Vol. 96, No. 1, 1980
September 16, 1980

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 370-376

POLYPEPTIDE COMPOSITION OF NUCLEAR ENVELOPE FOLLOWING THIOACETAMIDE-INDUCED NUCLEAR SWELLING

Gary A. Clawson, C. H. Woo, and Edward A. Smuckler *

Department of Pathology, University of California
School of Medicine, San Francisco, California 94143

Received July 28,1980

SUMMARY

Nuclear envelopes (NE)[1] were isolated from rat liver following thioacetamide-induced nuclear swelling. At 8 and 48 h intervals following thioacetamide treatment, periods which correspond to nuclear swelling phases, NE showed little change in polypeptide composition (although the polypeptide composition depended to some extent upon the isolation procedure used). Since the amount of NE protein increases by 30-40% and 70-80% at these times (respectively), the data suggest that concerted synthesis of a number of NE polypeptides occurred. We propose that triggered expansion of the NE may be an important first stage in the action of carcinogens.

INTRODUCTION

The nuclear envelope (NE) occupies a strategic position in cellular metabolism, regulating passage of macromolecules between nucleus and cytoplasm (1,2), although its role in selection of proteins for passage can be questioned. Morphologically, its singular structural features, including a double membrane and nuclear pores, make it unique among membranous systems. Recent biochemical studies have pointed to differences between NE and endoplasmic reticulum (ER). NE fatty acids behave independently from those of ER (3,4), enzymatic responses of NE are often differentiable from those of ER (3,5,6), and ER and NE protein kinases phosphorylate different polypeptides (7). Regarding functional aspects, many investigators have suggested a role for NE in the processing of RNA (8,9), and we have recently proposed that NE nucleoside triphosphatase activity, which somehow participates in nucleocytoplasmic RNA transport (10,11), may be involved in translocating RNA along the inner NE (or nuclear matrix elements) prior to export to the cytoplasm.

---

* To whom correspondence and requests for reprints should be addressed.

[1] Abbreviations used: NE, nuclear envelope; ER, endoplasmic reticulum; EDTA, ethylenediamminetetraacetic acid; TM buffer, 50 mM Tris-HCl (pH 7.6), 500 mM $MgCl_2$.

0006-291X/80/170370-07$01.00/0

The process of NE biogenesis may provide insight into its role in these processes. In this regard, rat liver following treatment with a low-dose (5 mg/100 g body weight) of the hepatocarcinogen thioacetamide provides an interesting system for studying NE formation (3). This treatment induces a biphasic nuclear swelling: At 8 and 18 h following treatment, nuclear surface area increases by 30-40%, and at 48 h it is increased by 70-80% (12). Additionally, the treatment is essentially non-toxic to animals, causing little cell death, division, or inflammatory response. To determine whether changes in NE polypeptide composition result from rapid NE expansion, we have examined rat liver NE following both thioacetamide-induced nuclear swelling phases.

MATERIALS AND METHODS

Male, Sprague-Dawley rats were used with body weights near 300 g. Thioacetamide-treated rats were given 5 mg thioacetamide/100 g body weight via stomach tube, and were sacrificed after various intervals.

Two populations of nuclei were isolated. Heavy nuclei were prepared by the method of Blobel and Potter (13). Light nuclei were isolated as previously described (3); briefly, light nuclei are the population of nuclei which entered a 1.8 M sucrose-buffer cushion but which did not sediment through 2.3 M sucrose-buffer, using the centrifugation conditions described (3,13).

Nuclear envelopes were prepared from purified nuclei using three different procedures. Method I was modified from the procedure of Harris and Milne (14). The modifications included; DNase I (RNase-free, Worthington Biochemical, Freehold, NJ) was added to 40 µg/ml, $MgCl_2$ was added to 0.1 mM, phenylmethylsulfonyl fluoride was added to 1 mM, and incubation was for 25 min at room temperature. A second digestion was performed for 10 min (with the same additions) but with DNase at 10 µg/ml. In method II, DNA was digested as in method I, and crude nuclear envelopes were then obtained by centrifugation at 17,500 rpm for 10 min at 4 C in an SS-34 rotor and Sorval centrifuge. The crude NE was rehomogenized in 20 mM Tris-EDTA (pH 7.6), and purified NE was isolated from discontinuous sucrose-buffer gradients (15). This method causes release of ribosomes from the nuclear surface. Method III was modified from that of Monneron (16). Nuclei from rat liver were resuspended by homogenization in 5 ml of 50% sucrose-TM buffer (50 mM Tris-HCl, pH 7.6; 500 mM $MgCl_2$). This suspension was overlayered with a 10 ml layer of 40% sucrose-TM buffer, and a linear 40-20% sucrose-TM buffer gradient, and purified NE was obtained from the gradients after centrifugation (16). This method effectively removes chromatin from the NE. NE isolated from the heavy nuclear population will be referred to as heavy NE, and light NE will refer to NE isolated from light nuclear populations.

NE polypeptides were examined on polyacrylamide slab gels as described by Laemmli (17). The gels were stained with coomassie brillant blue dye, and were destained by diffusion in 7% acetic acid. The gels were placed on slab gel backing sheets (Bio-Rad Laboratories, Richmond, CA) and air-dried. The dried gels were cut into 1-cm wide strips, and were scanned at 550 nm with a Gilford 240 spectrophotometer equipped with linear transport and servograph recorder. A filter of slit-width 50 µm was employed.

Chemical analyses were performed as previously described (10).

To examine the specificity of phosphorylation, NE isolated via method II was incubated with γ-^{32}P-ATP as described (18), proteins were separated by electrophoresis on 7% polyacrylamide gels, the gels were processed as described, and autoradiography was performed on dried gels using Kodak X-Omat-R x-ray film and exposure times of 4 to 14 days.

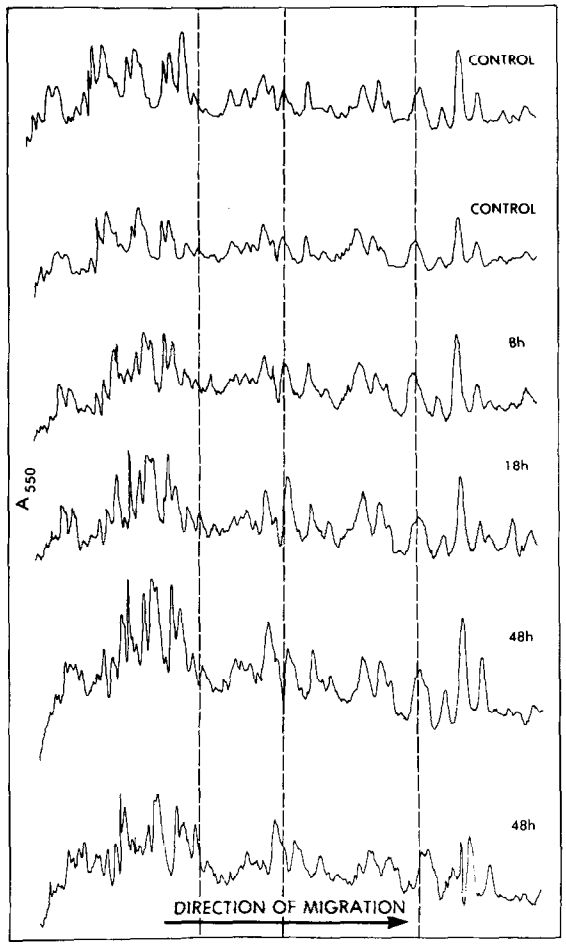

Figure 1. Densitometric scans of heavy NE polypeptides isolated from rat liver at intervals following thioacetamide treatment. Method I was used for isolation of NE, 7 μg of protein were applied to 10% polyacrylamide gels, and the gels were processed as described. The composition of heavy NE isolated via this procedure were (given as percent of dry weight of protein, phospholipid, RNA, and DNA, respectively): Control, 65.6, 21.1, 8.6, 4.7; 8 h, 66.1, 18.0, 11.4, and 4.5; 18 h, 60.2, 20.3, 14.7, and 4.8; 48 h, 60.0, 23.4, 13.2, and 3.4. Values are means of 2 or 3 determinations. The absorbance scale is 0.1 A_{550} units = 1/20 of the y-axis. Dashed lines, from left to right, represent the migration of phosphorylase B (94,000 daltons), bovine serum albumin (68,000 daltons), and ovalbumin (43,000 daltons). The bottom tracing is shifted slightly to the right.

RESULTS AND DISCUSSION

Nuclear envelopes were prepared from purified nuclei using three different methods, and their polypeptide compositions were examined using polyacrylamide gel electrophoresis (Figures 1-4). Although the polypeptide distributions of NE isolated by the various procedures showed some differences, 68,000-, 74,000-, and 78,000-dalton polypeptides were prominent in all preparations.

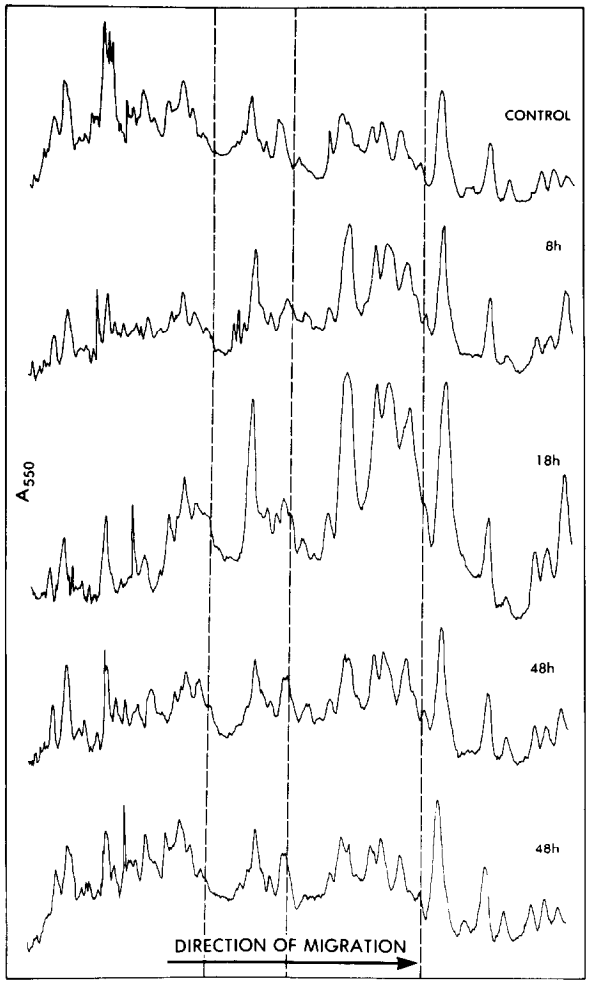

Figure 2. Densitometric scans of light NE polypeptides isolated from rat liver at intervals following thioacetamide treatment. Method I was used for isolation of NE, 7 µg of protein were applied to 10% polyacrylamide gels, and the gels were processed as described. The compositions of the light NE isolated via this procedure were (given as percent of dry weight of protein, phospholipid, RNA, and DNA, respectively): Control, 55.1, 30.8, 9.4, and 4.8; 8 h, 48.0, 38.9, 10.4, and 2.8; 18 h, 41.6, 36.2, 19.7, and 2.5; 48 h, 51.2, 33.7, 12.2, and 2.9. Values are means of 2 or 3 determinations (18 h is a single determination). The absorbance scale is 0.1 A_{550} units = 1/20 of the y-axis. Dashed lines denote the position of standards, as detailed in legend to Figure 1.

Also prominent were polypeptides of 57,000- and 54,000-dalton molecular weights.

Following thioacetamide treatment, there was little change in the overall polypeptide compositions of heavy NE. There was a proportional increase in the 57,000-dalton polypeptide in heavy NE isolated by all three methods. In heavy NE isolated via method I, there was a proportional increase in the 68,000-dalton polypeptide during the intial swelling phase (Figure 1), although at 48 h the polypeptide patterns were similar to those of control preparations.

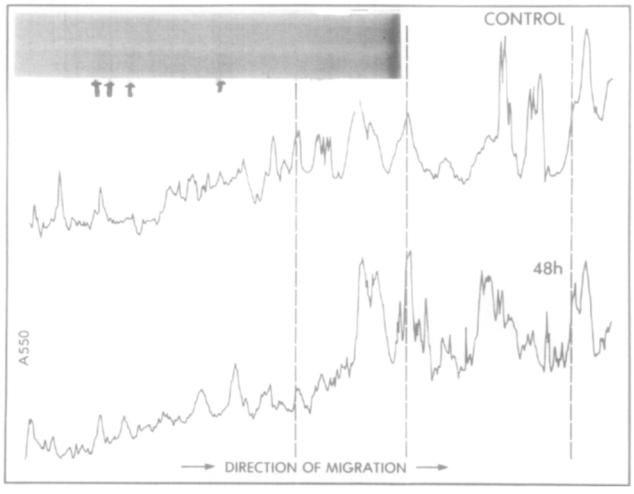

Figure 3. Densitometric scans of heavy NE polypeptides isolated from rat liver at intervals following thioacetamide treatment. Method II was used for isolation of NE, 7 µg of protein were applied to 7% polyacrylamide gels, and the gels were processed as described. The composition of NE isolated via this procedure was as previously reported (18). The absorbance scale is 0.1 A_{550} units = 1/8 of the y-axis. Dashed lines denote the position of standards, as detailed in the legend to Figure 1. Inset shows autoradiograms of ^{32}P-labeled polypeptides following phosphorylation experiments. Control and 48-h treated samples are shown, and arrows denote position of 3 major bands and lesser 68,000-dalton band.

With heavy NE isolated with method III, there was a proportional increase in the 54,000-dalton polypeptide (Figure 4). Some differences were observed in minor polypeptides of the NE preparations. For instance at 48 h after treatment, we observed a decrease in a minor protein with a molecular weight of about 48,500 daltons; this decrease may reflect a decrease of cytochrome P-450_{LM2}, previously identified as having a molecular weight of 48,700 daltons (19), since a 50% decrease of NE cytochrome P-450 occurs at 48 h following this treatment (3). Although this decrease was not evident in NE prepared via method III, we have reported that this method is not suitable for P-450 content analysis (20).

We also examined the specificity of phosphorylation of NE polypeptides using envelopes prepared via method II. NE suspensions were incubated with γ-^{32}P-ATP as described (18), and autoradiography was performed after polyacrylamide gel electrophoresis. Autoradiographs demonstrated a selective phosphorylation of 3 polypeptides, of approximate molecular weights 127,000, 120,000, and 112,000 daltons, with the 112,000-dalton band characteristically the heaviest (3 preparations; see inset to Figure 3). There was also a lesser phosphorylation of the 68,000-dalton polypeptide. This pattern was also found when NE isolated at 48 h following treatment were examined (also 3 preparations; see inset to Figure 3). Thus, although NE fractions enriched in the pore-complex

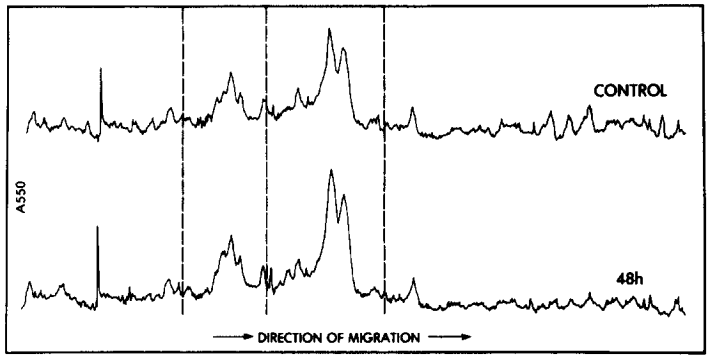

Figure 4. Densitometric scans of heavy NE polypeptides isolated from rat liver at intervals following thioacetamide treatment. Method III was used for isolation of NE, 7 µg of protein were applied to 10% polyacrylamide gels, and the gels were processed as described. The composition of NE isolated via this procedure was as previously reported (20). The absorbance scale is 0.1 A_{550} units = 1/8 of the y-axis. Dashed lines denote the position of standards, as detailed in legend to Figure 1.

lamina selectively phosphorylate the 68,000-dalton protein (7), it appears that other polypeptides may be preferentially phosphorylated when they are present in NE preparations.

We also employed method I to isolate light NE (Figure 2). We found a relative increase in the 57,000-dalton protein at 8 and 18 h following treatment, a relative increase in the 78,000-dalton protein, and a relative decrease in the 68,000-dalton polypeptide. These changes may relate to the first nuclear swelling phase (3), reflecting the significant shift of heavy nuclei to the light nuclear fraction at these times. The light NE polypeptides at 8, 18, and 48 h after treatment are quite similar to those of heavy NE, with little change in light NE polypeptides observed at 48 h, the time corresponding to the second nuclear swelling phase.

Since there are average increases of 30-40% and 70-80% in NE surface area (and protein) at 8 and 48 h (respectively) after thioacetamide treatment (3), the data suggest that the concerted synthesis of a number of NE polypeptides has taken place.

The initial nuclear swelling phase may be an integral phenomenon in the process of carcinogenesis. We note that carcinogens in general induce nuclear swelling (21). Triggered expansion of the NE would effectively disperse chromatin, possibly altering transcriptional controls, and may also facilitate interaction of carcinogenic (such as alkylating) agents with DNA. The altered transport of nuclear RNA observed after carcinogen treatment (22,23) may also reflect nuclear swelling, since we have previously noted that factors which induce nuclear swelling increase RNA transport in vitro (24). Further-

more, increased nuclear envelope surface area following nuclear swelling correlates with increased NE nucleoside triphosphatase activity (10), which appears to function in RNA transport.

ACKNOWLEDGEMENTS

This work was supported by USPHS grants CA21141 and AM19843

REFERENCES

1. Paine, P., Moore, L., and Horowitz, S. Nature 254, 109-114 (1975).
2. Feldherr, C., and Pomerantz, J. J. Cell Biol. 78, 168-175 (1978).
3. Clawson, G., Moody, D., James, J., and Smuckler, E. Cancer Res., in press.
4. Baker, R., and Chang, H. Biochem. J. 188, 153-161 (1980).
5. Fahl, W., and Jefcoate, C., and Kasper, C. J. Biol. Chem. 253, 3106-3113 (1978)
6. Bornstein, W., Levin, W., Thomas, P., Ryan, D., and Bresnick, E. Arch. Biochem. Biophys. 197, 436-446 (1980).
7. Lam, K., and Kasper, C. Biochemistry 18, 307-311 (1979).
8. Miller, T., Huang, C., and Pogo, O. J. Cell Biol. 76, 675-691 (1978).
9. Herlan, G., Eckert, W., Kaffenberger, W., and Wunderlich, F. Biochemistry 18, 1782-1788 (1979).
10. Clawson, G., James, J., Woo, C., Friend, D., Moody, D., and Smuckler, E. Biochemistry 19, 2748-2756 (1980).
11. Agutter, P., McCaldin, B., and McArdle, H. Biochem. J. 182, 811-819 (1979).
12. Olason, D., and Smuckler, E. Arch. Path. Lab. Med. 100, 415-418 (1976).
13. Blobel, G., and Potter, V. Science (Washington, DC) 154, 1662-1665 (1966).
14. Harris, J., and Milne, J. Biochem. Soc. Trans. 2, 1251-1253 (1974).
15. Steer, R., Wilson, M., and Ahmed, K. Exptl. Cell Res. 119, 403-406 (1979).
16. Monneron, A. Philos. Trans. R. Soc. London B. Biol. Sci. 268, 101-108 (1974).
17. Laemmli, U. Nature (London) 227, 680-685 (1970).
18. Clawson, G., Woo, C., and Smuckler, E. Biochem. Biophys. Res. Commun., in press.
19. Haugen, D., and Coon, M. J. Biol. Chem. 251, 7929-7939 (1976).
20. Clawson, G., Koplitz, M., Moody, D., and Smuckler, E. Cancer Res. 40, 75-79 (1980).
21. Agrelo, C. Toxicology 9, 21-27 (1978).
22. Shearer, R., and Smuckler, E. Cancer Res. 32, 339-342 (1972).
23. Shearer, R. Biochemistry 13, 1764-1767 (1974).
24. Clawson, G., Koplitz, M., Castler-Schechter, B., and Smuckler, E. Biochemistry 17, 3747-3752 (1978).