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Differential Induction by Cadmium of a Low-Complexity Ribonucleic Acid Class in Cadmium-Resistant and Cadmium-Sensitive Mammalian Cells†

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ABSTRACT: The Chinese hamster ovary (CHO) cell line and the subline Cd^{r}20F4 have been used to compare cadmium-induced ribonucleic acid (RNA) synthesis in cadmium-sensitive and cadmium-resistant cells, respectively. Gel electrophoresis of the cell-free translation products directed by polyadenylated [poly(A+)] messenger RNA (mRNA) from cadmium-induced Cd^{r}20F4 cells revealed four low molecular weight species (M_r 7000–21 000), including metallothionein, whose synthesis was not detected after translation of either cadmium-induced or uninduced CHO cell poly(A+) mRNA. At least two of these species were also detected after translation of an abundant 400-nucleotide (NT) RNA class purified from the cadmium-induced Cd^{r}20F4 cell RNA. Molecular hybridization of complementary deoxyribonucleic acid (cDNA) complementary to this abundant, cadmium-induced 400-NT RNA fraction indicates that the cadmium-induced RNA class possesses a total kinetic complexity of about 2000 NT's. At least half of these inducible sequences are also represented constitutively in less abundant RNA classes of both uninduced CHO and Cd^{r}20F4 cells. Induction of Cd^{r}20F4 cells with cadmium increases the cellular concentration of the 2000-NT-complexity RNA class to a level at least 2×10^3-fold greater than its constitutive level in uninduced Cd^{r}20F4 cells. Induction of CHO cells with cadmium increases the cellular concentration of a subset of the sequences in the 2000-NT-complexity class, but only to a level 100-fold over the constitutive level in uninduced CHO cells. The remainder of these sequences belongs to the least abundant CHO cell poly(A+) RNA class.

The Chinese hamster ovary (CHO) cell subline Cd^{r}20F4 is a stable, cadmium-resistant variant derived from the CHO line. It is distinguished from the parental CHO line by a 100-fold higher cadmium toxic threshold, a 50-fold or greater cadmium-inducible metallothionein synthesis rate, and a 50-fold or greater level of cadmium-inducible translatable metallothionein messenger ribonucleic acid (mRNA)[1] (Enger et al., 1981; Hildebrand et al., 1979).

Although the mechanism of cellular cadmium detoxification has not been established, metal-induced synthesis of cadmium-binding metallothioneins is thought to play an important role [Enger et al., 1980; Hildebrand et al., 1979; reviewed by Kägi & Nordberg (1979)]. Thus, the differences in both metallothionein synthesis and cadmium sensitivity between these two cell lines provide means for studying the molecular events involved in cellular metal detoxification.

In this context, we recently reported that the poly(A+) RNA from cadmium-induced Cd^{r}20F4 cells contains a highly abundant RNA class not present in the poly(A+) RNA from uninduced CHO cells (Walters et al., 1980). In this paper, we report the results of a more detailed examination of this cadmium-inducible RNA class. We describe (1) the cell-free translation products directed by the poly(A+) RNA from cadmium-induced and uninduced Cd^{r}20F4 and CHO cells, (2) the isolation of cDNA sequences enriched for sequences complementary to a cadmium-induced abundant poly(A+)

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[1] Abbreviations used: mRNA, messenger ribonucleic acid; poly(A+), polyadenylated; $NaDodSO_4$, sodium dodecyl sulfate; cDNA, complementary deoxyribonucleic acid; NT, nucleotide; tRNA, transfer RNA; EDTA, ethylenediaminetetraacetic acid; HAP, hydroxylapatite.

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RNA class, and (3) the hybridization of this cDNA with the poly(A+) RNA from cadmium-induced and uninduced Cd^r20F4 and CHO cells.

Materials and Methods

Cell Culture. We have previously described our methods for the growth and isolation of cadmium-resistant Chinese hamster cells (Hildebrand et al., 1979; Enger et al., 1981). Cells from line CHO or Cd^r20F4 were induced with 2.0 and 20 μM $CdCl_2$, respectively. Induction was for 4–6 h for the Cd^r20F4 cells and 10–12 h for CHO cells. These conditions resulted in the maximum levels of induced cellular thionein synthesis and translatable thionein mRNA. Poly(A+) cytoplasmic RNA was prepared from induced and uninduced cells as described by Enger & Tobey (1972) and Enger & Hanners (1978).

Electrophoresis of RNA. Poly(A+) RNA was prepared from a mixture of Cd^r20F4 cells grown in the presence of 20 μM $CdCl_2$ and 4 μCi/mL [^{3}H]uridine (New England Nuclear) for 6 h and an equal number of untreated Cd^r20F4 cells labeled for 6 h with 0.25 μCi/mL [2-^{14}C]uridine (New England Nuclear). Approximately 20 μg of this RNA mixture was loaded on a 100-mm 2% agarose gel cast in "E buffer" containing 20 mM methylmercury hydroxide as described by Bailey & Davidson (1976). Gels were electrophoresed until a bromphenol blue tracking dye was at the bottom of the gel. Following electrophoresis, the gel was stained in 20 μg/mL ethidium bromide for visualization of RNA and then sliced into 1.8-mm sections for radioactivity determination. RNA size standards were run in parallel and included the 4S, 5S, 18S, and 26S RNAs from sea urchin embryos; the 4S, 5S, 16S, and 23S RNAs from *Escherichia coli* cells; the 5.8S, 18S, and 28S rRNAs from CHO cells; bromegrass virus RNAs (the gift of Dr. P. Kaesberg); MS-2 virus RNA; and rabbit globin mRNA. For preparative electrophoresis, approximately 30 μg of the ^{3}H–^{14}C-labeled RNA mixture was added to 200 μg of poly(A+) RNA from cadmium-induced Cd^r20F4 cells. The mixture was then resolved by preparative electrophoresis (Zapisek et al., 1969) in a 20-cm 2% agarose gel cast in E buffer and 20 mM methylmercury hydroxide as described above. The RNA was eluted in 600-μL aliquots of which 75 μL was used for determination of radioactivity. The peak fractions of high ^{3}H to ^{14}C radioactivity (55–75) were pooled, tRNA was added to a final concentration of 20 μg/mL, and the mixture was precipitated by the addition of 2.5 volumes of ethanol at −20 °C overnight. Following centrifugation and resuspension of the RNA pellet, the sample was dialyzed against distilled water and then stored frozen at −20 °C.

Cell-Free Translation of mRNA. Poly(A+) RNA was translated in a wheat germ extract cell-free system with either [^{35}S]cysteine or [^{3}H]leucine. Following translation, the samples were reduced, carboxymethylated, and resolved in 15% $NaDodSO_4$–polyacrylamide gels as described previously (Enger et al., 1979). Translation products were detected by fluorography.

Hybridization of cDNA and Poly(A+) RNA. Poly(A+) RNA was prepared as described above and used to direct the synthesis of cDNA as previously described (Walters et al., 1979). Poly(A+) RNA and cDNA from the sources described below were mixed in a buffer containing 10 mM Tris (pH 7.5), 1.5 M NaCl, 2 mM EDTA, and 0.2% $NaDodSO_4$. Aliquots of 2–5 μL were heat denatured at 100 °C and incubated at 68 °C for times ranging from 30 s to 10 h. Longer incubations resulted in considerable RNA degradation as indicated by slower rates of hybridization upon remelting of hybridized samples and subsequent rehybridization. The ratio of RNA to cDNA varied from 20:1 to greater than 10^4:1, depending on the equivalent R_0t (ER_0t) value (Britten et al., 1974) desired. The specific radioactivity of the cDNA was routinely 1.6×10^7 cpm/μg. Hybrid formation was assayed by resistance to S_1 nuclease as previously described (Walters et al., 1979).

cDNA enriched for abundant, cadmium-inducible sequences ($cDNA_a$) was synthesized from the 400-NT RNA fraction purified by preparative gel electrophoresis (see above). Sequences complementary to those abundant in Cd^{2+}-induced Cd^r20F4 cell poly(A+) RNA were purified by the procedure of Alt et al. (1978). Briefly, cDNA was reacted to an ER_0t of 210 with a 10^3 excess of uninduced CHO cell poly(A+) RNA. The unreacted fraction was isolated by hydroxylapatite (HAP) chromatography and then reacted to an ER_0t of 0.07 with the poly(A+) RNA from Cd^{2+}-induced Cd^r20F4 cells. The double-strand fraction was again isolated on HAP and eluted; the RNA was hydrolyzed with 0.3 N NaOH and dialyzed against 0.5 M NH_4HCO_3 (two changes, 1 L each, 4 h per change) and 2% potassium acetate (2 L, overnight). Following dialysis, tRNA was added to a final concentration of 20 μg/mL, and the sample was precipitated with 2.5 volumes of ethanol at −20 °C overnight.

cDNA that reacted with the rapid- and slow-annealing components of induced CHO cell poly(A+) RNA in Figure 5B was purified from $cDNA_a$. The $cDNA_a$ was reacted with a 1400-fold excess of cadmium-induced CHO cell poly(A+) RNA. At an ER_0t of 20.0, the sample was chromatographed on HAP. The hybridized double-strand nucleic acid fraction ($cDNA_{0.4}$) and the unhybridized single-strand nucleic acid fraction ($cDNA_{0.12}$) were isolated, and the cDNA was purified as described above for the $cDNA_a$. These two subfractions of the $cDNA_a$ population each had reactivities of greater than 90% when reacted with poly(A+) RNA from cadmium-induced Cd^r20F4 cells to an ER_0t of 30. The synthesis of cDNA from chicken globin mRNA and its use as a kinetic standard have been described previously (Walters et al., 1979).

Results

We recently reported that the poly(A+) RNA from cadmium-induced Cd^r20F4 cells contains a highly abundant RNA class not found in the uninduced CHO cell RNA (Walters et al., 1980). This abundant class contains mRNA which encodes the synthesis of cadmium-binding proteins, metallothioneins (Griffith et al., 1980), and thus may be involved in the cadmium-resistant phenotype of the Cd^r20F4 cell. Therefore, it was of interest to determine whether these abundant mRNA sequences, and the proteins which they encode, are differentially regulated in the cadmium-resistant (line Cd^r20F4) and cadmium-sensitive cells (line CHO).

Molecular hybridization of cDNA with its template poly(A+) RNA indicates that cadmium-induced Cd^r20F4 cells contain an abundant RNA class not found in uninduced CHO cells. As shown in Figure 1B, these sequences comprise approximately 5% of total Cd^r20F4 cDNA and reacted within a range of ER_0t values of 10^{-2}–10^{-1}. There is no comparable transition in the reaction of CHO cDNA with its template RNA (Figure 1A).

We next determined the size distribution of the Cd^r20F4 RNA species which were both highly abundant and inducible by cadmium. A mixture of [^{3}H]uridine-labeled poly(A+) RNA from cadmium-induced Cd^r20F4 cells and [^{14}C]-uridine-labeled poly(A+) RNA from uninduced Cd^r20F4 cells (Figure 2A) was resolved by coelectrophoresis in methylmercury–agarose gels. Highly abundant, cadmium-induced RNA species were identified by the increase in the ratio of

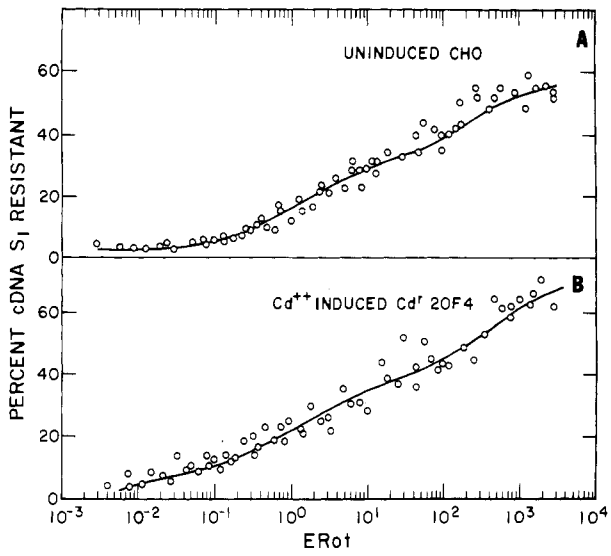

FIGURE 1: Hybridization of cDNA with poly(A+) RNA. Homologous hybridization of cDNA with its poly(A+) RNA templates. (A) Uninduced CHO cell RNA; (B) RNA from Cd^{r}20F4 cells induced 4 h with 20 μM $CdCl_2$.

^{3}H:^{14}C radioactivity. As shown in Figure 2A, a single peak of high ^{3}H:^{14}C radioactivity was resolved from the RNA mixture and migrated with RNA whose modal chain length was 400 nucleotides. An identical mixture of labeled RNA was added to 200 μg of poly(A+) RNA from cadmium-induced Cd^{r}20F4 cells and resolved on a preparative agarose gel (Figure 2B). Again, as in Figure 2A, a single peak of increased ^{3}H:^{14}C radioactivity was identified.

We have utilized the data in Figure 2 to independently determine the portion of the total poly(A+) RNA which represents cadmium-induced abundant sequences in Cd^{r}20F4 cells. As shown for the resolution of both RNA mixtures in Figure 2, the highest ^{3}H:^{14}C ratio of radioactivity in the 400-NT peak is about twice that of the other RNA size classes. Thus, the abundant, inducible RNA sequences comprise about half of the newly synthesized RNA mass in the 400-NT RNA fraction. Calculating from this fraction (50%), the total ^{3}H radioactivity loaded onto the gels, and the fraction of the total ^{3}H radioactivity which migrates in the 400-NT RNA fraction (see legend to Figure 2), we conclude that about 4% of the total newly synthesized RNA from cadmium-induced Cd^{r}20F4 cells represents cadmium-induced abundant RNA sequences. This value is very close to the 5% mass estimate independently derived by the cDNA:RNA molecular hybridization analyses presented in Figure 1 and indicates that the majority of the cadmium-induced abundant RNA sequences is contained in a single 400-NT size class.

The failure to detect more than one size class containing abundant, inducible RNAs could indicate either that a single mRNA species is induced or that all of the inducible sequences have a similar size. Thus, it was important to determine the kinetic complexity and mRNA coding capacity of the 400-NT RNA fraction. In order to determine the kinetic complexity of the 400-NT RNA class, we prepared a cDNA tracer ($cDNA_a$) which contained the sequences complementary to those present in high abundance in cadmium-induced Cd^{r}20F4 cells but which are not abundant in CHO cells. Poly(A+) RNA from cadmium-induced Cd^{r}20F4 cells was electrophoresed preparatively as in Figure 2B, and the 400-NT fraction was identified by the ^{3}H:^{14}C ratio of radioactivity of each aliquot. The purified "400-NT" RNA (modal size 400 NT's, range 300–700 NT's) was used as a template for the synthesis of cDNA. Those cDNA sequences which were hybridizable with uninduced CHO cell RNA were removed, and that portion of the remaining cDNA which was complementary to the most abundant RNA from cadmium-induced Cd^{r}20F4 cells was isolated (see Materials and Methods for additional details). As shown in Figure 3, 80% of the $cDNA_a$ tracer was hybridized by cadmium-induced Cd^{r}20F4 cell RNA by an ER_0t of 1.0. In contrast, only 4% of the $cDNA_a$ was hybridized by uninduced CHO cell RNA at an ER_0t of 1.0,

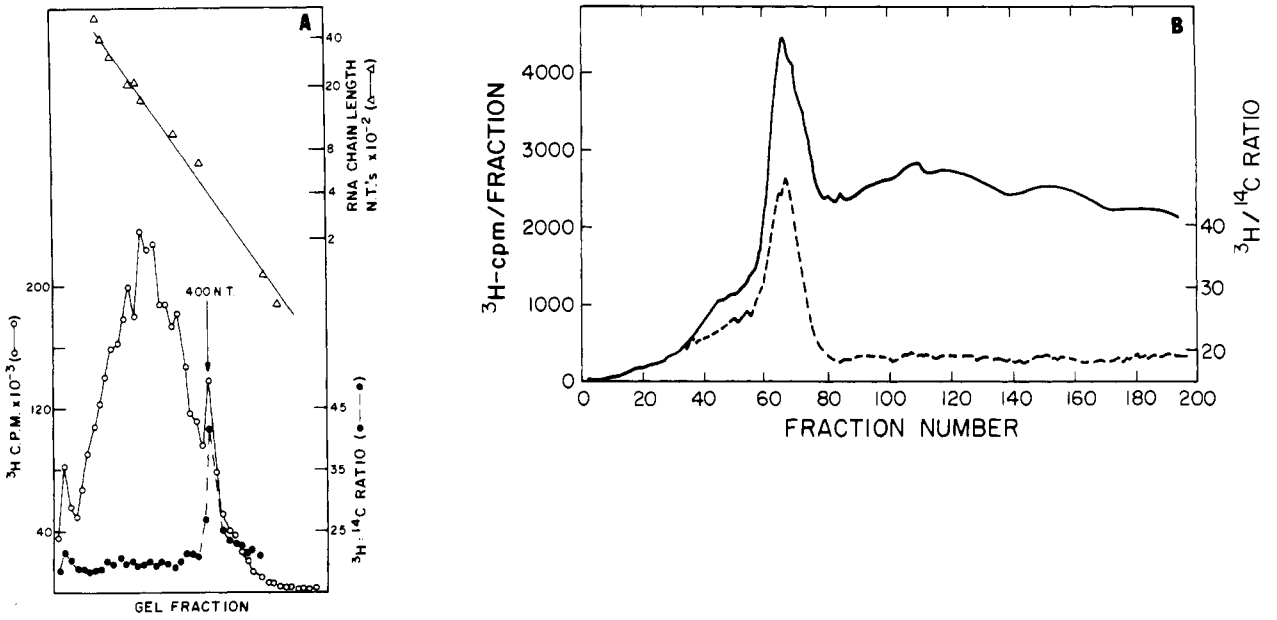

FIGURE 2: Methylmercury–agarose gel electrophoresis of Cd^{r}20F4 RNA. (A) A mixture of 10 μg each of ^{3}H-labeled poly(A+) RNA from induced Cd^{r}20F4 cells and ^{14}C-labeled poly(A+) RNA from uninduced Cd^{r}20F4 cells was electrophoresed at 50 V in a 100-mm 2% methylmercury–agarose gel. The relative migration of defined RNA species is shown in the upper portion of the panel. The 400-NT peak of ^{3}H radioactivity comprised 9% of the total [^{3}H]RNA. (B) Approximately 30 μg of the same ^{3}H–^{14}C-labeled RNA mixture from (A) above was combined with 200 μg of poly(A+) RNA from induced Cd^{r}20F4 cells and electrophoresed as described above in a 200-mm preparative chamber. Fractions (600 μL) were collected, and 75-μL aliquots were used for radioactivity determinations. Total ^{3}H radioactivity applied to the gel was 8 × 10^6 cpm. Fractions 55–75 were pooled, and the RNA was purified as described under Materials and Methods. Recovery of radioactivity in this fraction was ~7% of the total in each of three independent experiments.

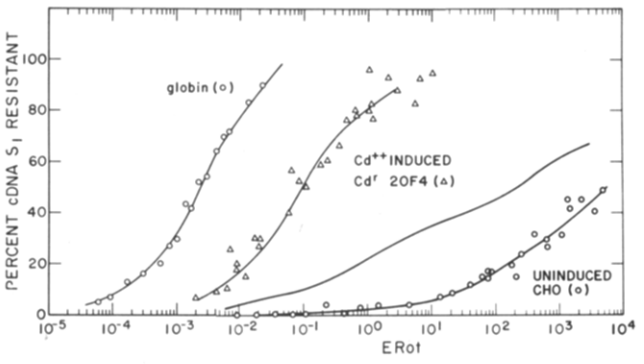

FIGURE 3: Hybridization of $cDNA_a$ with poly(A+) RNA. Hybridization of $cDNA_a$ (see Materials and Methods) with poly(A+) RNA from uninduced CHO or induced Cd^{r}20F4 cell RNA and the hybridization of cDNA and its chicken globin mRNA template. The reaction of Cd^{r}20F4 cDNA and its poly(A+) RNA template from Figure 1B is reproduced for comparison.

and 30% by an ER_0t of 700. Thus, this $cDNA_a$ tracer represents RNA sequences whose concentration is 10^4 higher in the induced Cd^{r}20F4 RNA than in CHO RNA.

The kinetic complexity of the $cDNA_a$ was determined by comparing the rate of its hybridization with cadmium-induced Cd^{r}20F4 cell RNA to that of the hybridization of cDNA synthesized from chicken globin mRNA and hybridized with its template (Figure 3). Calculating from the $ER_0t_{1/2}$ of these two reactions (0.04 and 0.0018 for $cDNA_a$ and chicken globin, respectively), and from the 5% of the total poly(A+) RNA estimated from Figures 1 and 2 to represent the inducible, abundant Cd^{r}20F4 sequences, we conclude that the total kinetic complexity of this class is about 2000 NT's and is sufficient to encode only a few low molecular weight proteins.

We confirmed this prediction by (1) examining the proteins whose synthesis were directed by the 400-NT RNA in a cell-free translation system and (2) comparing these proteins to those directed by the total poly(A+) RNA from induced and uninduced CHO and Cd^{r}20F4 cells. As shown in Figure 4A,B, poly(A+) RNA from cadmium-induced Cd^{r}20F4 cells directs the synthesis of four low molecular weight proteins (range of 7000–21 000) which are not directed by the RNA from uninduced CHO cells. [It should be noted that band 1, previously identified as metalothionein (Enger et al., 1979), likely contains two partially resolved isometallothioneins (C. E. Hildebrand and B. B. Griffith, unpublished experiments).] Thus, there are at least four mRNAs encoding low molecular weight proteins which are induced by cadmium in Cd^{r}20F4 cells to a sufficiently high abundance to detect their presence by cell-free translation.

We next examined the proteins directed by induced CHO cell and uninduced Cd^{r}20F4 cell RNAs. Densitometer tracings of fluorograms of the translation products (Figure 4C) indicate that none of the mRNAs for the four inducible proteins are present in these cells in sufficient abundance to detect their presence by cell-free translation.

The translation of the 400-NT RNA yielded the two smaller induction-specific proteins (Figure 4D) and possibly the two larger species as well. (Additional experiments will be required to confirm the latter due to the reduced concentration of the larger mRNA species in the size-fractionated 400-NT RNA population.) Thus, the limited coding capacity of the 400-NT RNA fraction as defined by molecular hybridization analysis is also confirmed by its translational activity.

The observation that cadmium induces a number of putative nonmetallothionein low molecular weight proteins raises the question of their identity and function. The most intensely

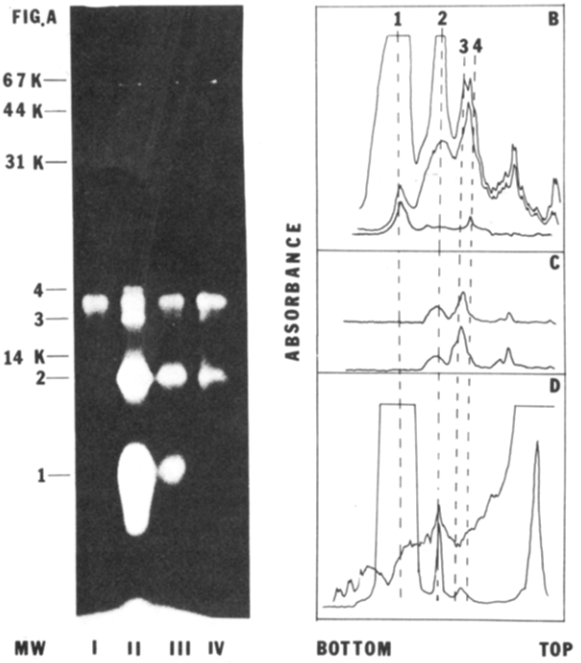

FIGURE 4: Cell-free translation of poly(A+) RNA. (A) Translation of poly(A+) RNA from uninduced CHO and induced Cd^{r}20F4 cells. Poly(A+) RNA from each of the two cell types was mixed to a constant RNA concentration of 370 μg/mL. The concentration of Cd^{r}20F4 RNA was 0 (I), 74 (II), 14.8 (III), and 7.4 μg/mL (IV). A total of 2.8 μg of RNA was translated in each reaction. Molecular weights are from parallel standards. (B) Densitometer tracings of the fluorographs from (A) above. Lower tracing, 0 μg/mL; middle tracing, 7.4 μg/mL; upper tracing, 74 μg/mL. The four unique induction-specific proteins are denoted by vertical lines. (C) Densitometer tracings of fluorograms of the translation products encoded by induced CHO cell RNA (upper tracing) and uninduced Cd^{r}20F4 cell RNA (lower tracing). (D) Densitometer tracings of fluorograms of the translation products encoded by the gel-resolved 400-NT RNA and labeled with either [^{35}S]cysteine (lower tracing) or [^{3}H]leucine (upper tracing).

labeled of these species, band 1, has been identified as metallothionein, and as pointed out above, it is likely a partially resolved mixture of the two isometallothioneins. However, the identity of the other three bands is presently unknown. One possibility is that the translation products in bands 2–4 are incompletely reduced and carboxymethylated metallothionein or aggregates of metallothionein. In this case, the response of cells to cadmium exposure would be less complex than suggested above. To address this possibility, we repeated the translation of the 400-NT RNA using radioactive leucine in place of cysteine. Since none of the nonvariant metallothioneins thus far sequenced contain leucine [see Kägi & Nordberg (1979) for a review], a differential labeling of these bands with leucine and cysteine would indicate that they are not related. The results of these experiments indicated no peak of radioactive leucine incorporation in band 1, consistent with its identification as metallothionein, while band 2 had a peak of leucine radioactivity. Thus, band 2 is likely not an artifact related to metallothionein. We conclude from these experiments that cadmium induces the synthesis of four low molecular weight proteins which include both metallothionein and nonmetallothionein species. Since we cannot detect these proteins in either uninduced CHO or Cd^{r}20F4 cells, or induced CHO cells, they must be either absent or present at significantly reduced levels.

To discriminate quantitatively between these alternatives, we used $cDNA_a$ to measure the concentration of its complementary sequences in uninduced Cd^{r}20F4 cell RNA (Figure 5A) and in induced CHO cell RNA (Figure 5B). In contrast

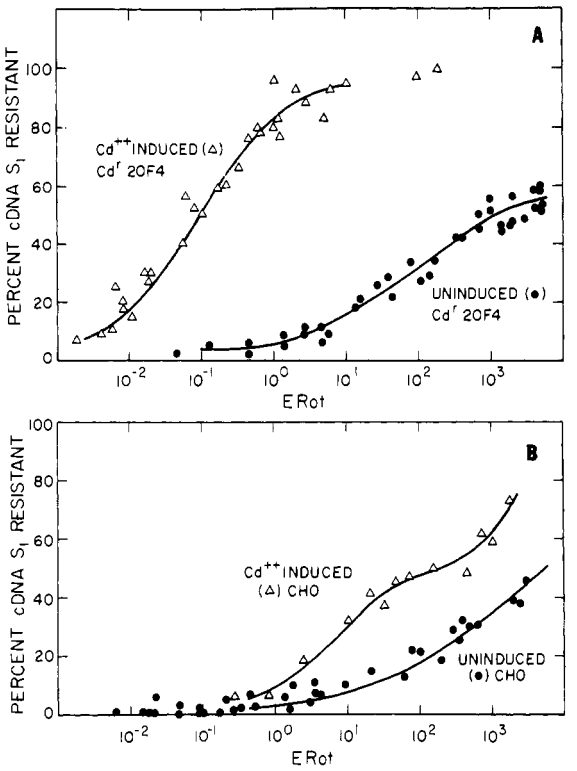

FIGURE 5: Hybridization of $cDNA_a$ with poly(A+) RNA from induced and uninduced Cd^{r}20F4 (A) and CHO (B) cells. Reaction of $cDNA_a$ with Cd^{2+}-induced Cd^{r}20F4 RNA is reproduced from Figure 3A.

to the reaction obtained with the induced Cd^{r}20F4 cell RNA, uninduced Cd^{r}20F4 RNA hybridized with $cDNA_a$ at a rate at least 2×10^3 slower. Only 55% of the $cDNA_a$ was in hybrid at an ER_0t of 5×10^3 when the reactions were terminated due to the thermal instability of the poly(A+) RNA. Thus, a 4-h exposure to 20 μM $CdCl_2$ increases the concentration of those RNA sequences complementary to $cDNA_a$ by at least 2×10^3-fold. It might noted here that the concentration of these sequences in uninduced Cd^{r}20F4 cells is about 8 times higher than that measured in uninduced CHO cells. The cause of this difference in constitutive expression is not known.

The induction of CHO cells with cadmium increases the concentration of only a subset of those sequences induced in Cd^{r}20F4 (Figure 5B). The reaction of $cDNA_a$ with poly(A+) RNA from induced CHO cells is described by a biphasic curve spanning more than 3.5 decades of ER_0t to achieve a 70% hybridization. We estimate that more than 4 decades of ER_0t would be required a 100% reaction. The reaction with induced Cd^{r}20F4 cell RNA, on the other hand, is qualitatively different. It is described by a monophasic curve in which greater than 90% hybridization can be achieved in slightly more than 2 decades of ER_0t, the same span of ER_0t requried to hybridize completely the chicken globin cDNA. These data indicate that there is little or no difference in the relative concentrations of the individual sequences induced by cadmium in Cd^{r}20F4 cells. However, there is a significant difference in the relative concentrations of the individual sequences comprising the two distinct abundance classes defining the reaction of $cDNA_a$ with induced CHO cell poly(A+) RNA.

We have confirmed this observation by purifying the two classes of sequences which differ in their kinetics of reaction with induced CHO cell RNA. The reaction of these two $cDNA_a$ subfractions with induced CHO RNA is shown in Figure 6. One component of the $cDNA_a$, $cDNA_{0.4}$, hybridizes with induced CHO RNA with the kinetics of the more rapidly

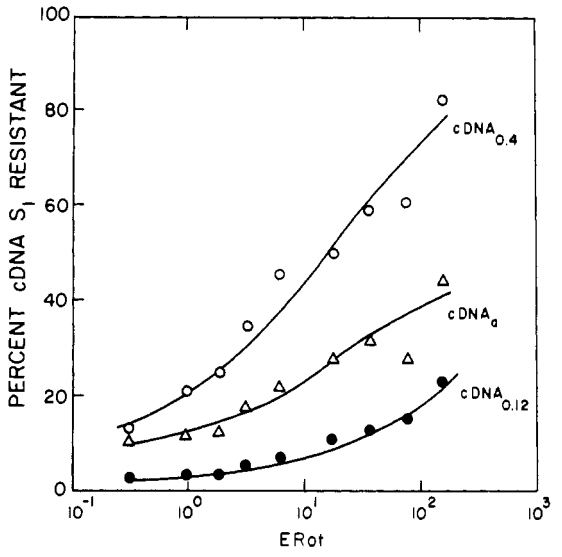

FIGURE 6: Hybridization of $cDNA_a$ sequences which are differentially regulated in CHO cells. $cDNA_a$ and its subfractions $cDNA_{0.12}$ and $cDNA_{0.4}$, isolated as described under Materials and Methods, were reacted with poly(A+) RNA from Cd^{2+}-induced CHO cells.

Table I: Reconstruction of $cDNA_a$ Annealing from $cDNA_{0.4}$ and $cDNA_{0.12}$ Fractions

	% hybrid[a]		% $cDNA_a$ hybrid	
ER_0t	$cDNA_{0.12}$	$cDNA_{0.4}$	predicted[b]	actual[a]
0.31	2	13	6	10
0.95	3	21	9	11
1.9	3	24	10	12
3.1	6	34	15	17
6.2	7	45	20	21
19	11	49	24	27
38	12	59	28	31
76	15	60	29	27
152	23	80	42	43

[a] Data taken from Figure 6. [b] Calculated from the relationship % hybrid $cDNA_a$ = (% $cDNA_{0.12}$ hybrid)(0.67) + (% $cDNA_{0.4}$ hybrid)(0.33).

reacting species of the biphasic curve in Figure 5B. The second component of the $cDNA_a$, $cDNA_{0.12}$, reacts much more slowly than either $cDNA_a$ alone or $cDNA_{0.4}$ and displays kinetics similar to those sequences reacting in the second transition of $cDNA_a$ in Figure 5B. That these results are not a consequence of alteration in the cDNA introduced during the fractionation process is shown by the following observations: (1) both tracers were greater than 90% reactive when hybridized with induced Cd^{r}20F4 RNA; (2) when the reactions of the two tracers are summed according to their representation in the $cDNA_a$ (33% for $cDNA_{0.4}$ and 67% for $cDNA_{0.12}$), the original $cDNA_a$ reaction curve is reproduced (Table I). We conclude, therefore, that (1) the components of $cDNA_a$ are induced to a different extent in CHO than in Cd^{r}20F4 and (2) that these two individual components of $cDNA_a$ are regulated differently in the two lines.

Previous work from this laboratory has shown that the induction of thionein mRNA synthesis by cadmium in Cd^{r}20F4 cells is dose dependent. The results reported here were obtained by inducing Cd^{r}20F4 cells at a cadmium concentration (20 μM) shown by these previous studies to maximally induce translatable thionein mRNA and cellular thionein synthesis. Induction of Cd^{r}20F4 at 2 μM $CdCl_2$, the concentration used to induce CHO cells, results in a 3-fold lower level of thionein synthesis and only a 6-fold lower level

of translatable thionein mRNA (M. D. Enger and C. E. Hildebrand, unpublished experiments). These differences are small in comparison to the 200-fold difference in the induced RNA levels in CHO and Cd^{r}20F4 cells. Furthermore, we have now obtained results identical with those reported here by inducing each of the two cell lines with 100 μM $ZnCl_2$ (J. K. Griffith and R. A. Walters, unpublished experiments). This latter regimen is nontoxic and induces the same maximal rates of thionein synthesis and thionein mRNA as the cadmium concentrations used here, 20 and 2 μM for Cd^{r}20F4 and CHO, respectively. Taken as a whole, these data indicate that the differences in induced RNA levels in CHO and Cd^{r}20F4 cells are not due to differences in the concentration of metal inducer but rather are an intrinsic property of the two cell lines. Since at least one of the proteins encoded by the induced mRNA, metallothionein, has been suggested to be involved in detoxifying or ameliorating the effects of metals, the difference in metal-induced RNA synthesis may provide an explanation for the difference in sensitivity to cadmium of these two cell lines.

Discussion

We have previously reported the presence of a low-complexity RNA class specific to cadmium-induced Cd^{r}20F4 Chinese hamster cells (Walters et al., 1980). This class includes mRNAs for the cadmium-binding proteins, metallothioneins (Griffith et al., 1980), thus suggesting a specific role for this RNA class in cadmium detoxification. The finding reported here that this RNA class is differentially regulated in cadmium-resistant and cadmium-sensitive cells is further evidence for this notion.

It has been previously proposed that metallothionein is a major factor responsible for cellular cadmium detoxification [Hildebrand et al., 1979; Enger et al., 1981; also see Kägi & Nordberg (1979) for a review]. While the data reported here are consistent with this view, the cell-free translation and cDNA hybridization analyses also suggest that the acquisition of the cadmium-resistant phenotype in cultured cells, in actuality, may be more complex. As shown in Figure 4, treatment of Cd^{r}20F4 cells with cadmium induces at least three low molecular weight nonmetallothionein species, in addition to metallothionein. These proteins are not detected after translation of induced CHO cell RNA. Likewise, we estimate from hybridization kinetics that the RNA class from which these proteins are translated has a complexity of 2000 NT's (Figure 3). This would be sufficient for encoding five metallothionein-sized mRNAs. In addition, this inducible 2000-NT-complexity RNA class is differentially regulated in the two cell lines. Some of its sequences are induced in both types of cells, although to different levels (Figure 5), while some sequences induced in Cd^{r}20F4 are among the least abundant RNA class measured in cadmium-induced CHO cells (Figure 6).

The suggestion that acquisition of a cadmium-resistant phenotype may involve factors other than (or in addition to) an enhanced capacity for metallothionein synthesis finds additional support in the data of Enger et al. (1981). Based on measurements of the rate and extent of accumulation of cadmium bound by metallothionein and nonmetallothionein cellular compartments of cadmium-sensitive and cadmium-resistant cells, they suggested a role for the nonmetallothionein compartment in determining cellular cadmium resistance. Accordingly, at least one other nonmetallothionein cadmium-binding protein has been identified in rat testis (Chen & Ganther, 1975), and cadmium has been shown to induce four higher molecular weight proteins (M_r 27 000–89 000) in chick and human fibroblasts (Levinson et al., 1980) and in Chinese hamster (C. E. Hildebrand and B. B. Griffith, unpublished experiments). Whether all of the cadmium-induced sequences are involved in metal detoxification or accommodation of the cells to metal is not known. However, the differential regulation of these two classes of sequences in cadmium-sensitive and cadmium-resistant cells would be consistent with such a role.

One means by which the Cd^{r}20F4 cells could acquire the increased capacity for the synthesis of these induction-specific RNAs is through the amplification of their respective structural genes. This mechanism is responsible for elevated dihydrofolate reductase levels in methotrexate-resistant cells (Alt et al., 1978) and for the increased synthesis of the first three enzymes in UMP anabolism in *N*-(phosphonacetyl)-2-aspartate resistant cells (Wahl et al., 1979). Such an explanation could also provide a possible explanation for the higher constitutive levels of these sequences found in the uninduced Cd^{r}20F4 cells. However, as shown below, gene amplification is not an obligatory requirement in accounting for the magnitude of the Cd^{r}20F4 cells' response to cadmium.

The poly(A+) RNA content of diploid Chinese hamster cells is 0.2 pg of RNA/cell (Enger & Tobey, 1972; M. D. Enger, unpublished experiments). With the assumption that 5% of this RNA respresents the induced, abundant 2000-NT-complexity class (Figures 1 and 2), there are 9×10^3 copies of each of five different average 400-NT-sized RNAs at steady state. Since the induction of this class occurs in 4 h under the described condtions, there are about 40 molecules of each mRNA species accumulated per minute per cell. This represents the initiation of one molecule per gene each 3 s. Eukaryotic RNA initiation rates of one initiation per 2–5 s have been estimated for blowfly larvae salivary gland rRNA (Griffith, 1978), one initiation per 3 s has been estimated to be required for sea urchin oocyte rRNA accumulation (Griffith & Humphreys, 1979; J. K. Griffith, B. B. Griffith, and T. D. Humphreys, unpublished experiments), and one initiation per 2–5 s has been estimated for ovalbumin, hemoglobin, and silk fibroin mRNA (Kafatos, 1972). Thus, the required rate of initiation of single-copy genes are within the range estimated for animal cells. In this context, it will be of great interest to determine whether or not these genes are indeed amplified in the cadmium-resistant Cd^{r}20F4 cells. Experiments to resolve these alternatives are now in progess.

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Interactions of Poly(N^{ϵ},N^{ϵ},N^{ϵ}-trimethyllysine) and Poly(lysine) with Polynucleotides: Circular Dichroism and A-T Sequence Selectivity†

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ABSTRACT: Complexes of $(Lys)_n$ and $[Lys(Me_3)]_n$ with natural and synthetic DNAs have been studied by CD as a function of ionic strength. In dilute EDTA, $(Lys)_n$ and $[Lys(Me_3)]_n$ produce the same distortions to the CD spectrum of calf thymus DNA at r (peptide residue/nucleotide residue) values < 0.6. At higher r values, the distortions are somewhat different. $[Lys(Me_3)]_n$ alters the conformation of some polynucleotides differently from $(Lys)_n$ under non-ψ conditions. Therefore, methylation of histones may serve to alter the structure of chromatin. At low ionic strength, $[Lys(Me_3)]_n$ and $(Lys)_n$ alter the viscosity of DNA to the same extent between r values of 0.0 and 1.0. In contrast to $(Lys)_n$–DNA, at high ionic strengths, $[Lys(Me_3)]_n$–DNA does not show ψ-type CD spectra. $(Lys)_n$ forms ψ^- structures with $(dA\text{-}dT)_n$ and $(dG\text{-}dC)_n$. $[Lys(Me_3)]_n$ forms ψ^- structures with $(dA\text{-}dT)_n$. Between 0.05 and 0.3 M NaCl, $[Lys(Me_3)]_n$ forms ψ^+ structures with $(dG\text{-}dC)_n$, while between 0.35 and 0.45 M NaCl, it forms a ψ^- structure with $(dG\text{-}dC)_n$. Neither $(Lys)_n$ nor $[Lys(Me_3)]_n$ forms ψ structures with $(dA)_n\cdot(dT)_n$ or $(dG)_n\cdot(dC)_n$. These results, in conjunction with the work of others on reconstitution of nucleosome-like particles from synthetic polynucleotides, suggest that the ability of DNA and histones to form nucleosomes is related to the formation of ψ structures. $(Lys)_n$ binds preferentially to $(dA)_n\cdot(dT)_n$ over $(dA\text{-}dT)_n$. $[Lys(Me_3)]_n$ binds to $(dA)_n\cdot(dT)_n$ and $(dA\text{-}dT)_n$ with equal affinity.

Methylated lysine residues are found in a variety of proteins, including histones H3 and H4 [see Paik & Kim (1980) for a review]. Interesting speculations about the functional effects of methylation of histones have been published (Brandt et al., 1975; Delange & Smith, 1975; Dixon et al., 1975; Paik & Kim, 1972). These speculations were a priori in nature since nothing was known about the effects of methylation on protein conformation or on protein–nucleic acid interactions. Therefore, we are studying the properties of methylated poly(L-lysine), $(Lys)_n$,[1] beginning with the limiting model poly(N^{ϵ},N^{ϵ},N^{ϵ}-trimethyl-L-lysine), $[Lys(Me_3)]_n$ (Granados & Bello, 1979, 1980). We have shown that $[Lys(Me_3)]_n$ stabilizes DNA against thermal denaturation more than $(Lys)_n$ does but is dissociated from nucleic acids at half the salt concentration that $(Lys)_n$ requires (Granados & Bello, 1980). The latter finding suggests that methylation of lysine weakens the electrostatic component of histone–DNA interactions.

Circular dichroism has been used to detect ψ structures in complexes of DNA with histones and polypeptides, in chromatin, and in nucleosomes, for DNA in organic solvents or salt solutions, and for DNA–metal complexes [see Cowman & Fasman (1980) and Shin & Eichhorn (1977) and references therein]. ψ spectra can be negative (ψ^-) or positive (ψ^+) in sign and are characterized by large extrema (some with mean nucleotide residue ellipicity as large as about 10^6 deg cm^2 dmol^{-1}) in the region 260–280 nm. The ψ spectra have been recognized as resulting from asymmetric aggregates. Reich et al. (1980) and Pyatigorskaya et al. (1978) have shown that ψ spectra result from differential scattering of right and left circularly polarized light. The scattering is the result of right- or left-handed superhelix formation, which may occur without a change in secondary structure (Reich et al., 1980; Potaman et al., 1981). We now report on ψ structures that are formed in complexes of $(Lys)_n$ and $[Lys(Me_3)]_n$ with nucleic acids, and we discuss their relationship to chromatin structure.

Experimental Procedure

Materials. (Lys·HBr) (50 000 daltons) was purchased from Sigma Chemical Co., and calf thymus DNA and polynucleotides were purchased from P-L Biochemicals. $[Lys(Me_3)]_n$ was prepared as previously described (Granados & Bello, 1979) except that methylation was done on $(Lys)_n$ in 0.02 M sodium borate (pH 9.2) instead of H_2O. The nucleic acids and polypeptides were dissolved in a pH 8.0 buffer containing 2×10^{-3} M sodium phosphate and 5.0×10^{-4} M EDTA or in EDTA only and were diluted with NaCl prior to direct mixing. The absorption and/or CD spectra of the

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[1] Abbreviations used: $(Lys)_n$, poly(L-lysine); $[Lys(Me_3)]_n$, poly(N^{ϵ},N^{ϵ},N^{ϵ}-trimethyl-L-lysine); EDTA, ethylenediaminetetraacetic acid.

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