

Inducibility of Metallothionein Throughout the Cell Cycle

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Received June 1984/Accepted 18 July 1984

Synchronized Chinese hamster cells were induced with ZnCl₂ at multiple stages of the cell cycle and labeled with [³⁵S]cysteine, and the ³⁵S-labeled proteins were isolated and separated into metallothionein and nonmetallothionein fractions. Metallothionein was found to be inducible in all stages of the cell cycle and in G₁-arrested cells.

Metallothioneins (MT) are cysteine-rich, low-molecular-weight proteins that are involved both in cellular detoxification of metals such as cadmium and mercury and in regulation of essential trace elements such as copper and zinc (1, 9). In addition to their role in metal homeostasis, MT have become increasingly important in the field of molecular biology. An MT promoter has been cloned and fused to other genes to allow metal-induced expression of those genes (5, 6). To establish optimal experimental conditions for such studies, it would be helpful to determine whether MT synthesis is induced throughout the entire division cycle or only in discrete portions of the cycle as occurs with tyrosine aminotransferase (4). Toward that goal, we have examined the ability of synchronized Chinese hamster cells to synthesize MT in response to exposure to zinc and have found that MT is inducible throughout the entire cell cycle and also in G₁ arrest.

A subclone of the cadmium-resistant 30F9 Chinese hamster cell (2), designated 30F9-6, was selected for its ability to withstand the multiple centrifugation steps involved in the synchronization procedures. Cells were grown in suspension culture in F10 medium supplemented with 15% neonate calf serum and antibiotics. The cells were synchronized either in the G₁ phase of the cell cycle via isoleucine deprivation (Ile⁻) (7) or in the very early S phase by a procedure combining Ile⁻ with hydroxyurea treatment (8). To quantify the rate of MT synthesis induced by treatment with zinc, samples of synchronized or exponentially growing 30F9-6 cells were treated with ZnCl₂ and labeled with [³⁵S]cysteine; the ³⁵S-labeled proteins were then chromatographed on a Sephadex G-75 column (3) as shown in Fig. 1A. The first peak to elute from the column (fractions 10 to 20) consisted of high-molecular-weight proteins, the middle peak represented MT, and the last peak to elute corresponded to unincorporated cysteine (3). The time course of MT synthesis in Zn-induced and uninduced cultures of 30F9-6 cells is shown in Fig. 1B. Very little MT was synthesized in the uninduced culture, but in the induced culture, the rate of synthesis of MT continued to increase during the entire course of the experiment. By 9 h postinduction, more than half of the protein-associated ³⁵S-label was incorporated into MT. Under these conditions, the synthesis of labeled non-MT proteins was not appreciably altered by exposure to zinc.

Because the mean duration of the division cycle of the 30F9-6 cell is ca 13.5 h, we could not utilize a 9-h induction period since even highly synchronized cells would become redistributed over the entire cell cycle by 9 h after release

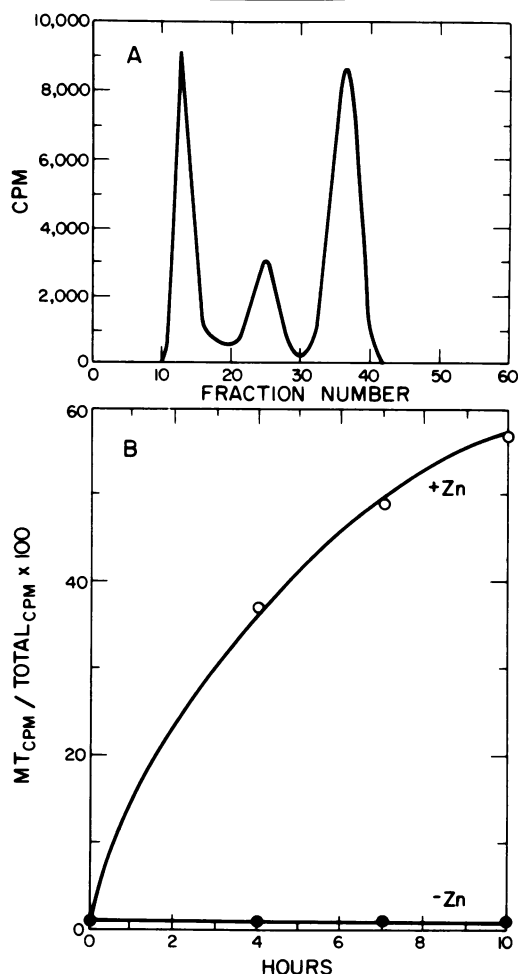


FIG. 1. (A) Elution profile of ³⁵S-labeled proteins that were obtained from synchronized 30F9-6 Chinese hamster cells and chromatographed on a Sephadex G-75 column. The cells were synchronized in G₁ by growth for 36 h in Ile⁻ medium (7); then a 100-ml sample of cells was placed in fresh, complete medium at *t* = 0. Six hours later, ZnCl₂ was added to the culture medium (to 100 μM), and 25 μCi of [³⁵S]cysteine (200 Ci/mmol; New England Nuclear Corp., Boston, Mass.) was added at 9 h. After the labeling period, the cells were harvested, the cytoplasmic fraction was isolated, and the ³⁵S-labeled proteins were analyzed on a Sephadex G-75 column as described previously (3). (B) [³⁵S]cysteine incorporation into MT (percentage of total [³⁵S]cysteine incorporated into protein) as a function of incubation period in medium containing 100 μM ZnCl₂. Radioactivity values represent counts per minute in a sample of 10⁶ cells.

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TABLE 1. MT synthesis in synchronized cultures of 30F9-6 Chinese hamster cells^a

Culture	Period of induction with ZnCl ₂ (h)	Labeling period (h)	Distribution of cells within the division cycle at the beginning of the labeling period (%)			MT (cpm)	Non-MT (cpm)	% MT ^b
			G ₁	S	G ₂ and M			
Exponential control	0-4	3-4	55	37	8	884	975	47.5
4 h post Ile ⁻ release	0-4	3-4	99	1	<1	491	759	39.3
4 h post Ile ⁻ /HU release	0-4	3-4	10	90	<1	541	982	35.5
7 h post Ile ⁻ release	3-7	6-7	74	26	<1	758	1,092	40.9
7 h post Ile ⁻ /HU release	3-7	6-7	2	53	45	1,452	1,702	46.4
10 h post Ile ⁻ release	6-10	9-10	41	54	5	1,002	1,696	37.1
10 h post Ile ⁻ /HU release	6-10	9-10	82	10	8	962	1,521	38.7
Unreleased Ile ⁻ (G ₁ -arrested) cells ^c	0-4	3-4	99	1	<1	1,288	3,260	23.6

^a Cultures of 30F9-6 cells were synchronized in either early interphase by Ile⁻ (7) or in mid interphase by combined Ile⁻ and hydroxyurea blocks (8). At various times after reversal of synchronization block, 100-ml cell samples were induced with 100 μM ZnCl₂ for 4 h and labeled with 0.25 μCi of [³⁵S]cysteine per ml during the final hour of the zinc induction period. Cells were then harvested, cytoplasmic fractions were prepared, and the ³⁵S-labeled proteins were analyzed via Sephadex G-75 chromatography (3). Radioactivity values represent counts per minute (cpm) per 10⁶ cells. Distributions of cells among the phases of the division cycle were determined in parallel cultures utilizing a procedure that combined flow cytometry, [³H]thymidine autoradiography, and cell number enumeration (7). HU, Hydroxyurea.

^b MT (cpm)/total protein (cpm) × 100.

^c The amount of [³⁵S]cysteine utilized in this experiment was increased by approximately fourfold over the amount utilized in the other experiments.

from synchronization block. Accordingly, we selected a 4-h induction period as an interval that would provide an acceptable quantity of radioactivity to be incorporated into cellular proteins under conditions in which a sufficient degree of synchrony was maintained. That is, cells were released from synchronization block, and at varying times thereafter, the cultures were treated with ZnCl₂ for incubation periods of 4 h each, with [³⁵S]cysteine being present during the final hour of the induction period.

The data obtained in our experiments indicate that G₁-, S-, or G₂-enriched populations of 30F9-6 cells can be readily induced to synthesize MT when treated with ZnCl₂ (Table 1). It is also probable that cells held in G₁ arrest through maintenance in Ile⁻ medium during the induction and labeling periods are also able to synthesize MT, although at a lower rate than that obtained with actively traversing G₁ cells (i.e., the "4 h post Ile⁻ release" culture in Table 1); the reduced capacity for synthesis of MT in G₁-arrested cells may result from alterations in patterns of protein synthesis attributable to the lack of availability of adequate quantities of isoleucine in the Ile⁻ medium. Induction of MT in the G₁-arrested 30F9-6 cells is reminiscent of the phenomenon in intact animals in which MT synthesis can be induced in tissues composed predominantly of G₁-arrested cells (e.g., liver, kidney) after administration of zinc salts (9).

Statistical analysis, utilizing the Regression and General Linear Models programs of the Statistical Analysis System, revealed that the MT synthesis data fit a model of the following general form: % MT synthesis = [a × (% cells in G₁ phase)] + [b × (% cells in S phase)] + [c × (% cells in G₂ and M)], where a, b, and c represent the relative rates of synthesis of MT in the various stages of the cell cycle. For the model in which the data from cultures 1 to 7 (Table 1) were included (the actively proliferating cultures), we obtained calculated values of a = 0.408 ± 0.026, b = 0.375 ± 0.038, and c = 0.585 ± 0.096. The overall correlation coefficient (R²) was 0.995. All of the observed values for MT synthesis, except for that of the exponentially growing culture, were well within the 95% confidence limits for the predicted values. (The exponentially growing culture exceeded the upper 95% confidence limit by only 2.4%.) Thus, it is evident that the inducibility of MT synthesis relative to the synthesis of other proteins that incorporate cysteine is

nearly equivalent in cells in the G₁ or S phases and slightly increased for cells in late interphase.

Given the need for precise regulation of essential trace elements plus the necessity for rapid induction of MT synthesis in cells exposed to high levels of cadmium or mercury, it is not surprising that MT is inducible throughout the cell cycle and in nonproliferating cells as well. Our results suggest that genes experimentally linked to the MT promoter also should be inducible throughout the entire cell cycle, which should allow one to activate genes in portions of the cycle where they are not normally expressed (e.g., activation of S phase genes in G₁, etc.).

We gratefully acknowledge the technical contributions of Judith G. Tesmer and the assistance of Jane M. Booker in analyzing the MT synthesis data. This work was performed under the auspices of the U.S. Department of Energy and the Los Alamos National Flow Cytometry and Sorting Research Resource funded by Public Health Service grant P41-RR01315-02 from the Division of Research Resources of the National Institutes of Health and by the Department of Energy.

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