Correlation between phosphorylated H1 histone and condensed chromatin in *Planococcus citri*

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Histones of the mealybug *Planococcus citri* have been isolated and characterised. Although no major differences were observed between the core histones of male and female insects, the pattern of H1 histones was significantly different between the sexes. A specific hyperphosphorylated H1 was found to be present only in male mealybugs, where the paternal chromosomes are condensed and transcriptionally inactive.

Mealybug *Chromatin organisation* *H1 phosphorylation* *Facultative heterochromatin*

1. INTRODUCTION

The phosphorylation of the lysine-rich histone H1 has been correlated with chromatin condensation at various levels of chromatin organisation [1–4]. Studies on actively dividing *Physarum polycephalum* cells [5], regenerating rat liver cells [6] and mammalian tumour cells during mid-exponential growth [7], have led to the proposal that H1 histone phosphorylation is associated with cell replication and is involved in the mitotic process [1,8]. In *Drosophila*, H1 phosphorylation has been implicated in the compaction of satellite DNA into constitutive heterochromatin [9].

I assess here the correlation between H1 phosphorylation and facultative heterochromatin [10]. The mealybug *Planococcus citri* provides a unique genetic system for such a study. The diploid chromosome number in these insects is 10, and there are no sex chromosomes [11]. In males, the paternally derived chromosomes appear condensed and are genetically inactive, whereas the maternal chromosomes are active or potentially active [12]. The inactive and active halves are spatially separate within the interphase nuclei in males. In females there is no distinction between paternal and maternal chromosomes [12]. Sex is apparently determined by whether inactivation of the paternal chromosome set occurs [13]. A very close correlation exists between genetic inactivity and the condensed heterochromatic state of the paternal chromosomes in males. Decondensation occurs in a few tissues and in such tissues genetic activity of the paternal set is restored. Experimental reactivation of the paternal set can be induced by treatment with polyanions like polystyrenesulfonate which interacts with basic proteins [14]. This supports the idea that histones play an important role in chromosome condensation.

As a beginning I have characterised and compared the chromosomal proteins of male and female mealybugs, and present the findings here.

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Abbreviations: SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; AP, alkaline phosphatase

2. MATERIALS AND METHODS

2.1. *Extraction and fractionation of histones*

Nuclei were isolated as in [15] from second instar male and female mealybugs. The insects were

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obtained from the Horticultural Experimental Station, Chethalli (Coorg), Karnataka, India. They were provisionally identified as *P. citri* by the Commonwealth Institute of Entomology, London.

Histones were isolated by the calcium phosphate gel method [16]. Chromatin was adsorbed onto the calcium phosphate gel. On elution with 0.8 M NaCl, H1 histone was obtained in a relatively pure form [17]. The core histones were eluted with 2 M NaCl [16,17]. Histones were electrophoresed on 12% polyacrylamide gels containing 6 M urea, 0.37% (w/v) Triton X-100 [18]. They were also electrophoresed on 10% polyacrylamide-SDS gels [19].

Histones were also extracted by treatment of nuclei with 0.25 N HCl. On treatment of the total histone preparation with 5% perchloric acid, the core histones were precipitated and the H1 was obtained in the supernatant [20].

2.2. *Alkaline phosphatase digestion*

Histones were incubated with alkaline phosphatase (*E. coli*) at a ratio of 1 mol enzyme per 4–10 mol histone [21]. All the incubations were in 0.01 M Tris–HCl (pH 8.0) at 37°C. Reactions were terminated by extensive dialysis against cold 0.9 N acetic acid after various time intervals.

3. RESULTS AND DISCUSSION

3.1. *Electrophoretic analysis of histones*

Histones were extracted by adsorption of chromatin onto calcium phosphate gel and selective elution with salt [16]. They were analysed on polyacrylamide-urea-Triton X-100 gels which allow for the resolution of both the individual histones and their variously modified forms [18]. The core histones exhibit four major bands corresponding to H2A, H2B, H3 and H4 (fig.1). The individual histones were identified by comparing the mobilities of the mealy bug histones with the corresponding proteins of rat liver and chicken erythrocytes. An electrophoretic comparison of the core histones of males and females reveals no major differences (fig.1).

However, the H1 histones were significantly different (fig.2). H1 histones from males were resolved on acrylamide gels into three major subfractions designated H1A, H1B and H1C, according to decreased relative electrophoretic mobility

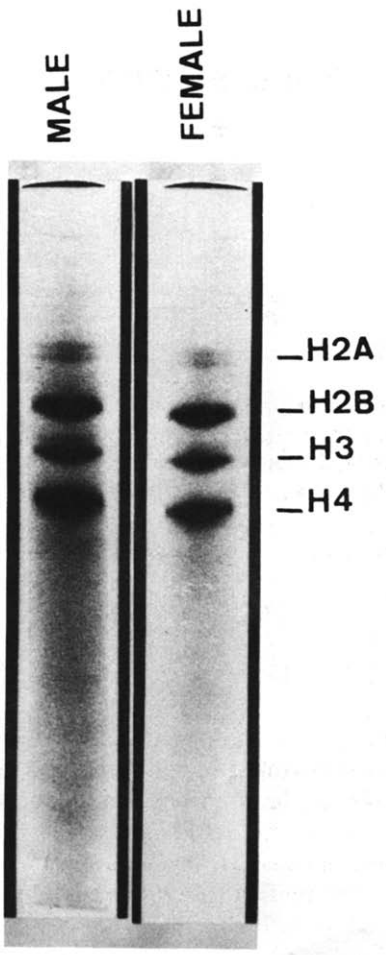

Fig.1. Electrophoretic analysis of core histones of female and male mealybugs. The 2 M NaCl eluate from mealybug nuclei was electrophoresed on 12 cm gels at 15 V/cm for 4–6 h as in [18]. After electrophoresis, the gels were stained with Amido Black (0.4% in acetic acid/methanol/water, 1:5:15, v/v) and destained by diffusion in the same solvent.

(fig.2A). On the other hand, H1 from females were resolved into only two bands corresponding to H1A and H1B; H1C was not detected (fig.2B). This pattern was reproducible and was seen with perchloric acid-extracted H1 histones as well (not shown).

H1 histones of both sexes are resolved on acrylamide-SDS gels into a single band (fig.2A,B). This indicated that the subfractions obtained on acrylamide-urea-Triton X-100 gels were modified forms of histone H1.

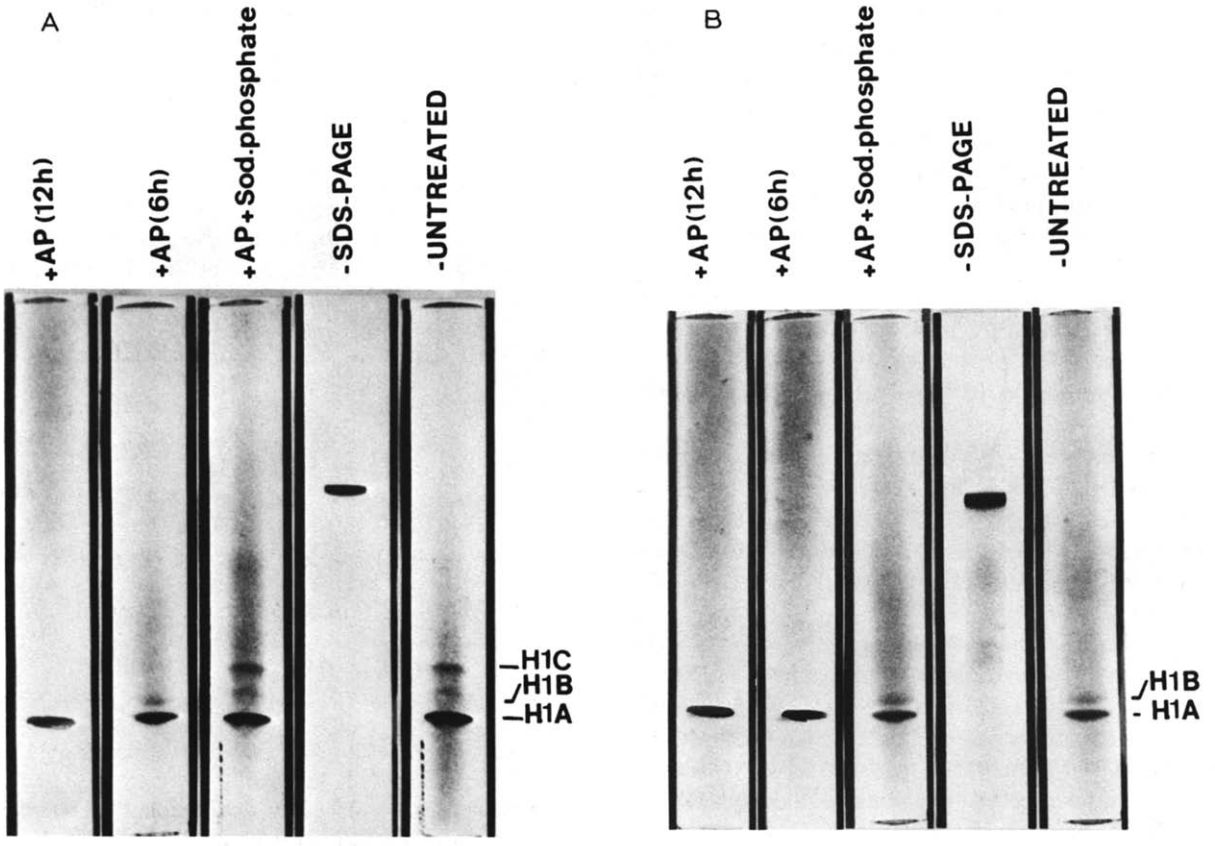

Fig.2. Electrophoresis of H1 histones from (A) males and (B) female insects, before (untreated) and after (AP 6 h and 12 h) alkaline phosphatase digestion. (AP + Sod. phosphate) Treated with alkaline phosphatase, but contained 10 mM phosphate in the incubation mixture. SDS–PAGE indicates the pattern on 10% acrylamide-SDS gels.

3.2. *Evidence for the presence of phosphorylated H1*

To check if the H1 subfractions were phosphorylated, the histones were treated with *E. coli* alkaline phosphatase for various lengths of time. As shown in fig.2 phosphatase treatment converts the slower migrating subfractions H1B and H1C into H1A. After 6 h (fig.2A) only the bands corresponding to H1A and H1B were seen and after 12 h (fig.2A) only H1A was present in males. In females (fig.2B) only H1A is observed after a 6 h treatment.

On the other hand, phosphatase treatment did not affect the mobility of any of the core histones indicating that these histones are not modified in either males or females. In addition, I have examined the mobilities of rat liver core histones and H1 histone and found that they were not affected by phosphatase treatment (not shown). The conversion of mealybug histone H1B and H1C into H1A was completely inhibited (fig.2A,B) when phosphatase treatment was done in the presence of 10 mM sodium phosphate buffer which inhibits *E. coli* alkaline phosphatase [22]. These experiments would argue against proteolysis and would indicate some phosphatase-sensitive modification as being responsible for the disappearance of mealy H1 subfractions. Poly ADP-ribosylation is another common modification of H1 histones [23]. I have considered the possibility that the two slower migrating bands might contain poly ADP-ribose. Phosphodiesterase which is a frequent contaminant of commercial preparations of alkaline phosphatase would remove poly ADP-ribose and give the observed electrophoretic pattern. To check if the subfractions contain poly ADP-ribose, the

H1 histones were treated with hydroxylamine which should labilize the poly ADP-ribose moieties [23]. Treatment for up to 2 h with 0.1 and 0.5 M hydroxylamine did not affect the mobility of H1 subfractions (data not shown).

It therefore appears that the multiplicity of H1 histone observed in *P. citri* is due to phosphorylation. After extensive alkaline phosphatase digestion, only the band corresponding to H1A is detected both in male and female insects. This indicates that H1C and H1B are the phosphorylated forms of H1A. The slower mobility of H1C when compared to H1B indicates that H1C is probably phosphorylated to a greater extent than H1B. Phosphate groups neutralise the basic charge on the histones so that the mobility of histones decreases under the conditions of electrophoresis (pH 4.6). While there is a difference between males and females in the level of H1 phosphorylation, the core histones are essentially similar in both sexes. Apart from the major H1 histone (H1A) which is apparently unmodified, a basal level of phosphorylation (H1B) is present in both male and female insects. A hyperphosphorylated form (H1C) occurs only in males. I have not been able to detect this form in females by the analysis presented above.

One cannot speculate as yet about the role of the basal level of phosphorylation in both sexes. However, it is the absence of H1C and the lack of interphase condensation of the paternal chromosomes in females which appear to be significant. I therefore suggest that in males, the hyperphosphorylated H1C is associated with the transcriptionally inactive, condensed paternal chromosome set. It is not unlikely that specific phosphorylation of H1 is one of the factors governing the condensation and possibly inactivation of paternal chromosomes in male mealybugs.

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