Acetylation and methylation patterns of core histones are modified after heat or arsenite treatment of *Drosophila* tissue culture cells

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ABSTRACT

Exposure of *Drosophila melanogaster* tissue culture cells to 37°C (heat shock) or to arsenite induces a severe deacetylation of core histones and blocks the methylation of histone H₃ and H₄. Heat shock induces the methylation of histone H_{2b}. These results are discussed in view of chromatin structure and function.

INTRODUCTION

Heat shock of *Drosophila melanogaster* is a highly suitable system for the elucidation of the mechanism(s) underlying the induction of specific genes (1,2). This stress leads to the synthesis of a number of RNAs (3-7), several of which code for new polypeptide chains, the heat shock proteins (8-10), while the synthesis of most other RNAs is repressed (3-7). This phenomenon appears to be general, as it has been observed in a great variety of organisms, from *E.coli* to man (11). Using *Drosophila melanogaster* tissue culture cells we have investigated the effects of heat shock on the pattern of acetylation and methylation of the core histones in an attempt to correlate the transcriptional changes in chromatin with possible histone modifications. We have found that heat shock induces a drastic deacetylation of the core histones which resumes slowly when the cells are brought back to 25°C. Furthermore, sodium arsenite which also induces the synthesis of the heat shock proteins (11,12-14) leads to a similar deacetylation of the histones. Heat shock induces the methylation of histone H_{2b} and blocks the methylation of histones H₃ and H₄. Arsenite treatment also blocks the methylation of histones H₃ and H₄, but only slightly increases the

methylation of histone H_{2b}. Implications of these results on the structure of chromatin after heat shock or arsenite treatment are discussed.

MATERIALS AND METHODS

Cell culture. The Drosophila melanogaster tissue culture cell line KCl61 was adapted to grow in suspension in D22 medium (15) containing 2% foetal calf serum (serum screened for viruses and mycoplasma, Gibco). The cells were grown in spinner flasks at 25°C. They had a generation time of about 15 hours and were adjusted to concentrations between $2 \cdot 10^6$ and $8 \cdot 10^6$ cell/ml.

(³⁵S) methionine labeling of proteins. Tissue culture cells were washed twice and concentrated approximately tenfold to reach about $5 \cdot 10^7$ cells/ml in D22 medium, minus yeast extract, lactalbumin hydrolysate, serum and methionine. Proteins were labeled with 50 to 100 µCi/ml L-(³⁵S) methionine (New England Nuclear or R.C. Amersham, 300 - 500 Ci/mmol) for different periods at 25 or 37°C. The cells were then harvested and washed once in complete D22 medium containing 1 mg/ml methionine.

Acetylation and methylation of proteins. Cells ($5 \cdot 10^7$ cells/ml) were labeled at 25°C or 37°C for 15 min with 100 µCi/ml (³H) acetic acid, sodium salt (New England Nuclear, 500 mCi/mmol) in D22 medium lacking yeast extract, lactalbumin hydrolysate and serum in presence or absence of 20 µg/ml cycloheximide or emetine. Cells ($5 \cdot 10^7$ cells/ml) were labeled at 25°C or 37°C for 30 min with 10 µCi/ml S-(methyl-³H) adenosyl-L-methionine (Amersham 85 Ci/mmol) in D22 medium lacking yeast extract lactalbumin hydrolysate, serum and methionine in presence or absence of 20 µg/ml cycloheximide or emetine (Sigma).

Chromatin isolation. Chromatin was isolated according to Hancock (16,17).

SDS-polyacrylamide gel electrophoresis of proteins. Pellets of chromatin were dissolved in SDS sample buffer (10% glycerol; 2.3% SDS; 62.5 mM Tris-HCl pH 6.8, traces of bromophenol blue) and heated at 100°C for 1 min. Proteins were electrophoresed in 12.5% polyacrylamide gels in SDS according to the methods of Laemmli (1970) (18) and Studier (1973) (19). Total proteins were

quantified by scanning wet coomassie stained gels with a Joyce-Loebl recording microdensitometer. The gels were dried and the labeled proteins were detected by fluorography (20). Labeled proteins were quantified by scanning the fluorographs (pre-flashed films were used) as described above. The area under the peak of each protein was measured using a numonic planimeter. Specific incorporation of tritium in the different proteins was calculated from the ratio of values of the labeled proteins to those of the stained proteins.

RESULTS

Acetylation of chromosomal proteins at 25°C or following a heat shock.

As (^3H) acetate can be actively metabolized into amino acid residues, e.g., glycine, (21) it was of importance to clearly define our conditions of labeling. Labeling was done in vivo at physiological pH during a short pulse (15 min) in presence or absence of protein synthesis. It is seen in Fig. 1 that, in vivo at 25°C, histones H_3 , $\text{H}_{2\text{b}}$, $\text{H}_{2\text{a}}$ and H_4 are highly acetylated as already described in other organisms (21). Our study will concern essentially these four histones. It is seen (Fig. 1) that 20 $\mu\text{g}/\text{ml}$ cycloheximide added 10 min before the labeling period had no effect upon the extent of acetylation of the histones, indicating that no metabolization of (^3H) acetate into amino acids had occurred during the 15 min pulse of labeling. Similar results were obtained if 50 $\mu\text{g}/\text{ml}$ of cycloheximide was used or if the drug was added 50 min before the labeling period. Under such conditions, mitosis and histones synthesis were completely inhibited and DNA synthesis was reduced (not shown). It is also of interest that a high amount of actinomycine D (5 $\mu\text{g}/\text{ml}$) had no effect upon this acetylation pattern. If the cells were exposed to a temperature of 37°C for one hour it is seen (Fig.1) that histones were no longer acetylated. This effect was not due to the presence of the heat shock proteins, as similar results were obtained in presence of 20 $\mu\text{g}/\text{ml}$ cycloheximide added 10 min before the heat shock, nor to indirect effects of heat shock on the processes involved in cell division. At 25°C, one hour after the temperature shift down, the acetylation of the histones

Figure 1 - Acetylation of chromosomal proteins at 25°C or following a heat shock

Cells were labeled with (^3H) acetate and chromatin was isolated as described in Materials and Methods. A fluorograph of a 12.5% SDS polyacrylamide gel is presented. a) labeling 15 min at 25°C. b) as a) but 20 $\mu\text{g/ml}$ of cycloheximide was added 10 min before the labeling. c) as a) but 5 $\mu\text{g/ml}$ actinomycine D was added 10 min before the labeling. d) labeling 15 min at 25°C. e) labeling during the last 15 min of a one hour heat shock. f) as e) but 20 $\mu\text{g/ml}$ of cycloheximide was added 10 min before the heat shock. g) labeling at 25°C, 1 hour after a one hour heat shock. h) as g) but in presence of 20 $\mu\text{g/ml}$ cycloheximide added 10 min prior to the heat shock. i) (^{35}S) methionine labeled chromosomal proteins from heat shocked cells. It must be pointed out that tracks g and h are overexposed if compared to tracks d, e and f in order to show the acetylation of the heat shock proteins.

occurred again normally; although, at this time, the rate of acetylation was weaker than before the heat shock (for quantitative results see Fig. 2) and the heat shock proteins present in chromatin were acetylated. Cycloheximide added 10 min before the heat shock decreased, at 25°C one hour after the heat treatment, the recovery of the acetylation of the histones (for quantitative results, see Fig. 2). When cycloheximide is added 10 min before the labeling at 25°C one hour after the heat treatment, no effect is detected upon the extent of acetylation of the core

Figure 2 - Time-course of acetylation of the histones

Cells were labeled with (^3H) acetate at different periods: before, during and after the heat shock and chromatin was isolated as described in Materials and Methods. Specific incorporation of (^3H) acetate was determined by dividing the amount of label incorporated in each histone by the amount of the corresponding coomassie stained histone as described in Materials and Methods. ○○ : Cells labeled with (^3H) acetate for 15 min in presence of 20 $\mu\text{g/ml}$ cycloheximide added 10 min before the labeling. ●● : Cells labeled with (^3H) acetate for 15 min but in this case cycloheximide (20 $\mu\text{g/ml}$) was added 10 min before the heat shock (arrows in the figure). The black bar in the absciss between 1 and 2 represents the heat shock period.

histones; however in this case no heat shock proteins were found acetylated (not shown). Similar results were obtained if emetine was used instead of cycloheximide (not shown).

We have performed quantitative experiments in order to determine the specific incorporation of (^3H) acetate in the histones during heat shock treatment. It is seen in Fig. 2 that the rate of acetylation of the different histones (standardized to an equal amount of histones) is only 15% of its normal value when the labeling is performed during the last 15 min of a one hour heat shock. This phenomenon is insensitive to cycloheximide or emetine and lasts at least during the first half hour at 25°C after the heat shock. Additional experiments have shown that during the first half hour at 37°C the rate of acetylation of the histones is already affected. Later at 25°C , the rate of acetylation of the histones grows rapidly and reaches a normal value about 2 hours after the end of the heat treatment. If

Figure 3 -

A. Pulse-chase experiment

Cells were labeled with (^3H) acetate for 15 min at 25°C and subsequently chased in a complete D_{22} medium in presence of 5mM acetate either at 25°C or at 37°C . After 0, 30 min and 1 hour of chase, cells were pelleted and chromatin was isolated as described in Materials and Methods. Specific incorporation of (^3H) acetate was determined as described in Materials and Methods and in the legend of Fig. 2. The percentage of (^3H) acetate remaining associated with the different histones was calculated by dividing the values obtained after 30 min or 1 hour of chase by the amount of (^3H) acetate present in the histones just after the labeling. $\nabla\nabla$: histone H_3 , chase at 37°C . $\blacktriangledown\blacktriangledown$: histone H_3 , chase at 25°C . $\triangle\triangle$: histones $\text{H}_{2a} + 2b$ chase at 37°C . $\blacktriangle\blacktriangle$: histones $\text{H}_{2a} + 2b$, chase at 25°C . $\circ\circ$: histone H_4 , chase at 37°C . $\bullet\bullet$: histone H_4 , chase at 25°C .

B. Variation of the degree of histone acetylation during the heat shock treatment

Data presented in Fig. 2 and 3 were used to calculate the results shown in this figure. Results are presented in percent of the total acetylated histones (in our conditions of labeling) in normal cell conditions of growth at 25°C . An histogram of the values obtained after every hour of the processus is presented. Calculations were performed using the following formula:

$A_{t=n} = X \cdot A_{t=n-1} / 100 + R_{t=n} (100 - X) / 100$ where $A_{t=n}$ is the percentage of the total acetylated histones at time $t=n$, and X is the percentage of histones remaining labeled after a 1 hour chase (stability of the labeling, see figure 3). We have fixed the value of X to an average of 30%. R is the percentage of the specific incorporation of (^3H) acetate at different times t_{1-n} and is proportional to the rate of acetylation of the histones. Values of R_t are chosen at the end of each hour and are an average of the values obtained with the different histones (see

figure 2). 1) Continuous line represents the histogram calculated when cells are shifted back to 25°C after a 1 hour heat shock. 2) Broken line represents a similar condition but in this case 20 µg/ml cycloheximide was added 10 min before the heat shock. 3) Dotted lines represent the values obtained when the cells are kept at 37°C. The one hour heat shock (1) and (2) was performed between time $t=1$ and 2 and is symbolized by a black bar.

cycloheximide or emetine is added 10 min before the heat shock (see the arrow in Fig. 2), the rate of acetylation of the histones at 25°C after the heat shock also increases, but slower than in the normal case and reaches a maximum 1 hour after the end of the heat treatment and subsequently declines.

It has already been described that acetylation is not a stable modification of proteins (22,23). Pulse-chase experiments were performed in order to study the turn-over of (^3H) acetate in the core histones. Cells were labeled for 15 min at 25°C as usual and were chased in presence of 5 mM acetate at 25 or 37°C. It is seen in Fig. 3A that at 25°C, 70% of (^3H) acetate present in the histones is chased within one hour. At 37°C, a similar result is obtained except in the case of histone H_3 which retains slightly more of its acetate than at 25°C (45% instead of 35%).

Results presented in Fig. 2 and 3A were used to calculate a theoretical histogram describing the variation of the percentage of the total acetylated histones. Results are presented in Fig. 3B. It is seen in this figure that heat shock induces a dramatic deacetylation of the potentially "acetylatable core histones". After one hour at 37°C this deacetylation was calculated to be about 60% in the case of H_4 , H_{2b} and H_{2a} . When the temperature is shifted back to 25°C this lack of acetylation was gradually reduced, until 3 hours after the heat shock almost all of the "potentially acetylatable" histones were again acetylated. When an inhibitor of protein synthesis was added 10 min before the heat shock, no recovery of acetylation of the histones was found. An even more dramatic result is obtained if the cells are kept at 37°C. It must be pointed out that if the calculation is performed in the case of H_3 a deacetylation of about 50% is found after one hour at 37°C.

Figure 4 - Methylation of chromosomal proteins at 25°C or following a heat shock

Cells were labeled for 30 min with S-(methyl-³H)adenosyl-methionine and chromatin was isolated as described in Materials and Methods. A fluorograph of a 12.5% SDS polyacrylamide gel is presented. a) labeling 30 min at 25°C. b) as a) but 20 µg/ml cycloheximide was added 10 min before the labeling. c) as a) but 5 µg/ml actinomycin D was added 10 min before the labeling. d) labeling 30 min at 25°C. e) labeling during the last 30 min of a one hour heat shock. f) as e) but 20 µg/ml of cycloheximide was added 10 min before the heat shock. g) labeling at 25°C, 1 hour after a one hour heat shock. h) as g) but in presence of 20 µg/ml cycloheximide added 10 min prior to the heat shock. i) (³⁵S)methionine labeled chromosomal proteins from heat shocked cells. It must be pointed out that tracks g and h are overexposed if compared to tracks d, e and f in order to show the methylation of HSP 70.

Methylation of chromosomal proteins at 25°C or following a heat shock. Using S-adenosyl-L-methylmethionine as a methyl donor, it has been shown (21) that the methylation of proteins is insensitive to inhibitors of translation indicating that S-adenosyl-L-methylmethionine acts only as a methylating agent of the proteins, but did not participate in the protein synthesis (24). It is seen in Fig. 4 that, in vivo at 25°C, histones such as H₃ and H₄ are highly methylated as already described in other organisms (21,24). This particular pattern of methylation was

Figure 5 - Time-course of methylation of the histones

Cells were labeled with S-(methyl- ^3H)adenosylmethionine at different periods: before, during and after the heat shock and chromatin was isolated as described in Materials and Methods. Specific incorporation of S-(methyl- ^3H)adenosylmethionine was determined by dividing the amount of label incorporated in each histone by the amount of the corresponding coomassie stained histone as described in Materials and Methods. ○○ : cells labeled with S-(methyl- ^3H)adenosylmethionine for 30 min. ●● : cells labeled with S-(methyl- ^3H)adenosylmethionine for 30 min but in this case 20 $\mu\text{g}/\text{ml}$ cycloheximide was added 10 min before the heat shock (arrow in the figure). The black bar in the absciss between 1 and 2 represents the heat shock period.

found to be insensitive to cycloheximide (20 or 50 $\mu\text{g}/\text{ml}$) or actinomycine D (5 $\mu\text{g}/\text{ml}$) added 10 or 50 min before the labeling. These experiments suggest that under our conditons of labeling the methylation of the core histones is independent on the processes involved in cell division. When cells were exposed to a temperature of 37°C for one hour, it is seen in Fig. 4, that histones H₃ and H₄ were no longer methylated and H_{2b} was found methylated. Cycloheximide had no effect upon this result, suggesting that the heat shock proteins were not involved in this phenomenon. When cells are brought back to 25°C, one hour after the temperature shift down, the pattern of methylation of the core histones is rather complex : H_{2b} is still being methylated and H₃ and H₄ are remethylated. Cycloheximide, added 10 min before the heat shock abolishes the recovery of the rate of methylation of H₃ and H₄ (for quantitative results, see Fig. 5). Similar results were obtained with an other inhibitor of protein synthe-

sis : emetine, instead of cycloheximide (data not shown). It is interesting to point out that the major heat shock protein : HSP 70 is methylated at 25°C after a one hour heat shock.

Results presented in Fig. 5 show that the rate of methylation of H₃ and H₄ is only 10% of its normal value during heat shock. This phenomenon is insensitive to cycloheximide or emetine. At 25°C, immediately after heat shock, the rate of methylation of H₃ and H₄ increases and reaches about 50% of its normal value 2 hours after the end of the heat shock. Cycloheximide or emetine added 10 min before the heat treatment (see the arrow in Fig. 5) abolishes this recovery; although, even in presence of cycloheximide a temporary slight recovery occurs during the first 30 min after the heat treatment. In contrast, the rate of methylation of H_{2b} shows a stimulation of at least 10 times with a maximum at 25°C just after the end of the one hour heat shock. 2 hours after the end of the heat treatment the rate of methylation of H_{2b} is still about 40% of its maximal value. The kinetic of the rate of methylation of H_{2b} is unaffected by cycloheximide or emetine. By using the same conditions of labeling, pulse-chase experiments were performed as described in the case of acetylation (Fig. 3A). Within two hours of chase, at 25°C or 37°C, in presence of 1 mg/ml methionine, no detectable chase of S-(methyl-³H) adenosyl methionine was detected at the level of histones H₃ and H₄ (data not shown).

We have taken advantage of the fact that low doses of arsenite were able to induce the synthesis of some of the heat shock proteins at the normal temperature (13,14). It is seen in Fig. 6 that when cells are incubated 4 hours with 5.10⁻⁵ M sodium arsenite, the acetylation of histones H₃, H_{2a}, H_{2b} and H₄ and the methylation of histones H₃ and H₄ are completely abolished. In contrast to the heat shock, the rate of methylation of H_{2b} is only weakly increased by sodium arsenite.

DISCUSSION

In Drosophila melanogaster tissue culture cells, heat shock induces transcriptional changes which are highly divers, ranging from the rapid induction of heat shock mRNAs, the almost unchanged synthesis of histone mRNAs to a drastic sup-

Figure 6 - Effect of sodium arsenite on the acetylation and methylation pattern of the core histones

Cells (5.10^6 cells/ml) were either incubated at 25°C or treated for 4 hours with 5.10^{-5}M arsenite at 25°C . After this treatment, the cells were concentrated to 5.10^7 cells/ml and labeled at 25°C with (^3H) acetate or with S-(methyl- ^3H)adenosylmethionine for respectively 15 and 30 min and chromatin was isolated as described in Materials and Methods. A fluorograph of a 12.5% SDS-polyacrylamide gel is presented. a) Cells labeled with (^3H) acetate at 25°C . b) as a) but after arsenite treatment. c) Cells labeled with S-(methyl- ^3H)adenosylmethionine at 25°C . d) as c) but after arsenite treatment.

pression of the synthesis of most other mRNAs and ribosomal RNA (with the exception of 5S RNA which is synthesized but not processed and tRNA which is not affected) (1-7). Altogether these events lead to a decrease of ^3H uridine incorporation in RNA of 70% as a result of the heat shock (25). Heat shock also blocks cell division and induces a gradual decrease of DNA synthesis (26) and an asynchrony of the synthesis of the histones (27). In these cells, in normal conditions of growth at 25°C , histones H_3 , H_{2a} , H_{2b} and H_4 are acetylated and histones H_3 and H_4 (but not H_{2a} and H_{2b}) are methylated as already described in other organisms (21,24). It has been found that after one hour at 37°C (heat shock) the core histones H_3 , H_{2a} , H_{2b} and H_4 are no longer acetylated, H_3 and H_4 are no longer methylated and a strong methylation of H_{2b} is induced. In order to rule out that the modifications of the pattern of acetylation and methylation of the core histones described above are an indirect consequence of the heat shock at the level of the processes dependent on DNA synthesis or cell division, control experiments have been performed. In *Drosophila* cells $20 \mu\text{g/ml}$

cycloheximide or emetine completely inhibit protein synthesis within 10 min (25); under such conditions, mitosis and histones synthesis are totally inhibited and DNA synthesis is reduced. Control experiments show that when protein or RNA synthesis is inhibited at 25°C, acetylation or methylation of the core histones is not affected. Similar results are obtained when cycloheximide is added 50 minutes before the labeling period or when 50 µg/ml of cycloheximide is used. At 37°C, the lack of acetylation of the histones as well as the induction of the methylation of histone H_{2b} was also found independent on protein synthesis. These results were therefore not due to the inhibition of mitosis by heat shock nor to a direct effect of temperature upon the rate of the enzymes responsible for these acetylations and methylations, as similar effects (except in case of methylation of H_{2b}) were observed when cells were exposed for four hours to 5.10⁻⁵M sodium arsenite, a compound which induces essentially the high molecular weight heat shock proteins (12, 13,14, Arrigo unpublished results). The literature is well documented concerning the pattern of acetylation of the core histones which has been found independent on cell division, histone or DNA synthesis (22,28). Furthermore, Sanders et al. (1973) (29) have shown that acetylation of histones can occur in mature (non dividing) erythrocytes. Much less informations are available concerning the methylation of H₃ and H₄. Although, inhibition of protein synthesis does not affect the methylation of these two histones (28).

Kinetic experiments indicate that the rate of acetylation of histones slowly recovers at 25°C after a heat treatment to reach its normal level two hours after a one hour heat shock. If an inhibitor of protein synthesis (and therefore of mitosis) is added ten minutes before the heat shock, at 25°C after the heat treatment no recovery of the rate of acetylation of the histones was found, although it should be pointed out that during the first hour at 25°C a slight temporary recovery occurs. Similar observations are made concerning the recovery of the methylation of histone H₃ and H₄. These experiments also suggest that both heat shock and sodium arsenite inactivate the histone acetylase(s) and methylase(s) (specific for H₃ and H₄) so that protein syn-

thesis is required during and after the "stress" treatment to recover these activities. Whether the heat shock proteins or the newly made acetylase(s) and methylase(s) are essential for this recovery is not known. Note that at 25°C after heat shock different events are well temporally correlated: the migration of the heat shock proteins from the nucleus to the cytoplasm (25,26,30), the reinitiation of transcription of the non heat shock genes (25, 31), the reacylation of the core histones, the remethylation of histones H₃ and H₄ and the decrease of methylation of histone H_{2b}. In every case, the reinitiation processes require the synthesis of proteins during and after the heat shock (25,31).

It has been found that the turn-over of the acetylation of the core histones is very rapid in Drosophila cells, as already observed in other systems (22,32). Thus, in our conditons of labeling, a heat shock of one hour results in a deacetylation of about 60% of the core histones. When cells are kept at 37°C or in presence of an inhibitor of protein synthesis this deacetylation is even more drastic as no recovery of acetylation of the core histones occurs. In presence of arsenite a similar effect is probably occurring, although no quantitative data were obtained. What could be the effect of such a deacetylation on the structure or template activity of chromatin ? Acetylation is a post-synthetic modification of the four nucleosomal core histones whose exact biological role is unknown at present; although correlations exist between histones acetylation and transcription (28, 33) or chromatin solubilisation and indirectly gene activation (34). Histone acetylation is thought to reduce nucleosome oligomer interactions, implying that chromatin becomes less compact and easier to transcribe (34). This assumption is interesting as I have already noticed that chromatin becomes more difficult to shear after heat shock (26). Rapid reversible acetylation may over a period of time involve all chromatin (34). Other experiments suggest that the continuous redistribution of DNA sequences with respect to rapidly acetylated chromatin is itself dependent upon the coordinate and reversible acetylation of rapidly acetylated histones (35). In view of these suggestions, our results may indicate that 60% of the chromatin containing rapidly acetylated histones before the heat shock will contain

deacetylated histones after one hour at 37°C, and this could be enough to induce a gradual compaction of chromatin during the heat treatment. Whether such a compaction of chromatin induced by heat or arsenite could be helpful in order to prevent chromosomal damages and/or to maintain a reduced transcriptional level of the non heat shock genes remain to be shown. Most of the heat shock proteins are found labeled by (³H)acetate at 25°C after a heat shock. As this labeling is dependent on the protein synthesis, it is unclear whether this effect reflects a true acetylation or incorporation of labeled amino acids in the proteins.

It has been found that methylation is a rather stable protein modification, confirming the results of Wang *et al.*, 1982 (36) who estimate an half life *in vivo* of about 65 hours. These results suggest that very few histones H₃ and H₄ may be demethylated after a one hour heat shock or after arsenite treatment. The methylation of HSP 70 occurring at 25°C after a one hour heat shock has already been described by Wang *et al.*, 1982 (36) in chicken fibroblasts. More intriguing is the strong methylation of histone H_{2b} after heat shock. This phenomenon which is independent on the synthesis of proteins is reversible and disappears at 25°C, 3 to 4 hours after heat shock. Under our conditions of labeling arsenite only weakly increases the methylation of H_{2b}. At present, no clear biological function has been assigned to the methylation of histones (in particularly histones H₃ and H₄). Concerning the methylation of H_{2b} at 37°C, it is interesting to note the 3 to 5 fold increase in the synthesis of this histone during heat shock (27). One hypothesis could be that methylation of H_{2b} at 37°C may enhance the compaction of chromatin similarly to H_{2b} from sea urchin sperm (37), or acts as a memory of the heat treatment.

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