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# THE EFFECT OF HYDROCORTISONE TREATMENT ON THE IN VIVO PHOSPHORYLATION OF A SUBGROUP OF NON-HISTONE NUCLEAR PROTEINS IN THE MOUSE LYMPHOSARCOMA P1798

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## Summary

The in vivo phosphorylation of a small subgroup of non-histone nuclear proteins, similar to the high mobility group (HMG) proteins described by Goodwin and Johns (Methods in Cell Biology (1977) Vol. XVI, eds. G. Stein, J. Stein and L. J. Kleinsmith, pp. 257-267, Academic Press, New York), was studied in the P1798 mouse lymphosarcoma. A single injection of cortisol, resulting in a 50% reduction in tumor mass of the sensitive strain of this tumor, caused a marked suppression in phosphorylation of at least three of these proteins, independent of any apparent change in protein concentration. No such change was observed in the cortisol-resistant strain, and it is suggested that the effect of cortisol in causing regression of this tumor might be mediated via changes in phosphorylation of specific nuclear proteins.

## Introduction

Goodwin and Johns (1) have reported the isolation of a small number of nuclear proteins which are extractable into 0.35 M NaCl and which remain in solution when the 0.35 M NaCl extract is made 2% (w/v) with respect to trichloroacetic acid. These proteins, which were originally isolated from calf thymus nuclei, have been designated HMG (high mobility group) proteins based on their electrophoretic mobilities in 20% polyacrylamide gels, and their possible role as structural proteins in chromatin has been postulated (1). More recently, Javaherian et al (2) have shown that the $HMG_1$ and $HMG_2$ proteins of this group are able to alter the configuration of the DNA double helix and have suggested that this modification might affect transcription. This paper demonstrates that in the P1798 lymphosarcoma regressing after a single injection of cortisol, there is a marked suppression of phosphorylation of a small subgroup of nuclear non-histone proteins isolated in the same manner as the HMG proteins described by Goodwin and Johns. While others have identified the presence of HMG proteins in a variety of tissues and species (3-7), to our knowledge this is the first report dealing with their in vivo phosphorylation. However, Kleinsmith (8) has presented convincing evidence for the phosphorylation of other nuclear proteins and suggests that changes in phosphorylation may be related to control of cell proliferation.

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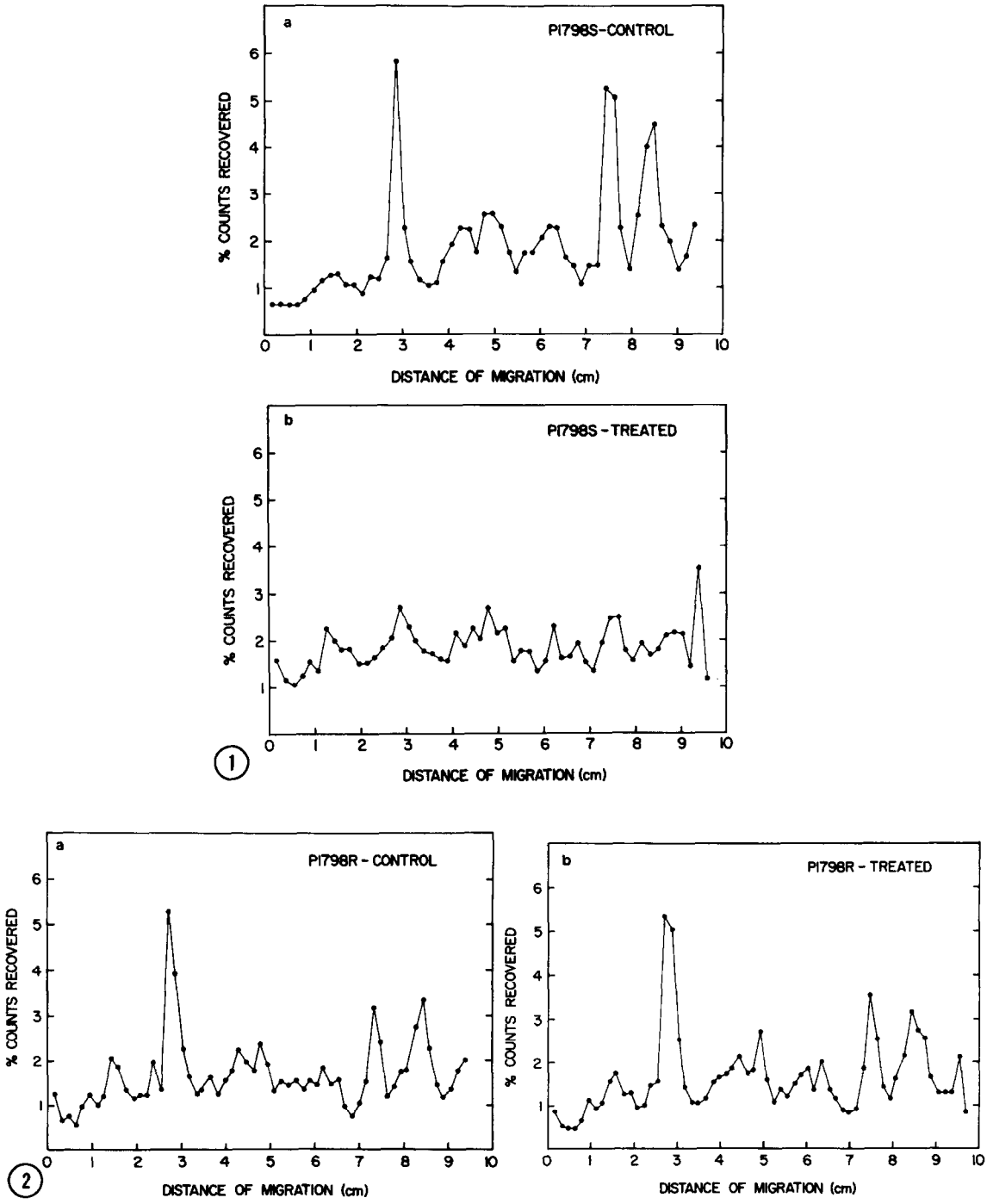


Figure 1. Phosphorylation of HMG proteins isolated from the (A) untreated control and (B) cortisol-treated sensitive strain of the P1798 lymphosarcoma. 100 $\mu$g of the isolated protein was subjected to SDS - 10% polyacrylamide gel electrophoresis and the gels sliced and counted. Data are presented

## Methods

Mice bearing the 18-day old cortisol-sensitive or -resistant lines of the mouse lymphosarcoma P1798 were each injected intraperitoneally with 7.5 mCi of [$^{32}$P] dipotassium phosphate in 0.5 ml of 0.9% NaCl solution one hour prior to sacrifice. When phosphorylation was studied after cortisol treatment, mice were injected subcutaneously with a single dose of hydrocortisone acetate (50 mg/kg) 24 hours before sacrifice; this treatment produced a 50% reduction in the weight of the sensitive tumor, but had no effect on the resistant tumor. Mice were killed by cervical dislocation, the tumors rapidly removed, weighed and homogenized in 4 volumes of ice-cold 0.25 M sucrose - 0.003 M calcium acetate solution. All subsequent steps were carried out at 0-4°. A purified nuclear preparation was obtained essentially according to Chauveau et al (9). The HMG proteins were extracted from the nuclear pellet by the method of Goodwin and Johns (1) and finally analyzed on SDS - 10% polyacrylamide gels according to Laemmli (10). Freshly prepared phenylmethylsulfonylfluoride (50 mM in isopropanol) was added to all the isolation buffers at a final concentration of 0.5 mM.

The gels were stained in 0.05% Coomassie Blue and destained electrophoretically prior to scanning at 600 nm in a Gilford recording spectrophotometer. To determine the distribution of radioactivity, the gels were cut into slices of approximately 1.7 mm and counted in 5 ml ACS scintillant using a Beckman LS-100 C scintillation spectrometer. Protein in the original sample was determined by the method of Lowry et al (11).

## Results

As can be seen from Figures 1A and 2A, phosphorylation of several HMG proteins occurs in the growing P1798 lymphosarcoma. In contrast, the phosphorylation of at least three of these proteins, corresponding to areas of the gel at 2.8, 7.6 and 8.5 cm, is markedly suppressed in tumors regressing after a single injection of cortisol (Figure 1B). In addition, we have consistently observed a slight enhancement ($\sim$75%) in the phosphorylation of a protein in the 1.2 - 1.5 cm region of the gel. These changes occurred without any obvious change in the protein pattern as observed from the densitometric tracing (data not shown). Importantly, no such changes in phosphorylation were noted in the resistant tumor treated with cortisol (Figure 2B). We feel this is significant inasmuch as the phosphorylation profile is essentially identical in the untreated controls of both the sensitive and resistant tumors.

By standardization of the gel system with known molecular weight protein markers, it was determined that the three peaks whose phosphorylation was suppressed by cortisol treatment corresponded to proteins of molecular weight 64000, 21000 and

as the % counts recovered from the gel. Specific radioactivity was 157,000 and 71,000 cpm/mg protein for control and treated, respectively.

Figure 2. Phosphorylation of HMG proteins isolated from the (A) untreated control and (B) cortisol-treated resistant strain of the P1798 lymphosarcoma. Details are as in Figure 1. Specific radioactivity was 244,000 and 301,000 cpm/mg protein for control and treated, respectively.

17000, respectively. The very slow moving protein whose phosphorylation was increased upon cortisol treatment, has a molecular weight of roughly 90000. That none of these peaks corresponded to histone $H_1$, a possible contaminant of this system, was established by isolating $H_1$ from the tumor (12, 13), and electrophoresing it in parallel with the HMG preparation.

## Discussion

There is considerable evidence supporting a role for non-histone nuclear proteins in regulating transcription in eukaryotic cells (8), although the exact mechanism whereby this is accomplished is still unclear. However, it is known that alterations in protein - DNA binding do occur as a result of changes in the extent of acetylation, phosphorylation, or methylation of these regulatory proteins (14). This study has demonstrated a striking decrease in the phosphorylation of a specific group of non-histone nuclear proteins, the so-called HMG proteins, in the P1798 lymphosarcoma regressing after cortisol treatment and it is tempting to relate impaired phosphorylation of certain of these proteins with inhibition of tumor growth. No such inhibition of phosphorylation was noted in the cortisol-resistant strain of this tumor (Figure 2B).

Cho-Chung et al (15) have reported that when nuclei from regressing DMBA-induced mammary tumors are incubated with ATP, the phosphorylation of a specific group of basic proteins is increased when compared to growing tumors. Although the isolation procedure we have used here is entirely different than the histone-type procedure reported by Cho-Chung et al (15), it is possible that the slow-moving peak whose phosphorylation is enhanced after treatment of the cortisol-sensitive tumor is related to the "regression-associated protein" described by these workers. The three other proteins whose phosphorylation is suppressed by cortisol appear to be unique, and current work is aimed at determining if they play a direct role in the mediation of cortisol-induced regression. Alternatively, it is possible that the effect we have observed is not due to cortisol *per se*, but rather, is a general phenomenon associated with inhibition of cell growth; this possibility is also under active investigation. We have not addressed ourselves to the problem of how this suppression of phosphorylation seen after cortisol treatment occurs; however, several potential explanations exist: (1) changes in the concentration or activity of the specific kinase(s) responsible for phosphorylation of these proteins; (2) changes in phosphatase activity; (3) changes in ATP pool sizes which might differentially affect particular kinases depending on their respective $K_m$ values. Although determination of the exact mechanism would be of considerable interest, we have no particular insight at this time as to which of the above possibilities is most likely.

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