Changes in the methylation pattern of core histones during heat-shock in *Drosophila* cells

R.Camato and R.M.Tanguay*

Génétique Moléculaire et Humaine, Centre Hospitalier de l'Université Laval and Département de Médecine, Université Laval, Ste Foy, P.Q., Canada G1V 4G2

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The post-translational methylation of histones in the presence of inhibitors of protein synthesis, was studied during a heat-shock to *Drosophila* cells in culture. In control cells (23°C), both histones H3 and H4 are methylated. After heat-shock (37°C), there is a dramatic reduction in the methylation of H3 and an increase in the methylation of another core histone identified as H2B. These changes in the pattern of methylation vary with the temperature of the heat-shock. The increased methylation of H2B is also observed in arsenite-treated cells but the methylation of H3 is unchanged, being similar to that observed in control cells. Inhibition of synthesis of heat-shock proteins has no effect on the methylation changes, suggesting that heat-shock proteins are not directly involved in the methylation reaction. These changes could be involved in the extensive transcriptional regulation occurring in these cells during heat-shock.

Key words: heat-shock/histone/methylation/arsenite/*Drosophila* cells

Introduction

The heat-shock response first reported in *Drosophila* (Ritossa, 1962) is characterized by extensive regulation of gene expression operating both at the transcriptional and translational levels (for review, see Ashburner and Bonner, 1979). In salivary glands, a brief heat-shock induces the rapid appearance of a limited number of new puffs at the heat-shock loci and a concomitant regression of most of the puffs active prior to the shock (Ashburner, 1970; Tissières *et al.*, 1974). At the molecular level, the heat-shock induces a rapid and dramatic increase in the rate of transcription of heat-shock (HS) genes and a parallel decrease in the transcription of previously active genes (Findley and Pederson, 1981). Changes at the chromatin level are also noted as shown by the increased sensitivity of HS genes to DNase I in nuclei of heat-treated cells (Wu *et al.*, 1979) as well as by the accumulation of RNA polymerase II in HS loci (Plagens *et al.*, 1976) and its depletion from the inactivated loci (Jamrich *et al.*, 1977). Little is known, however, about the mechanism(s) of induction of the heat-shock response or about the molecular changes at the chromatin level responsible for the coordinated activation of HS genes and repression of previously active genes.

Post-translational modifications of histones have been previously correlated either with the active or inactive state of chromatin (Allfrey, 1977; for review, see Isenberg, 1979). In *Tetrahymena*, heat-shock or deciliation lead to an increased phosphorylation of histone H1 (Glover *et al.*, 1981). Here we report important changes in the pattern of methylation of

some of the core histones upon heat-shock or arsenite treatment.

Results

Methylation pattern of histones upon heat-shock

When Kc cells are heat-shocked at 37°C, we observe drastic changes in the methylation pattern of two core histones, H2B and H3. In control (23°C) nuclei, both H3 and H4 are methylated (Figure 1B, lane 1). In contrast, heat-shocked cells show a decreased methylation of H3 and an increase in the methylation of a band co-migrating with histone H2B (Figure 1B, lane 1). The methylation pattern of H4 is not altered. To confirm the identity of the hypermethylated band co-migrating with H2B, total histones were acid-extracted from nuclei. As shown in Figure 1B, lane 2, the methylation pat-

Fig. 1. SDS-polyacrylamide gel electrophoresis (12%) analysis of L-[methyl-³H]methionine-labeled proteins. (A) Coomassie blue stained gel of nuclear proteins (lane 1) or acid extracted histones (lane 2) at 23°C or 37°C. (B) Corresponding fluorogram. The equivalent of 3 x 10⁶ cells were loaded in each lane. Cycloheximide and chloramphenicol were present to prevent labeling due to protein synthesis. (Exposure 1 week).

*To whom reprint requests should be sent.

Fig. 2. Comparative methylation of histones in control (lane 1), arsenite-treated (lane 2), or heat-shocked cells (lane 3). (A) Coomassie-blue stained gel and (B) corresponding fluorogram. In the heat-shock sample (lane 3), protein synthesis inhibitors were added prior to the heat-shock to prevent the synthesis of heat-shock-induced proteins. In lanes 1 and 2, the inhibitors were added 1 h after treatment and cells were labelled as described in Materials and methods. Histones from 5×10^6 cells were loaded in each lane (Exposure 3 days).

tern of acid-extracted histones is identical to that of total nuclear protein suggesting that the hypermethylated band is indeed histone H2B.

Methylation pattern of histones after arsenite treatment

A heat-shock mimicking response can be elicited by a variety of agents interfering with cellular functions (reviewed in Ashburner and Bonner, 1979). It was therefore of interest to study the methylation of histones after an arsenite treatment known to induce the synthesis of some of the heat-shock proteins (Johnston *et al.*, 1980; Vincent and Tanguay, 1982). Under these conditions, we observe an increased methylation of H2B comparable to that noted in heat-shocked cells (Figure 2B, lane 2). However, the decreased methylation of H3 observed upon heat-shock is not seen in arsenite-treated cells.

Variation in histone methylation patterns with increasing temperatures

We have previously shown the heat-dependent translocation to the nuclear fraction of cellular cytoskeletal proteins and heat-shock proteins in these cells (Tanguay and Vincent, 1982; Vincent and Tanguay, 1982). Studies at various temperatures from 27°C to 39°C also show the heat dependency of the methylation patterns of H2B and H3 (Figure 3). The lowest temperature which induced an increase in the methylation of H2B is 33°C (Figure 3B). On the other hand, the significant decrease in the methylation of H3 occurs at 35°C (Figure 3B). The most pronounced overall changes in the methylation patterns are observed at 37°C (Figure 3).

Pre-existing histone H2B is methylated

The synthesis of histone H2B is activated by heat-shock in Kc cells (Sanders, 1981). This increased synthesis of one of the core histones is regulated at the transcriptional level (Tanguay *et al.*, in preparation). To find out if pre-existing histone H2B was methylated upon heat shock, cells were heat-treated in the presence of inhibitors of protein synthesis to prevent the heat-shock-induced synthesis of H2B. As shown in Figure 2 lane 3, the methylation pattern of histones under these conditions is similar to that observed in cells where the synthesis of new H2B was not prevented (Figures 1 and 3) indicating that pre-existing histones are subject to the methylation changes. It is not known if newly induced H2B is methylated during heat-shock.

Discussion

A non-coordinated transcriptionally regulated expression of histone genes has been previously shown to occur during heat-shock in *Drosophila* Kc cells (Sanders, 1981; Tanguay *et al.*, in preparation). The rate of synthesis of H2B is increased while that of the other core histones is decreased. As reported here, this altered expression of the histone genes is accompanied by extensive changes in the pattern of methylation of some of the core histones: the methylation of H2B increases while that of H3 decreases. The increased methylation of H2B starting at 33°C (Figure 3) coincides with a similar increase in the rate of synthesis of H2B which also starts at 33°C and is highest at 37°C (Tanguay *et al.*, in preparation). However, there does not seem to be a direct link between the increased synthesis of H2B and its increased methylation: firstly, the increased methylation of H2B is seen during an arsenite treatment (Figure 2) which does not induce the increased synthesis of H2B observed during heat-shock (Tanguay *et al.*, in preparation). Secondly, the changes in the methylation pattern of histones are also observed under conditions where a new synthesis of histones is prevented (Figure 2 lane 3). Of course, the possibility that newly synthesized H2B induced by heat-shock is also methylated cannot be excluded from the data of these experiments. Furthermore, the fact that the heat-induced changes in the methylation patterns of histones are observed even under conditions where synthesis of heat-shock proteins is prevented (by adding the inhibitors prior to the heat-shock) indicates that the heat-shock proteins are not directly involved in the methylation reaction. This suggests that the observed changes are not a consequence of the expression of HS genes but rather could be linked to events occurring in the early phases of the heat-shock response. This is consistent with preliminary data (not shown) demonstrating that the changes in the pattern of histone methylation are observed within 10–15 min after the initial

Fig. 3. Temperature dependency of histone methylation. (A) Coomassie blue stained gel of nuclear proteins and (B) corresponding fluorogram. The experimental procedure is the same as in Figure 1. 5×10^6 nuclei were loaded in each lane. (Exposure 3 days).

tion of the heat-shock.

Finally, the changed methylation pattern observed in the absence of new protein synthesis also indicates that the reaction is catalysed by pre-existing methylases whose activity is modified by the heat-shock or arsenite treatment. Both cytosolic- (Paik and Kim, 1980) and chromatin-bound methylases (Wallwork *et al.*, 1977) have been reported to be involved in the methylation of histones but the nature of the enzymes involved and the type of modification of histone H2B occurring during heat-shock and arsenite treatment are presently unknown.

While the exact significance of the changes in the pattern of synthesis (Sanders, 1981) and methylation of histones upon heat-shock is still unclear, the changes in the post-translational modifications of core histones reported here could be involved in structural reorganization of chromatin and could be related to the intensive changes observed in the transcriptional activity of these cells under these conditions. We previously observed that the induction of heat-shock proteins in these cells starts at temperatures between 30°C and 33°C (Vincent and Tanguay, 1982; Tanguay *et al.*, in preparation) while the methylation changes occur mainly at temperatures above 33°C. Since, in these temperature dependency experiments, the induction of heat-shock proteins occurs at lower temperatures than the changes in the methylation of H2B and H3, it seems that these changes in methylation are not related

to the induction of heat-shock proteins. Besides the activation of HS genes, the heat-shock response in *Drosophila* is characterized by a regression of previously active puffs (Ashburner, 1970) and a reduction in the synthesis of normal proteins (Tissières *et al.*, 1974; McKenzie *et al.*, 1975). In the temperature-dependent induction of heat-shock proteins mentioned above, it was also noted that the reduction in the synthesis of normal proteins starts at 35°C and is very pronounced at 37°C. This coincides with the temperatures where the increased methylation of H2B and the decreased methylation of H3 occur. Thus, the reduction in the synthesis of normal proteins and the changes in the methylation of core histones, could possibly be related. Furthermore, the findings (Figure 2) that the methylation changes are different in heat-shocked and in arsenite-treated cells is intriguing since the induction of some heat-shock proteins under these conditions is not accompanied by the drastic reduction of normal protein synthesis observed upon heat-shock.

While the biological significance of histone methylation remains unclear, there have been suggestions for its function in chromatin condensation (Paik and Kim, 1980). Methylated histones seem to have an increased affinity for anionic molecules such as DNA in chromatin (Byvoet and Baxter, 1975). Thus, the changes in the methylation pattern of histones during heat-shock coupled with the changes in the phosphorylation patterns of H2A and H4 recently described

(Glover, 1982) could be involved in the chromatin reorganization which is likely to occur during the activation of HS loci and the inactivation of the other cellular genes during heat-shock.

Materials and methods

Cell culture and heat-shock and arsenite treatments

A *D. melanogaster* Kc cell line obtained from G.Echalier (Paris), was grown at 23°C in D-22 medium with 10% foetal calf serum (Echalier and Ohanessian, 1970). The heat-shock or arsenite treatments were performed as previously described (Tanguay and Vincent, 1982). Briefly, cells were washed in D-22 medium without yeastolate, lactalbumin, and serum (D-22⁻) and preincubated in the same medium for 1 h at 23°C (control), 37°C (heat-shock) or in the presence of 50 µM sodium arsenite (arsenite treatment) at 23°C.

Labelling of proteins with L-[methyl-³H]methionine

Following 1 h of preincubation at 23°C, 37°C, or with 50 µM sodium arsenite at 23°C, cells were further incubated for 1 h in the presence of cycloheximide (100 µg/ml) and chloramphenicol (40 µg/ml) to prevent protein synthesis during each treatment. L-[methyl-³H]methionine (80 Ci/mmol, New England Nuclear) was then added at 20–50 µCi/10⁷ cells and cells were further incubated for 2 h in the same conditions. Cells were harvested by low-speed centrifugation and washed in D-22⁻ containing cycloheximide, chloramphenicol, and 0.1 mM phenylmethylsulfonyl fluoride (PMSF; 0.1 mM) as described by Wang *et al.* (1981). In the experiments described in Figure 2, lane 3, both protein synthesis inhibitors were added prior to the heat shock treatment to prevent the synthesis of heat-shock proteins.

Cell fractionation and histone extraction

Nuclei were isolated as previously described (Tanguay and Vincent, 1982). Histones were extracted from these nuclei with 0.3 N HCl in the presence of 0.2 mM PMSF as a protease inhibitor. 1 ml of 0.3 N HCl was added to 10⁸ nuclei and the extraction was carried out for 1 h at 0°C with frequent mixing. After centrifugation at 16 000 g (10 min), the pellet was reextracted with 0.5 volume of the same solution. The histones from the combined supernatants were precipitated with cold trichloroacetic acid (25%, w/v). The pellet was washed with cold acetone and the proteins solubilized in SDS buffer (Laemmli, 1970).

Gel electrophoresis

Proteins were separated on 12% or 15% SDS-polyacrylamide gels using the Thomas and Kornberg (1975) buffer system. Gels were stained with 0.16% Coomassie blue (Serva Blau R), photographed, destained, and processed for fluorography either with EN³HANCE (New England Nuclear) or according to the procedure of Bonner and Laskey (1974). Dried gels were exposed to Kodak XH or XAR-5 films.

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