ANALYSIS OF CHROMATIN REPEAT UNITS IN LOGARITHMICALLY AND STATIONARY GROWING CELLS OF PARAMECIUM AURELIA AND TETRAHYMENA PYRIFORMIS

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SUMMARY

We have used micrococcal nuclease as a probe of the repeating structure of chromatin isolated from the macronuclei of logarithmically and stationary grown Paramecium aurelia and Tetrahymena pyriformis. For both these lower eukaryotes, the monomer size is shown to vary depending on the stage in the growth cycle. P. aurelia exhibits a monomer size of 153±7 bp and 178±6 bp and T. pyriformis 207±10 bp and 230±10 bp in logarithmic and stationary cells, respectively. Both exhibit a nucleosome size of 140 bp. We discuss the possible association of these changes with histone content and nuclear activity changes, and also a possible reason for the divergence from the size pattern of monomer repeats seen in lower eukaryotes by T. pyriformis.

INTRODUCTION

Recent reports have indicated that there is a division between lower and higher eukaryotes with regard to the size of the monomer, the basic repeat unit of chromatin. Higher eukaryotes generally exhibit a monomer size of approximately 200 base pairs (bp), comprising a 140 bp nuclease resistant core particle (nucleosome) and a 60 bp spacer or linker region of DNA (1,2,3,4). Reports for the lower eukaryotes are usually within a range of 150-170 bp, also generally with a core particle size of a 140 bp, and a variable spacer length (5,6,7,8,9, 10,11,12).

This evolutionary divergence may also be related to differences in H1 content or structure, as H1 seems to be intimately associated with the DNA of the spacer region (13). Noll (7) and Morris (14) have suggested a causal relationship between the evolution of H1 structure (lysine-arginine content) and the spacer DNA

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length. Also, Morris (14) has shown that the monomer size of chicken liver and erythrocyte is variable and has suggested that this may be related to their H1 and H5 content.

In this paper we examine changes in the monomer repeat size of macronuclei isolated from P. aurelia and T. pyriformis, related to the cell's position in its growth cycle. Nuclear activity varies during the growth of a cell, as demonstrated by changes in ribosomal RNA content in T. pyriformis (15) and in its extreme for the reduced number of polysomes formed in P. aurelia senescence, (Sundararaman and Cummings, in press). Recent work in other systems suggests that nuclear activity states are reflected in the monomer repeat lengths, and similar trends are reported here (14,20).

METHODS

Growth of Cells and Isolation of Nuclei

P. aurelia, stock 513, was grown and harvested (after 24 & 72 hrs) as described by Tait and Cummings (16). T. pyriformis (stock HSM) were grown on 1% proteose peptone, and harvested after 14 hrs. and 40 hrs. in a similar way to P. aurelia. Macronuclei were prepared from both organisms by the method described by Cummings (in press in Methods in Cell Biology) using 6% gum arabic and 0.03% Deoxycholate and Nonidet P40 for logarithmically grown P. aurelia and 0.04% DOC and NP40 for stationary cells. No detergents were used with T. pyriformis.

Digestion of Nuclei with Micrococcal Nuclease

This method is basically that of Seale (17). All nuclei were treated in the same manner. HeLa nuclei were prepared by the method described by Seale (17). Nuclei were digested using the requisite amount of Sigma micrococcal nuclease (0.1 units/10 A_{260} units of nuclei measured in 1% SDS) and then treated with Proteinase K (10 µg/ml, EM Biochemicals).

Polyacrylamide gel Electrophoresis

The DNA samples were heat denatured by boiling for 5 minutes, with immediate cooling, and then run on 4% PAGE gels (acrylamide-biacrylamide 19:1 containing tris-borate electrophoresis buffer (18), in 6M urea, at 200V for 1½-2 hrs. The gels were loaded with 0.5-1.5 A_{260} units and were subsequently stained with 2 µg/ml ethidium bromide in water, illuminated with two Blak- Ray lamps (U-V products XX15) and photographed through a red filter.

RESULTS

The digestion of macronuclei isolated from P. aurelia with micrococcal nuclease generates a regular series of DNA fragments, whose repeat length is shorter than that shown for HeLa nuclei (figure 1). Thus, P. aurelia, like other lower eukaryotes, N. crassa, A. nidulans, S. cerevisiae, has a monomer

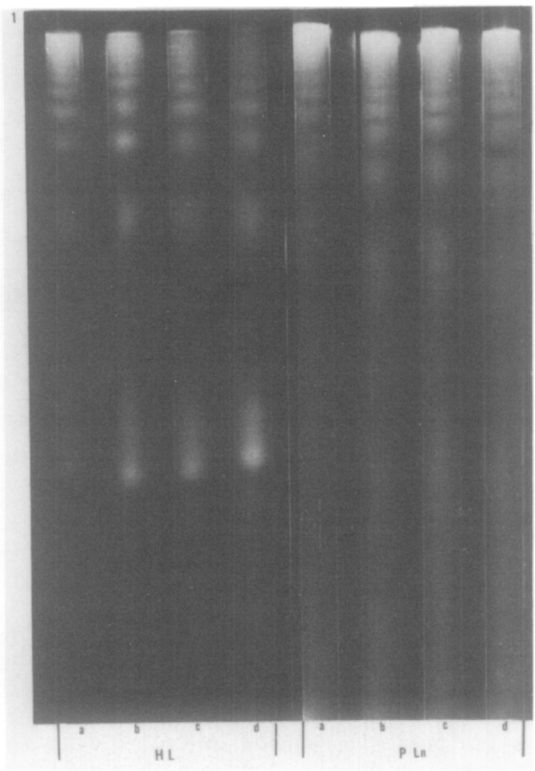

Figure 1: Time course of micrococcal nuclease nuclease digestion of nuclei from logarithmically growing P. aurelia and HeLa nuclei.

Nuclei were digested with 0.1 units/10_{260} units of micrococcal nuclease for a, 30 sec; b, 60 sec; c, 120 sec; d, 240 sec at 37°C. Samples were processed as described in experimental procedures and electrophoresed on 4% PAGE gels, containing 6M urea, for 2 hrs. at 200V. HL= HeLa cells, P.Ln=Logarithmic P. aurelia cells.

repeat size fewer than 200 bp. The size of the micrococcal nuclease fragments were determined by co-electrophoresis with HIND II and III restriction fragments estimated by Danna and Nathans (19) and the HeLa repeats were allocated the value assigned by Compton et al. (4). Nuclei from logarithmically growing P. aurelia have a repeat size of 153±7 bp. Figure 1 shows a time course digestion of P. aurelia and HeLa cell nuclei; the DNA from both sources was heat denatured prior to electrophoresis on 5% polyacrylamide-urea gels (see Methods). Figure 1 indicates a change in the repeat pattern after 30 sec. in the P. aurelia samples (also see Table 1) (a phenomenon also noted by Johnson et al. (12), and Thomas

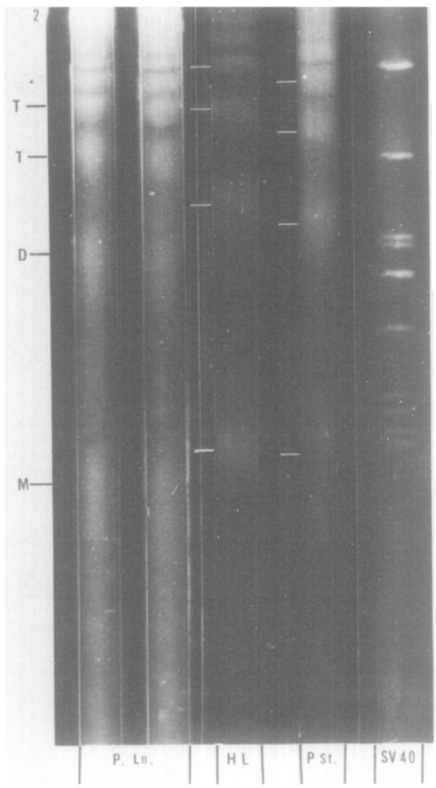

Figure 2: Micrococcal nuclease digests of logarithmic and stationary P. aurelia and HeLa nuclei.

Digest were carried out as described in Figure 1.
P.Ln = Logarithmic P. aurelia cells
P.St. = Stationary P. aurelia cells
HL = HeLa cells
SV40 = HIND II and III restriction fragments of SV40
Monomer (M) Dimer (D) Trimer (T) and Tetramer (T) bands are indicated.

and Thompson (20), and that the monomer and core particles (140 bp) are more diffuse and less easily visualized when compared to the HeLa digests. This may be another facet of the endogenous nuclease activity. The percentage of TCA soluble material for a limit digest was determined to be 50% in logarithmically grown P. aurelia, similar to other eukaryotes (data not shown).

Figure 2 shows the chromatin digestion patterns obtained for logarithmically growing and stationary P. aurelia nuclei. This shows the non-coincident

Table 1: The size (base pairs) of DNA fragments produced by micrococcal nuclease digestion of logarithmic and stationary cells from P. aurelia and T. pyriformis.

Band Number	P. aurelia Logarithmic 30 second[a]	P. aurelia Logarithmic 240 second[b]	P. aurelia Stationary 480 second[b]	T. pyriformis Logarithmic 180 second[c]	T. pyriformis Stationary 180 second[c]
1.	-	170	190	185	240
2.	340	320	340	440	460
3.	520	480	550	620	680
4.	700	660	750	830	
5.	800	780	860		
6.	1030	880			
7.		1030			
Av. monomer size (bp)	168±8	153±7	178±6	207±10	230±10

The sizes of the DNA bands were measured at their midpoints and are taken from

a. Figure 1

b. Figure 2

c. Figure 3

migration of the two P. aurelia samples and the HeLa nuclei digest. That this is not due to variable exonucleolytic action or corresponding multiples is seen by the fact that the out-of-phase situation occurs throughout the repeat pattern. The monomer repeat size for stationary cells of P. aurelia (Table 1) was estimated to be 178± bp.

The change in monomer repeat size depending on the stage of the growth cycle was also observed in T. pyriformis (Figure 3, Table 1). Early logarithmically grown cells were determined to have a repeat size of approximately 207±10 bp and stationary cells one of approximately 230±10 bp. It is difficult to assign definitive values to T. pyriformis because the bands produced are very broad and diffuse and therefore difficult to measure. This was also seen by Frado et al. (10) who also indicated the observed absence of large oligomer repeats in the digest, as we confirm here.

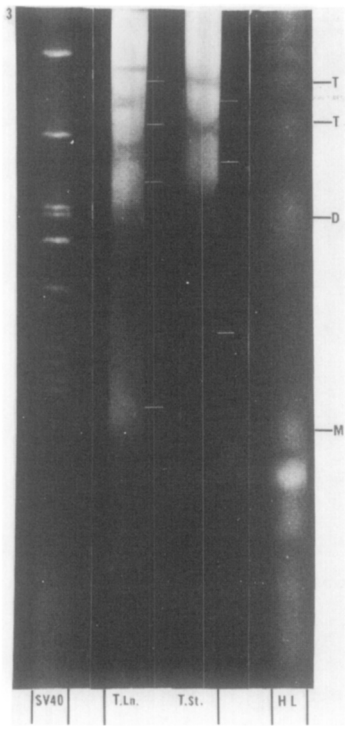

Figure 3: Micrococcal nuclease digests of nuclei from Logarithmically and Stationary growing T. pyriformis and HeLa cells.

Digests were carried out as described in Figure 1.
T.Ln. = Logarithmic T. pyriformis cells
T.St. = Stationary T. pyriformis cells
HL = HeLa cells
SV40 = HIND II and III fragments of SV40
Monomer (M) Dimer (D) Trimer (T) and Tetramer (T) bands are indicated.

DISCUSSION

The present results indicate that the chromatin of P. aurelia is packaged in a way similar to that of most other lower eukaryotes, producing a monomer repeat size of 153±8 bp in logarithmically growing cells, which is comparable to most of the other reports for lower eukaryotes. T. pyriformis, on the other hand has a larger monomer repeat size, in both logarithmic and stationary cells, falling in the same size range as higher eukaryotes. Other reports concerning the repeat size for T. pyriformis are given by Gorovsky and Keevert (9), 155 bp,

and Frado et al. (10), approximately 205 bp. Our value is in the same region as that reported by Frado et al. Gorovsky and Keevert state that their digests of T. pyriformis nuclei had mobilities similar to calf thymus nuclear digests, and so their value may represent an underestimation of the monomer repeat size. Tetrahymena is reported to have an abnormal complement of histones (21) and this may account for its dissimilarity with other lower eukaryotes. Stylonychia mytilus also has an apparently large monomer repeat size, 220 bp (11), which may be due to the use of starved cells, or a result of some abnormalities in its histone complement.

We also report a change in monomer size dependent on the stage in the growth cycle of both P. aurelia and T. pyriformis. Accompanying the cellular changes that occur during the growth cycle, (e.g., T. pyriformis loses 25% of its ribosomes when changing from logarithmically growing cells to a starved state (15), there is a concomitant change in chromatin conformation reflected in a 25 bp (P. aurelia) and 23 bp (T. pyriformis) increase in the monomer repeat size. There have been several reports recently of different repeat sizes within the same animal (14,20,22). These changes have been correlated with changes in histone complement (particularly H1 and H5) and activity states of the respective genomes. Variations in H1 during development and cellular differentiation have been reported extensively (23,24), and it is perhaps possible that similar variability may occur during the growth cycle. The spacer size variation may be due to changes in acetylation or phosphorylation (20), to changes in primary structure, e.g., lysine and arginine content (14), or even to a change in the percentage of the H1 molecule able to associate fully with the spacer region.

Values for the number of lysine and arginine residues of H1 in stationary P. aurelia and T. pyriformis are approximately 40 (25) and 75 (21), respectively. These correlate well with the number of base pairs in the spacer regions of stationary nuclei, 33 bp and 90 bp, respectively. This correlation has been noted by Noll (7) and Morris (8) in fungi, and Morris (14) in chicken liver and erythrocytes. So analysis of the H1 lysine-arginine content in logarithmically

growing cells might give an indication as to where the observed changes in monomer size might originate.

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REFERENCES

1. Hewish, D.R. and Burgoyne, L.A. (1973). Biochem. Biophys. Res. Commun. 52, 504-510.
2. Noll, M. (1974). Nature 251, 249-251.
3. Olins, A.L. and Olins, D.E. (1974). Science 183, 330-332.
4. Compton, J.L., Bellard, M. and Chambon, P. (1976). PNAS (USA) 73, 4382-4386.
5. Lohr, D. and VanHolde, K.G. (1975). Science 188, 165-166.
6. Thomas, J.O. and Furber, V. (1976). FEBS Letters 66, 274-280.
7. Noll, M. (1976). Cell 8, 349-355.
8. Morris, N.R. (1976). Cell 8, 357-363.
9. Gorovsky, M.A. and Keevert, J.B. (1975). PNAS (USA) 72, 3536-3540.
10. Frado, L.-L.Y., Annunziato, A.T. and Woodcock, C.L.F. (1977). Biochim. Biophys. Acta 475, 514-520.
11. Lipps, H.J. and Morris, N.R. (1977). Biochem. Biophys. Res. Commun. 74, 230-234.
12. Johnson, E.M., Littau, V.C., Allfrey, V.C., Bradbury, E.M. and Matthews, H.R. (1976). Nuc. Acid Res. 3, 3313-3329.
13. Noll, M. and Kornberg, R.D. (1977). J. Mol. Biol. 109, 393-404.
14. Morris, N.R. (1976). Cell 9, 627-632.
15. Hallberg, R.L. and Bruns, P.J. (1976). J. Cell Biol. 71, 383-394.
16. Tait, A. and Cummings, D.J. (1975). Biochim. Biophys. Acta 378, 282-295.
17. Seale, R.L. (1976). Cell 9, 423-429.
18. Peacock, A.C. and Dingman, C.W. (1967). Biochemistry 6, 1818-1827.
19. Danna, K. and Nathans, D. (1971). PNAS (USA) 68, 2913-2917.
20. Thomas, J.O. and Thompson, R.J. (1977). Cell 10, 633-640.
21. Johmann, C.A. and Gorovsky, M.A. (1976). Biochemistry 15, 1249-1256.
22. Spadafora, D., Bellard, M., Compton, J.L. and Chambon, P. (1976). FEBS Letters 69, 281-285.
23. Seale, R.L. and Aronson, A.I. (1973). J. Mol. Biol. 75, 647-658.
24. Shires, A., Carpenter, M.P. and Chalkley, R. (1975) PNAS (USA) 72, 2714-2718.
25. Isaaks, R.E. and Santos, B.G. (1973). J. Protozool. 20, 482-489.