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Phosphorylation of Histones in Cells Treated with Hypertonic and Acidic Media

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Factors in the extracellular environment, specifically hypertonic or acidic growth media, are shown to alter the modification of histones in several cell lines. For histone 2A, changes in modification were visible in the mass pattern and were found to be primarily changes in phosphorylation. The increased modification of the core histones was quickly reversed when cells were returned to normal medium.

In the cell nucleus, posttranscriptional modifications of histones by phosphorylation, acetylation, methylation, ubiquitination, and poly(ADP) ribosylation, and perhaps by other means as well, may affect various activities such as transcription, replication and chromosome condensation (2, 3).

During studies on the quantitation of H2A phosphorylation and acetylation (5), it was found that certain environmental factors, namely, hypertonic and low-pH media, led to substantial changes in the extent of H2A modification, whereas no changes were visible by mass shifts in the other histones. In this paper, we show that most, if not all, of the increase in modification is phosphorylation, describe the factors that cause the increase, and suggest that the phenomenon may be physiologically significant.

Induction of histone 2A modification. During the course of experiments on histone acetylation and phosphorylation, we noticed that incubation of cells in certain media led to noticeable changes in the ratio of the unmodified (b_0) and modified (b_1) forms of H2A. Further investigation revealed that two different environmental factors, ionic strength and pH, could lead to an increase of H2A modification as judged by mass shifts on stained gels. We were not able to observe any mass shifts in other histones.

Figure 1 shows the extent of this change. Typically, b_1 H2A accounts for about 25 to 35% of total H2A in L1210 in exponential growth. A 2-h treatment with 100 mM KCl added to regular growth medium increased the percentage of modified H2A to 75%. Likewise a 2-h treatment with medium adjusted to pH 5.5 also led to an increase in modified H2A to 74%. Intermediate values of KCl or pH gave intermediate values of modification.

Other electrolytes were tested for their ability to increase H2A modification. The effectiveness of the salts tested was found to be in the order KCl = NaCl > $LiSO_4$ > $MgSO_4$ > $CaCl_2$. These results suggested that increased H2A modification could be a reaction to hyperosmotic media. To test this, cells were incubated with nonionic molecules of various molecular weights. Nonelectrolytes larger than 150 daltons led to increased H2A modification whereas smaller ones did not (Fig. 2). The smaller ones included ethylene glycol, dimethyl sulfoxide, and glycerol, which are commonly used as cryoprotective agents and, hence, rapidly penetrate cells. The larger nonelectrolytes, such as sorbitol, increased H2A modification as well as did the electrolytes.

The changes in H2A modification induced by tonicity or pH were rapid, with most of the change occurring within 15 min but with further increases for up to 2 h. The change was then gradually reversed in both cases, probably as cells lost viability from the extended treatment. The increased modification of H2A induced by either method was rapidly reversed when cells were returned to normal media; within 15 min, the b_1 H2A had returned to about 25%.

Addition of fresh serum, fetal or normal, as well as depletion of various amino acids from the medium did not increase modification of H2As. Also, the addition of mellitin, a 26-amino-acid polypeptide which dramatically increases Na entry into quiescent 3T3 cells (7, 8), of 12-*O*-tetradecanoylphorbol-13-acetate, or of ouabain, which interferes with the Na-K pump (4), failed to induce modification of H2As.

Induction of histone modification in other mammalian cells. After we observed that hyperosmotic media could induce modification in H2A of L1210 cells, we investigated some mammalian cells of human or mouse origin to study the generality of the phenomenon. The human leukemic cells, HL60, K562, and U937, as well as the mouse erythroleukemic cells, C19, were subjected to treatment with 100 mM KCl or 300 mM sorbitol for 1 h. Histone gels of control and treated cells clearly showed that treatment with KCl resulted in conversion of unmodified form b_0 to modified form b_1. The extent of the modification induced by KCl varied in the various cell lines tested. The human line HL60 showed the least relative increase in modification, perhaps because H2A.1 of the untreated cells is already more extensively modified than those of the other cell lines (unpublished data). H2A.1 of the human line U937 exhibited the most extensive conversion of b_0 to b_1. These results indicate that induction of H2A modification by hyperosmotic media is a general phenomenon.

Identification of modification as phosphorylation. Because of the rather specific mass effect on histone 2A, the identity of the H2A modification was suspected to be phosphorylation since acetylation would be expected to affect the mass pattern of all core histones (3, 5). To verify this, however, L1210 cells were treated with 100 μg of cycloheximide per ml for 10 min to inhibit protein synthesis before addition of KCl and [^{14}C]acetate or KCl and $^{33}PO_4$ to the culture.

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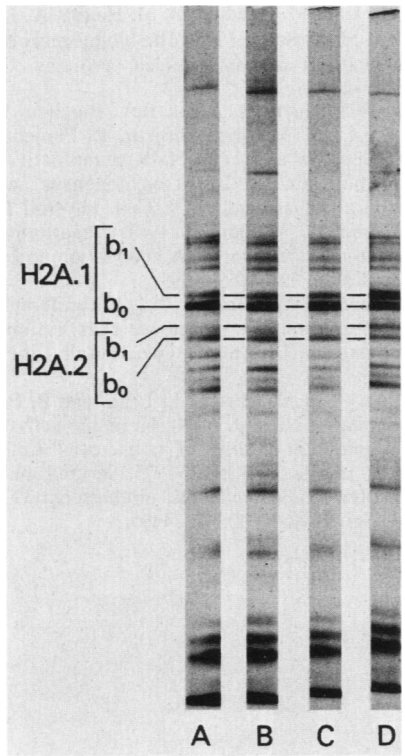

FIG. 1. Effect of KCl concentration on H2A modification. KCl (3 M) was added to cultures of L1210 cells in log phase to final concentrations of 30 mM (gel B), 100 mM (gel C), or 200 mM (gel D). Gel A is the untreated control. After 2 h at 37°C, the cells were pelleted and nuclei were prepared, washed with 330 mM NaCl, and extracted with HCl. Histones were electrophoresed as described elsewhere (1).

Although there was a large conversion from b_0 to b_1 H2A in stained gels, fluorographs of the same gels did not show incorporation of [^{14}C]acetate. In contrast, when the cells were treated with KCl and $^{33}PO_4$, the modified forms of the H2As, except the Z variant, exhibited increased incorporation of $^{33}PO_4$. In addition, the fluorographs revealed $^{33}PO_4$ incorporation in the modified forms of H3 and H4, although no mass changes could be detected in stained gels. Because H4 and H3 are primarily acetylated and are phosphorylated to less than 1% in the control state, increases in phosphorylation of these histones would be hidden in the mass of the acetylated forms. Since the phosphorylated form accounts for about 33% of the modified (b_1 + b_2) H2A and, therefore, 7 to 10% of the total H2A in control cells, a two- to threefold increase in phosphorylated H2A would be visible as an increase in the relative amount even in the presence of acetylated H2A (6).

Although both KCl treatment and low pH caused $^{33}PO_4$ incorporation to increase at least severalfold into most of the phosphorylated proteins on this gel, the one major exception was histone 1. The low pH greatly increased the $^{33}PO_4$ incorporation of histone 1, but KCl treatment led to a slight increase. So even though the effects of these two treatments on histone phosphorylation were similar, they were not identical. In addition, there could be differences with other proteins not separated on these gels.

Possible physiological significance. Does this increased phosphorylation of histones and possibly other proteins have any physiological significance? In this regard, it is interesting that when histones were extracted directly from mouse liver, spleen, and kidneys (1), we observed that although both liver and spleen had considerably more b_0 than b_1 H2A.1, in kidneys, b_1 H2A.1 was substantially increased relative to b_0. Since kidneys are the organs involved in the salt and osmotic balances of the body and are involved in removing excess materials, it was notable that kidney H2A showed that same

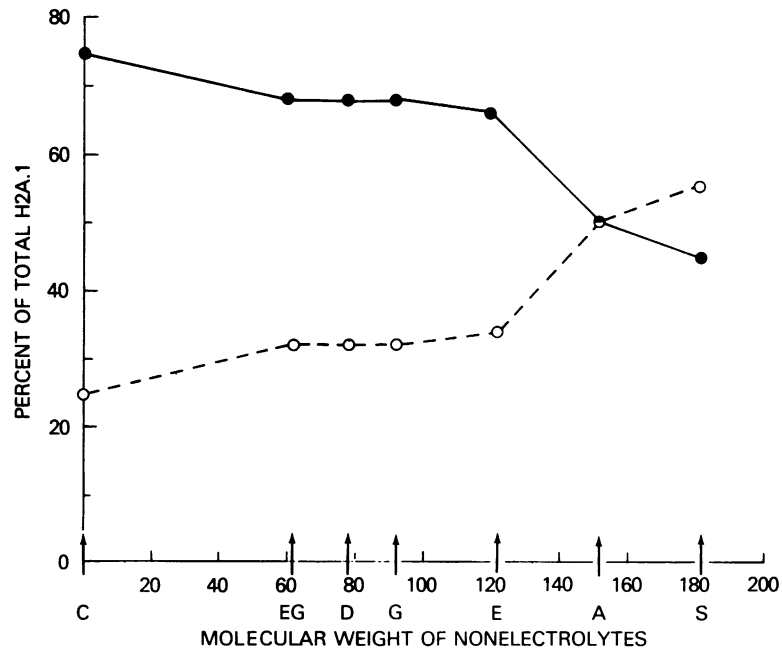

FIG. 2. Effect of nonelectrolytes on H2A modification. L1210 cells were prelabeled overnight with [^{3}H]lysine and then treated with medium containing the indicated compound at 300 mM for 1 h at 37°C. Histones were prepared and analyzed (1). The b_0, b_1, and b_2 forms of H2A.1 were quantitated as described elsewhere (5). C, Control; EG, ethylene glycol (M_w = 62); D, dimethyl sulfoxide (M_w = 78); G, glycerin (M_w = 92); E, erythritol (M_w = 122); A, adonitol (M_w = 152); and S, sorbitol (M_w = 182). Closed circles, b_0 form; open circles, b_1 + b_2 forms.

increased modification as did tissue culture cells treated with hypertonic or low-pH conditions.

To maximize the effects, these phenomena were studied with pH's or tonicities near the edge of cell tolerance. However, significant changes in phosphorylation were induced by less drastic conditions. Each 0.5-pH-unit decrease in the growth medium, down to pH 6.0, induced a 10-percentage-point increase in the fraction of modified H2A. This result suggests that there could be significant changes in histone phosphorylation during the routine growth of cells in culture and further suggests that this phenomenon may be physiologically relevant.

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