MOLECULAR AND CELLULAR BIOLOGY, Feb. 1989, p. 847–850
0270-7306/89/020847-04$02.00/0

Vol. 9, No. 2

Enhancement of Major Histocompatibility Class I Protein Synthesis by DNA Damage in Cultured Human Fibroblasts and Keratinocytes

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Received 26 July 1988/Accepted 18 October 1988

Exposure of primary human fibroblasts or simian virus 40-transformed human keratinocytes to several different classes of DNA damage, including UV light C (254 nm), resulted in a rapid increase in the expression of human major histocompatibility class I (MHC-I) proteins. MHC-I induction was also detected after exposure to low doses of the protein synthesis inhibitor cycloheximide, suggesting that MHC-I induction by DNA damage may be a component in a derepressible cellular SOS pathway.

Exposure of eucaryotic cells to DNA-damaging agents induces several distinct cellular responses, including DNA repair, arrest of DNA synthesis, increased replication of latent papovaviruses, increased expression of endogenous retroviruses, and increased synthesis of specific cellular proteins (6, 9–11, 13, 14, 17). We used high-resolution two-dimensional protein gel electrophoresis (HR2D) to study patterns of change in cellular protein synthesis induced after exposure of cultured human fibroblasts and keratinocytes to several DNA-damaging regimens. Changes in the rate of synthesis of individual polypeptides were quantitatively evaluated by use of the QUEST computer system (5), which is designed to accurately quantitate and match HR2D patterns. We compared the effects of UV light C (UVC) (254 nm) and the metabolically activated form of the chemical carcinogen benzo(*a*)pyrene, benzo(*a*)pyrene-*trans*-7,8-dihydrodiol-9,10-epoxide (BPDE), in the normal fetal lung fibroblast strain LI58 as well as in three fibroblast strains with an increased sensitivity to DNA damage: GM5509 (xeroderma pigmentosum group A) (8), GM1389 (xeroderma pigmentosum variant) (3), and GM1915 (a familial hypercholesterolemia hyperlipoproteinemia strain defective for UVC- and BPDE-induced unscheduled DNA repair synthesis) (7). In addition, we studied the effects elicited in the simian virus 40 DNA-transformed human keratinocyte line SVK14 (16) by UVC, BPDE, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), and 7,12-dimethyl-benz(*a*)anthracene (DMBA). To test whether DNA damage-inducible proteins are under negative regulation, we also compared the inducible patterns of change elicited by DNA damage with the changes induced by single low doses of the protein synthesis inhibitor cycloheximide (CHX). Cell cultures were treated with single doses of each drug or modality and pulse-labeled with [^{35}S]methionine at intervals up to 24 h after exposure, and the radiolabeled proteins were resolved on standardized HR2D gels (20 by 20 cm) (4, 5). Sets of autoradiographic images from individual experiments were then quantitated by use of the QUEST system (5).

Initially, we examined the effects of UVC and CHX on human major histocompatibility class I (MHC-I) expression after exposure of quiescent, confluent cultures of LI58 cells. Shown in Fig. 1 are the regions containing MHC-I from the HR2D patterns of total cell lysates prepared from either control or treated cells. UVC (compare Fig. 1A with C and D) and CHX (compare Fig. 1A and B) both induced the synthesis of MHC-I within 4 h. Quantitative data on the behavior of the six marked MHC-I products are listed in Table 1, series A. Induction of MHC-I in resting cultures of nondividing LI58 cells further suggests that the increase in the rate of synthesis is not a secondary consequence of DNA damage or CHX-induced arrest of DNA synthesis.

The identities of the MHC-I products marked in Fig. 1 were determined by direct immunoprecipitation of MHC-I products from CHX-induced LI58 cells by the anti-framework MHC-I rabbit serum K-455 (12). The HR2D pattern of immunoprecipitated MHC-I products (Fig. 2A) was used to identify the corresponding MHC-I products detected in the total cell lysate pattern. To align the two patterns, we mixed the MHC-I immunoprecipitate directly with the total cell lysate prepared from the same cells and separated them by HR2D (Fig. 2B). The products marked in the total cell lysate (e.g., Fig. 1B) aligned directly with the MHC-I products immunoprecipitated by the K-455 serum. The positions of the three major CHX-inducible MHC-I products detected in the total cell lysate pattern are indicated in both Fig. 1B and Fig. 2B by the letter "c."

To further test whether MHC-I induction by DNA damage in human fibroblasts is dependent on specific DNA lesions, we examined the effects of both UVC and BPDE in exponentially growing cultures of four human fibroblast strains: LI58, GM5509, GM1389, and GM1915 (Table 1, series B). Both UVC and BPDE induced MHC-I expression in LI58 cells within 4 h, indicating that the triggering signal for induction is not restricted to DNA lesions induced by UVC damage. UVC treatment of exponentially growing LI58 cells induced higher levels of MHC-I induction than did UVC treatment of quiescent LI58 cells (compare series A and B in Table 1). The UDS-defective lines GM5509 and GM1915 and the UDS-competent, postreplication repair-defective line GM1389 exhibited highly altered patterns of MHC-I induction. In particular, higher average ratios of MHC-I induction in these strains (Table 1, series B) suggest a correlation between an increased sensitivity to DNA damage and the extent of MHC-I induction.

Finally, we examined patterns of MHC-I expression induced in the simian virus 40 DNA-transformed human keratinocyte line SVK14 (16) after exposure to several different DNA-damaging regimens (Table 1, series C). Al-

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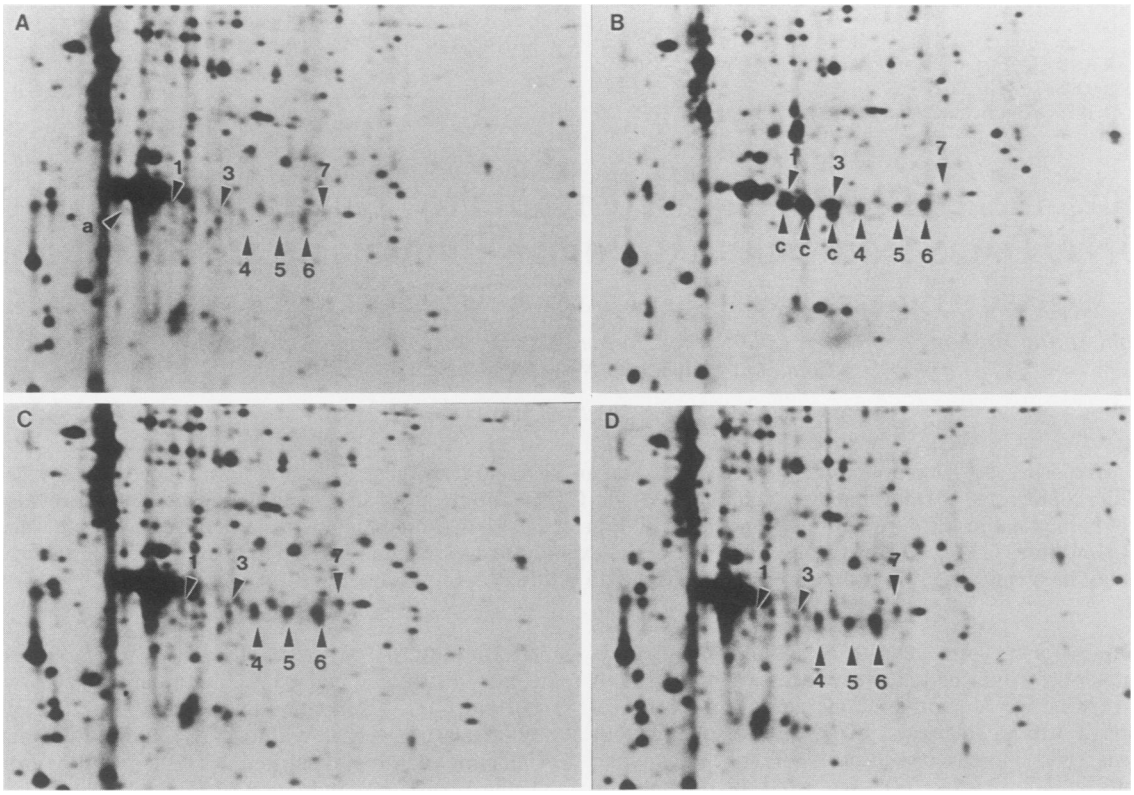

FIG. 1. Induction of MHC-I synthesis in quiescent LI58 human fibroblast cells. Each panel shows the region containing MHC-I from the HR2D pattern of total cell lysates prepared from [^{35}S]methionine-labeled confluent LI58 cells. Radiolabeled lysates were separated first on isoelectric focusing gels with a broad range of ampholytes (ph 3.5 to 10) and then on 10% acrylamide second-dimension gels. (A) Control cells plated and labeled in parallel with treated cells. (B) CHX (1 μg/ml)-treated cells labeled at 4 to 8 h after exposure. (C) UVC (1 J/m^2)-treated cells labeled at 4 to 8 h. (D) UVC (5 J/m^2)-treated cells labeled at 4 to 8 h. Cell labeling, sample preparation, and HR2D were carried out by previously published procedures (4, 5), The numbered arrowheads refer to the same numbered products designated in Fig. 2 and listed in Table 1, series A to C. a, Actin; c, three major CHX-induced polypeptides within the MHC-I series.

TABLE 1. Relative expression of MHC-I after DNA damage

Series	Cells	Regimen (dose)	Relative expression of MHC-I product[a]:						Avg ratio
			1	3	4	5	6	7	
A[b,c]	LI58	UVC (1.0 J/m^2)	1.23	2.04	3.75	2.67	1.93	1.55	2.19
		UVC (2.0 J/m^2)	1.84	2.86	3.93	3.89	2.89	2.44	2.97
		UVC (5.0 J/m^2)	1.39	2.36	4.59	3.50	2.80	2.18	2.80
		UVC (20.0 J/m^2)	1.30	2.40	5.77	3.31	3.67	1.81	3.04
		CHX (1.0 μg/ml)	19.32	67.40	3.94	3.92	2.21	1.52	16.38
B[c,d]	LI58	UVC (1.0 J/m^2)	1.44	19.74	3.90	3.64	3.12	8.73	6.76
		BPDE (0.1 μg/ml)	1.65	10.70	0.87	0.63	0.59	4.07	3.08
	GM5509	UVC (1.0 J/m^2)	1.43	35.00	9.56	10.50	4.50	10.49	11.91
		BPDE (0.1 μg/ml)	1.37	48.20	6.62	10.68	ND	4.86	14.34
	GM1389	UVC (1.0 J/m^2)	1.72	38.40	5.25	9.12	6.25	12.92	12.27
		BPDE (0.1 μg/ml)	1.73	42.60	4.18	8.88	5.56	14.55	12.91
	GM1915	UVC (1.0 J/m^2)	0.87	19.00	5.76	7.25	3.18	4.73	6.76
		BPDE (0.1 μg/ml)	0.96	7.55	2.41	7.56	3.62	3.59	4.28
C[c,d]	SVK14	UVC (3.0 J/m^2)	1.23	7.12	1.17	ND[e]	ND	1.17	2.67
		UVA (13.5 J/cm^2)	1.55	7.17	0.74	ND	ND	1.10	2.64
		BPDE (0.5 μg/ml)	1.81	1.53	0.85	ND	ND	1.60	1.44
		MNNG (5.0 μg/ml)	1.51	1.97	1.75	ND	ND	1.11	1.58
		DMBA (0.5 μg/ml)	1.21	1.15	1.14	ND	ND	1.20	1.17
		CHX (1.0 μg/ml)	65.72	25.54	1.87	ND	ND	0.76	23.47
		HU (5 mM)	0.68	0.32	1.02	ND	ND	1.17	0.79

[a] Expressed as the ratio of counts (parts per million loaded per gel) of individual polypeptides for treated versus nontreated control cells. The numbering of individual MHC-I products refers to designation used in Fig. 1 and 2.

[b] Quantitation of MHC-I products detected in total cell lysate patterns of quiescent cell cultures of LI58 cells.

[c] Exposures to UVC, UV light A (UVA), BPDE, CHX, and hydroxyurea (HU) were carried out as previously described (14). MNNG and DMBA were dissolved in dimethyl sulfoxide (final concentration, 0.1%) in the culture medium.

[d] Quantitation of MHC-I products detected in total cell lysate patterns of exponentially growing cell cultures.

[e] ND, Not detected in total cell lysates.

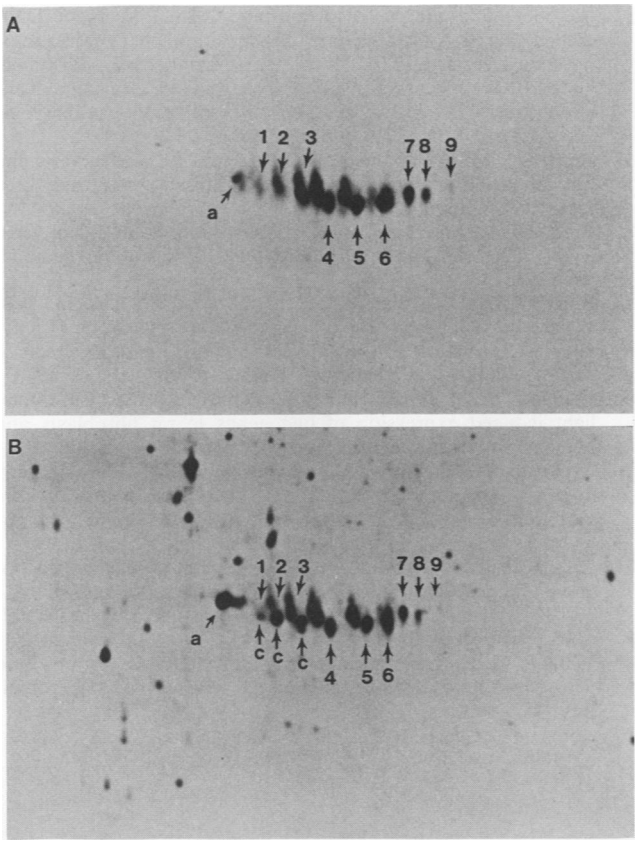

FIG. 2. Identification of MHC-I products detected in the total cell lysate pattern of [^{35}S]methionine-labeled LI58 cells. Each panel shows the same region of the HR2D pattern obtained from the following samples. (A) Immunoprecipitation by K-455 serum of MHC-I products from CHX (1.0 μg/ml)-treated LI58 cells labeled at 4 to 8 h after drug addition (12). (B) Alignment of the immunoprecipitate shown in panel A with the total cell lysate stock prepared from the same cells. The immunoprecipitate (10 μl) was mixed directly with 200,000 dpm of the stock sample, and the components were separated by HR2D. The numbered arrowheads indicate the positions of the major individual clusters of MHC-I products detected in the K-455 immunoprecipitate. a, Actin; c, major CHX-induced proteins detected in the total cell lysate pattern of LI58 cells (Fig. 1B). Cell labeling, immunoprecipitations, sample preparation, and HR2D were carried out by previously published procedures (4, 5).

though the overall number of K-455-reactive MHC-I products detected in total cell lysate patterns of this transformed cell type was lower than that for any of the primary fibroblast strains tested, all DNA-damaging regimens tested, as well as CHX, induced MHC-I. Induction of MHC-I was also observed after treatment with UV light A (345 to 440 nm), although the dose used was greater than 1,000 times that of UVC. The metabolic inhibitor hydorxyurea (5 mM) alone did not induce an overall increase in MHC-I expression, again indicating that arrest of DNA synthesis is insufficient for MHC-I induction. Time course studies (data not shown) demonstrated a persistence of these effects for 24 h after exposure.

Our data demonstrate the MHC-I induction is a part of the cellular stress response elicited in human fibroblasts and keratinocytes by several classes of DNA-damaging agents. The ability of CHX to induce MHC-I suggests that this induction may serve as a probe for the action of a stress response pathway in mammalian cells under negative regulatory control. Recent studies indicate that mouse *H-2*, *H-K*, and *H-D* mRNAs are transcriptionally induced by protein synthesis inhibitors, including CHX (R. Zeff and S. Nathenson, personal communication), and that RNA synthesis inhibitors can synergize with gamma interferon in the transcriptional induction of MHC-I in human K562 leukemia cells (2). These findings are consistent with the hypothesis that transcription of MHC-I genes may be regulated, in part, by labile transcriptional repressors. The precise molecular mechanisms, however, underlying DNA damage and CHX induction of MHC-I, the specific MHC-I alleles induced, and the possible effects on posttranslational modifications, have not been determined. CHX could act through differential inhibition of the synthesis of specific repressors or through increased stabilization of specific transcripts, although this mode of action may not be equivalent to that of DNA damage. Following DNA damage we have observed no significant decrease in overall cellular protein synthesis and do not believe that the overall inhibition of protein synthesis is sufficient to induce MHC-I. By analogy with *recA*-dependent cleavage of *lexA*-repressed *din* genes in *Escherichia coli* (18), damage of cellular DNA in eucaryotes could physically trigger derepression of a specific subset of cellular genes through the direct action of an activated *recA*-like molecule.

Previous studies in primary human fibroblasts and SVK14 cells indicated that UVC and CHX can induce several proteins in common, including the secreted factor EPIF (6) and c-*myc* (15). Recently, we reported that CHX, actinomycin D, and UVC all induced the increased replication of latent polyomavirus in semipermissive transformed rodent cells (14), an effect which is a marker for the action of a DNA damage-inducible *trans*-acting signal (10). The functional significance of inducible responses to DNA damage, however, is poorly understood. One possible consequence of DNA damage induction of MHC-I is the targeting of damaged cells for immune recognition and somatic cell selection. Studies indicating that immunogenic tumor variants, derived by treatment of nonimmunogenic, spontaneous murine tumors with MNNG, express elevated levels of MHC-I (1) support this hypothesis. In summary we have obtained evidence for the induction of MHC-I products in human fibroblasts and keratinocytes by several classes of DNA-damaging agents, including UVC, alkylating agents, and chemical carcinogens, suggesting a possible relationship between the DNA damage-monitoring systems operating in mammalian cells and components of the cellular immune system.

We thank J. D. Watson for support, R. DeMars for LI58 cells, B. Lane for SVK14 cells, D. Busbee for GM1915 cells, P. Peterson for K-455 serum, M. Mathews for comments on the manuscript, and C. Chang and P. Myers for technical assistance.

This work was supported by grants from the National Institutes of Health and the Cancer Research Institute to J.I.G.

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