Chromatosomes are not produced from *Tetrahymena* chromatin by micrococcal nuclease digestion

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Summary. The subnucleosomal organization of *Tetrahymena* chromatin, which has an unusual H1 histone, was investigated by NaCl extraction and micrococcal nuclease digestion of nuclei. It was found that *Tetrahymena* histone H1 is extracted with 0.35 M NaCl, whereas bovine thyroid H1 is not. Micrococcal nuclease digestion of *Tetrahymena* nuclei did not yield chromatosomes as a stable intermediate, whereas digestion of bovine thyroid nuclei did.
Key words. Tetrahymena; chromatin; chromatosome; histone H1.

It is now widely accepted that linker histones have a central conserved region, that this region forms a globular part in each molecule [1], and that the globular part is involved in the locking of two full turns of DNA around the core histone octamer [2]. However, most of the structural studies of linker histones carried out so far have been on those from higher eukaryotes. In yeast and *Trypanosoma brucei brucei* [3] no H1 histone has been found as yet. In the protozoan *Tetrahymena*, another lower eukaryote, histone H1 has been shown to have no sequence homology with H1 histones of higher eukaryotes [4, 5]. One feature of the *Tetrahymena* H1 histone is that it is a small protein with a relatively small net charge as compared with H1 histones of higher eukaryotes. Another feature is the apparent absence of the central globular domain. It is interesting to examine the characteristics of a chromatin with such an unusual H1 histone. The results of salt extraction and micrococcal nuclease digestion of *Tetrahymena* nuclei are reported here.

Materials and methods

Preparation of Tetrahymena nuclei. Nuclei were prepared from cultures of *Tetrahymena pyriformis* amicronucleate strain GL in the early stationary phase as described earlier [6], except that 2.1 M sucrose, 20 mM Tris-HCl, 1 mM $MgCl_2$, 3 mM $CaCl_2$, pH 6.5 was used in place of 1.9 M sucrose, 20 mM Tris-HCl, 1 mM $MgCl_2$, 3 mM $CaCl_2$, pH 6.5 in the final purification step.

Preparation of bovine thyroid nuclei. Bovine thyroid nuclei were prepared from a frozen gland (Nippon Ham, Osaka Japan). It was sliced and homogenized in 10 volumes of 0.25 M sucrose, 50 mM Tris-HCl, 25 mM KCl, 5 mM $MgCl_2$, pH 7.0 (homogenization buffer) containing 1 mM phenylmethanesulfonyl fluoride (PMSF). After filtration through four layers of gauze the homogenate was centrifuged at 1000 × g for 10 min. Pelleted nuclei were washed successively with homogenization buffer containing 1 mM PMSF twice, homogenization buffer containing 0.1 % Triton X-100 and 0.1 mM PMSF once, and homogenization buffer twice.

Salt extraction. Nuclei were suspended in 10 mM Tris-HCl, pH 7.5 containing 0.15–0.35 M NaCl to give a

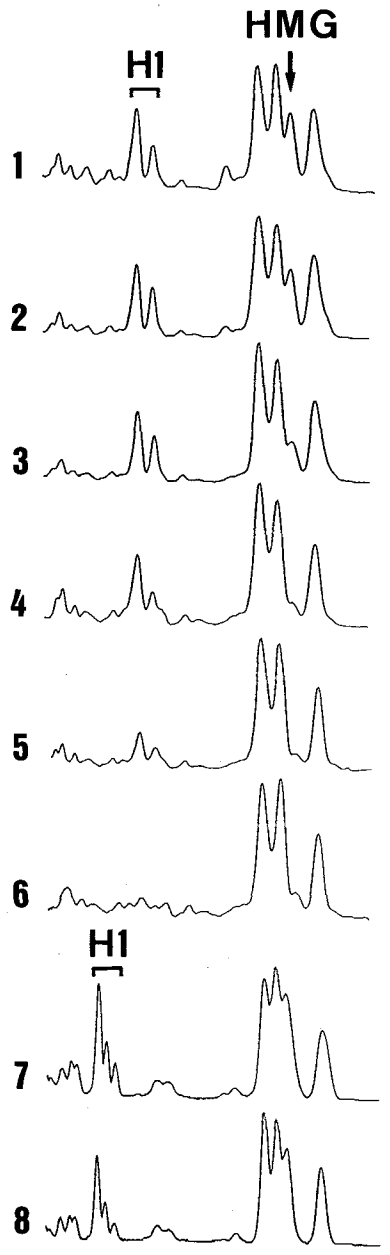

Figure 1. SDS-polyacrylamide gel electrophoresis of nuclear proteins. Proteins of whole nuclei (1, 7) and nuclei washed with 0.15 M (2), 0.20 M (3), 0.25 M (4), 0.30 M (5) or 0.35 M (6, 8) NaCl in 10 mM Tris-HCl, pH 7.5 were electrophoresed on 15% polyacrylamide gels. *Tetrahymena*, 1–6; bovine thyroid, 7, 8. Densitometric tracings of relevant regions only are shown. The positions of *Tetrahymena* and bovine H1 histones and the *Tetrahymena* HMG protein are indicated in the figure. Other major peaks correspond to core histones. Migration is from left to right.

concentration of 0.11–0.15 mg chromatin DNA/ml. After 10-min extraction at 0 °C, they were pelleted by centrifugation at 1000 × g for 10 min. Extraction was repeated once. Proteins in the residual chromatin as well as in unextracted nuclei were analyzed in 15% SDS-polyacrylamide gels [7]. Gels were stained with Coomassie brilliant blue R250 and scanned at 530 nm with a densitometer (Joyce-Loeble, model Chromoscan 3, Tyne & Wear, England).

Micrococcal nuclease digestion. Nuclei or residual chromatin after salt extraction were dispersed in 60 mM NaCl, 7 mM Tris-HCl, 7 mM $MgCl_2$, 1 mM $CaCl_2$, pH 7.5 at ca 0.15 mg chromatin DNA/ml [8]. *Tetrahymena* nuclei were digested at 37 °C with 20 U/ml micrococcal nuclease (Worthington Biochemical, USA). Bovine thyroid nuclei were digested at 37 °C with 40 U/ml micrococcal nuclease. Samples were withdrawn and the digestion stopped at intervals. DNA was isolated and electrophoresed on 7% polyacrylamide gels [9]. *Tetrahymena* nuclei extracted with 0.35 M NaCl could not be dispersed evenly in the buffer initially, but by the time the first sample was withdrawn the digestion mixture had become an even suspension as a result of the digestion process.

Results

One feature of the H1 histone of *Tetrahymena* is that it is charged less positively than H1 histones of higher eukaryotes. Because the interaction between an H1 histone and the rest of the chromatin is mostly ionic [10], there was a possibility that the *Tetrahymena* H1 histone is bound to chromatin less firmly than H1 histones of higher eukaryotes. This was examined by salt extraction of nuclei.

When *Tetrahymena* nuclei were washed with 0.15 M to 0.35 M NaCl, it was found that the HMG proteins were extracted almost completely by 0.25 M NaCl (fig. 1). A half or more of the H1 histone is extracted by 0.3 M NaCl, and all of it is extracted by 0.35 M NaCl. In contrast, only a small portion, if any, of bovine thyroid H1 is extracted by 0.35 M NaCl under the conditions employed.

Another feature of the *Tetrahymena* H1 histone that can be inferred from the primary structure is the lack of the central globular region [4, 5]. In higher eukaryotes, the central globular part of an H1 molecule is located at the DNA exit point of the nucleosome and gives DNA some resistance to micrococcal nuclease digestion there [2]. Thus chromatosomes are produced from chromatin of higher eukaryotes as an intermediate of micrococcal nuclease digestion.

When *Tetrahymena* nuclei were digested with micrococcal nuclease and DNA isolated from the digests was electrophoresed on a polyacrylamide gel, no discrete band of chromatosomal size DNA was seen (fig. 2). The presence of the HMG proteins does not seem to be responsible for the failure of micrococcal nuclease to give chromatosomes, because removal of the HMG proteins by washing the nuclei with 0.25 M NaCl did not change the result. As controls, *Tetrahymena* chromatin depleted of both the H1 histone and HMG proteins, by washing the nuclei with 0.35 M NaCl, and bovine thyroid nuclei, were also digested with micrococcal nuclease. The electropherograms of DNA isolated from the digests show that in the former case core particles were the only stable intermediate, whereas with thyroid nuclei chromatosomes as well as core particles were formed.

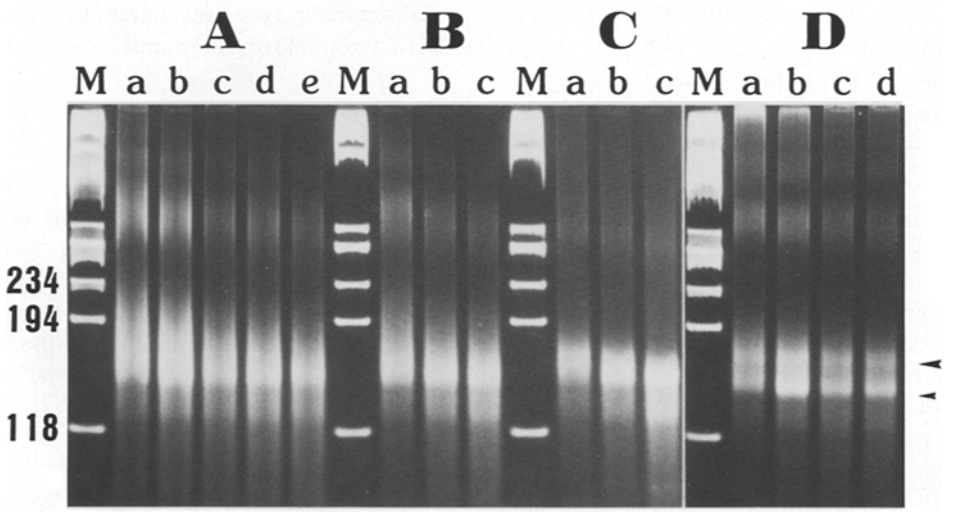

Figure 2. Polyacrylamide gel electrophoresis of DNA isolated from micrococcal nuclease digests. DNA isolated from micrococcal nuclease digests of *Tetrahymena* nuclei (A), *Tetrahymena* nuclei washed with 0.25 M NaCl (B), *Tetrahymena* nuclei washed with 0.35 M NaCl (C), and bovine thyroid nuclei (D) was electrophoresed on 7% polyacrylamide gels. Digestion times vary from a to e. M denotes markers (Hae III-fragments of øX174 DNA), the sizes of which are shown in base pairs on the left. The large and small arrowheads on the right indicate the positions of 168 bp (chromatosomal size) and 146 bp (core particle size) DNA fragments, respectively.

Discussion

The salt extraction experiments showed that the H1 histone of *Tetrahymena* is bound less firmly than the bovine H1 histone. A hydrophoby comparison between *Tetrahymena* H1 and human H1b has suggested that the *Tetrahymena* C-terminal region (residues 51-165) corresponds to the human C-terminal region (residues 108-218)[4]. Because the net charge difference between these proteins is large in this region (+ 31 for *Tetrahymena*, + 44 for human), the difference in salt extractability between *Tetrahymena* and bovine H1 histones may be due to the difference in charge density in the C-terminal regions of these proteins if the modes of association of histone H1 are similar.

As expected from the lack of the globular region in the *Tetrahymena* H1 histone[4, 5], micrococcal nuclease digestion failed to give chromatosomes. Two interpretations are possible for this, i.e. 1) histone H1 is present at the DNA exit point on the nucleosome, but nevertheless does not provide resistance to micrococcal nuclease trimming there, or 2) it does not exist at the DNA exit point, i.e. *Tetrahymena* histone H1 is not a linker histone. At present, data are not available to discriminate between these possibilities. If the latter is the case it follows that in at least three unicellular eukaryotes, i.e. *Tetrahymena, Trypanosoma*[3] and yeast, the presence of typical linker histones and chromatosomes is questionable.

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A marine mollusc provides the first example of in vivo storage of prostaglandins: Prostaglandin-1,15-lactones

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Summary. Prostaglandin- (PG) 1,15-lactones and, in smaller amounts, free acids, were isolated from both the mantle and the dorso-lateral appendices of the opisthobranch mollusc *Tethys fimbria.* In vivo conversion of PGs into the corresponding lactones and accumulation of PGE_2- and PGE_3-1,15-lactones in the appendages were shown. The detachment of these appendages from the molested mollusc caused the in vivo conversion of PGE_2- and PGE_3-lactones back to PGE_2 and PGE_3 respectively, thus providing the first example of a mechanism by which prostaglandins can be stored and, when needed, released.
Key words. Prostaglandins; prostaglandin-1,15-lactones; prostaglandin biosynthesis; marine eicosanoids; opisthobranch molluscs.

Prostaglandins are well-known mediators of several biological responses in both vertebrate and invertebrate organisms[1]. They are not pre-formed mediators and their biosynthesis from a common precursor like arachidonic acid generally occurs de novo following chemical, immunological or mechanical stimulation[1]. However, during our study of the chemical defenses of opisthobranch molluscs[2], we came across novel natural prostaglandin (PG) derivatives whose structure suggests a role as PG precursors.

The species belonging to the opisthobranch molluscan family Tethyidae, when molested, release a possibly defensive mucous secretion and easily detach their dorso-lateral appendages (cerata), which then contract and continue to release mucus for many hours without decomposing[3]. From the mantle and the cerata of one of these species, *Tethys fimbria*[4], we isolated a series of PG-1,15-lactones (fig. 1)[5, 6]. High pressure liquid chromatography (HPLC) analysis of extracts from both the mantle and the cerata of the mollusc also revealed the presence of peaks co-eluting with PGE_2 and PGE_3 standards (fig. 2, table 1), and proton nuclear magnetic resonance (NMR) analysis of these components confirmed this finding (unpublished results). The co-existence of PG-1,15-lactones and PG free acids raised questions regarding: 1) their biogenetic relationship, and 2) which of the two classes of compounds, if any, has a biological role in *T. fimbria.*