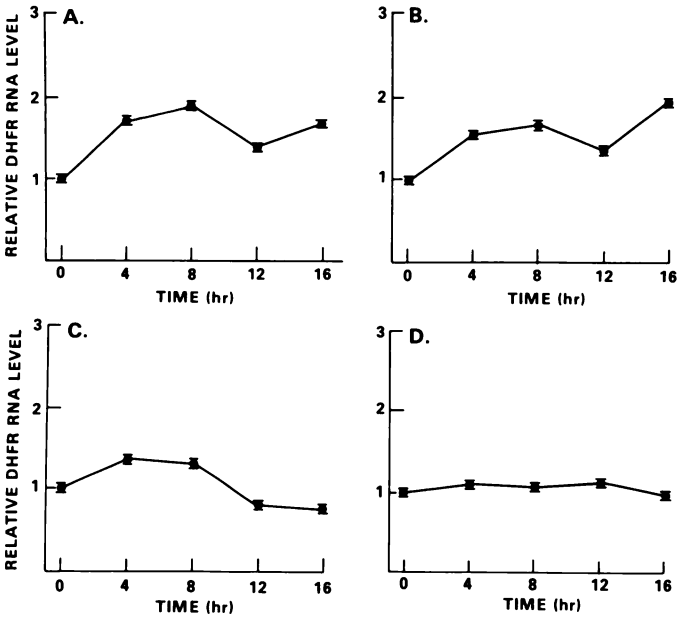

Fig. 5. DHFR RNA in amino acid-stimulated minigene-transfected cells. DHFR minigene-transfected cells were grown in amino acid-depleted medium and then stimulated to grow as described under Materials and Methods. At $t = 0$, the medium was changed to stimulating medium, and at various times the cells were harvested and total cellular RNA was isolated by disruption in guanidine isothiocyanate and fractionation in CsCl (16). The total cellular RNA from various time points was denatured in formaldehyde, dotted onto nitrocellulose paper (4), and hybridized to ^{32}P-labeled human DHFR cDNA pHD84 (23), and the amount of radioactivity bound to each sample was determined. Each point represents the average of two samples containing 8 μg of total cellular RNA. The DHFR RNA level is relative to the level in the amino acid-deprived state, $t = 0$. A, Hdhfr (clone 3A); B, Hdhfr (clone 6A); C, HdhfrΔ5′ − 113 (clone 11); D, Hdhfr Δ3′ + (728–2593) (pooled cells).

TABLE 2

Induction of DHFR levels by amino acids and serum

DHFR minigene with clone number	-Fold induction amino acid[a]	Average fold induction amino acid[b]	-Fold induction serum[c]	Average fold induction serum
Hdhfr				
3A	3.2 ± 0.2		3.3 ± 0.1	
5A	4.9 ± 0.8	4.0	2.4 ± 0.1	3.6
6A	4.2 ± 0.2		3.6 ± 0.2	
7A	3.5 ± 0.3		3.7 ± 1.7	
HdhfrΔ5′ − 322				
1A	5.6 ± 0.3		3.9 ± 0.8	
3A	4.1 ± 0.4	4.5	2.2 ± 0.2	3.4
5A	3.7 ± 0.3		4.0 ± 0.6	
HdhfrΔ5′ − 113				
1	3.1 ± 0.3		8.1 ± 0.5	
2	1.9 ± 0.1	2.0	4.6 ± 0.3	4.7
11	1.1 ± 0.1		1.3 ± 0.1	
HdhfrΔ3′ + 728				
1A			2.3 ± 0.4	
2A	2.1 ± 0.1	6.7	3.8 ± 0.5	3.0
3A	6.2 ± 0.4			
4A	11.7 ± 0.5		2.9 ± 0.4	
HdhfrΔ3′ + (728-2593)				
1	3.3 ± 0.1			
2	2.4 ± 0.4	2.6		
5	2.6 ± 0.6			
6	2.3 ± 0.1			
pMTVdhfr				
12A	0.7 ± 0.1	0.8	1.0 ± 0.1	0.9
18A	0.9 ± 0.1		0.8 ± 0.2	
pMTVdhfr + intron pooled		1.1 ± 0.1		1.1 ± 0.1

[a] Fold induction for clonal cell lines transfected with each minigene construct were determined as described in Table 1.

[b] Determined from the average of several clonal lines or the average of a pooled population of cells transfected with a DHFR minigene.

[c] From Ref. 16.

expression system in order to analyze the nucleotide sequences involved in serum modulation of DHFR expression (16). In the present study, using a similar approach, we have attempted to determine which DHFR nucleotide sequences are responsible for its regulation following release from amino acid deprivation and, perhaps, to better understand the mechanisms involved in this regulation.

In order to determine whether the two DHFR growth modulators are functioning through the same or different mechanisms, a comparison of results from serum and amino acid induction of DHFR levels in cells transfected with different DHFR minigene constructs was made. This comparison, which is shown in Table 2, indicated that there were important similarities between the two growth modulators. In both systems, enzyme levels in cell transfected with the human DHFR minigene Hdhfr were inducible following release from growth inhibition, whereas levels in cells transfected with pMTVdhfr were not. Significant differences in induction from the complete minigene by both amino acids and serum were found when nucleotide sequences to within 113 bp 5′ to the start of transcription were deleted. Removal of these nucleotide sequences eliminated approximately half of the amino acid-mediated induction of DHFR levels and caused the serum-induced responsiveness to become variable among different clonal cell lines. This hypervariable effect may be caused by the juxtaposition of different CHO sequences close to the DHFR promoter in the different cell lines.

The effect of 5′ minigene deletion constructs on amino acid and serum induction of DHFR levels suggested that there is transcriptional regulation under both conditions. Although an earlier study did not find evidence of transcriptional modulation of DHFR levels during amino acid deprivation, the conditions used in the two studies were not identical. In the earlier cellular study, amino acid-deprived cells were compared to exponentially growing cells in which only a small fraction of the cells would be in S phase and exhibit the transcriptional modulation of DHFR associated with DNA synthesis. Thus, the differences in the two studies may reflect differences in the proportion of cells in S phase under the two experimental conditions. Since both serum and amino acid modulation resulted in cell synchronization, and since both treatments appeared to exert their effects through similar 5′ sequences, it is possible that any cell synchronization technique may cause similar modulation of the DHFR gene through similar 5′ nucleotide sequences.

Additional structure function studies will be required to determine the function of the DHFR gene sequences between 322 and 113 bp 5′ to the start of transcription. This region of the DHFR gene has been shown to contain nuclease-hypersensitive sites (25). This region does not, however, include the repeated DNA sequences immediately 5′ to the start of transcription (21, 26) which in the mouse contains Sp1 transcription factor binding sites (27) and which is critical for transcription *in vitro*. The mouse, and presumably the human, DHFR

promoter is apparently complex, with electrophoretic variant nucleosomes (28) and bidirectional transcription (13, 29, 30).

While analysis of 5′ minigene deletions indicated similarities between serum and amino acid modulation of DHFR, analysis of 3′ minigene deletions indicated that there were significant differences as well. Clonal cell lines transfected with HdhfrΔ3′ + 728, a minigene which eliminated the DHFR polyadenylation site principally used in CHO cells, resulted in a hypervariable response to amino acid induction. This hypervariability may reflect the presence of CHO sequences which vary from clone to clone in the DHFR transcript. Furthermore, amino acid stimulation of cells transfected with the minigene HdhfrΔ3′ + (728–2593), which eliminated sequences within the 3′ nontranslated region of the minigene, caused only 50% of the increase in DHFR protein levels compared to cells transfected with the complete minigene. Although these 3′ nontranslated DHFR sequences appeared to influence the modulation of DHFR levels during amino acid deprivation, they did not appear to influence DHFR levels in response to serum stimulation. Previous studies demonstrated that the replacement of 3′ DHFR DNA sequences with hamster or viral sequences can affect DHFR regulation by contact inhibition (31). The mechanism through which 3′ sequences act to influence gene expression is not clear. Although it is possible that these 3′ DHFR sequences affect transcription, it is more likely that their influence is on RNA processing, transport, stability, or translational efficiency.

In this study, critical regions of the DHFR gene were surveyed for responsiveness to amino acid stimulation. These studies indicated that nucleotide sequences both 5′ to the start of transcription and in the 3′ nontranslated portion of the DHFR gene were involved in the amino acid induction of the DHFR levels. These studies suggested that both transcriptional and post-transcriptional mechanisms are involved the amino acid modulation of the DHFR gene.

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