HISTONE ANALYSIS IN *VOLVOX*

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1. Introduction

Histones are basic proteins associated with the chromosomal complex, and are thought to play some role in gene action at the level of cellular differentiation [1]. *Volvox*, with its two distinct cell types, somatic and reproductive, represents cellular differentiation at the simplest level. Nucleic acids have been isolated from this organism [2], but we know of no investigations dealing with the demonstration of histones in *Volvox*.

We now report that proteins have been isolated from *Volvox carteri* which are histones by the following criteria: preparative behavior, amino acid profile, absence of tryptophan, ultraviolet absorption, and electrophoretic pattern. Although no apparent variation in electrophoretic pattern was found in three developmental stages of the life cycle, some quantitative differences were noted. In comparing the histones of calf thymus and *V. carteri*, similar electrophoretic patterns were observed; in addition, quantitative differences were seen between some of the histone bands of the two organisms.

2. Methods

The organism used in this study was *Volvox carteri f. nagariensis* Iyengar, strain HK-10 [3]. Axenic cultures were grown in medium devised by Provasoli and Pintner. A detailed description of the culture techniques and the life cycle was presented by Starr [4].

The histone content was determined at three stages: 1) immediately after the release of young spheroids, 2) before cleavage of the gonidia, and 3) before release of young spheroids. Samples of each stage were collected on filter paper and the wet weight determined. All subsequent steps were carried out at 0°–4°C.

The histone extraction procedure was a modification of Bonner et al. [5]. About 6 g wet weight were ground in a mortar and pestle in 0.05 M Tris–HCl–0.01 M $MgCl_2$, pH 7.6. This mixture was centrifuged at 2000 *g* to obtain an initial cytoplasmic supernatant and a nuclear-rich pellet. This pellet was resuspended in the grinding

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medium, centrifuged at 10 000 *g* three times, and then extracted for 30 min after the addition of 1/4 volume 1N H_2SO_4. After centrifugation, the supernatant was mixed with four volumes of ethanol, and the precipitated protein was dried in a vacuum dessicator overnight.

Electrophoresis was carried out using the method of Reisfeld et al. [6], as modified by Bonner et al. [5]. After electrophoresis, the gels were stained with Amido Black, and the absorption profiles of the gels were obtained with a Photovolt densitometer (420 mμ).

The presence of tryptophan was tested for in a portion of the sample by the Hopkins–Cole method. The ultraviolet absorption was determined from 300 mμ to 200 mμ on a Cary 14 recording spectrophotometer using a 1 mg/ml calf thymus histone sample (Sigma) for comparison. Amino acid profiles were obtained with a Spinco Model 120C automatic acid analyzer set up for single column analysis. After performic acid oxidation, cysteine was analyzed as cysteic acid.

3. Results and discussion

Absorption spectra (fig. 1) of proteins extracted from *V. carteri* were found to be essentially identical to the spectrum of standard calf thymus histone (Sigma), which absorbs strongly in the 220–230 mμ

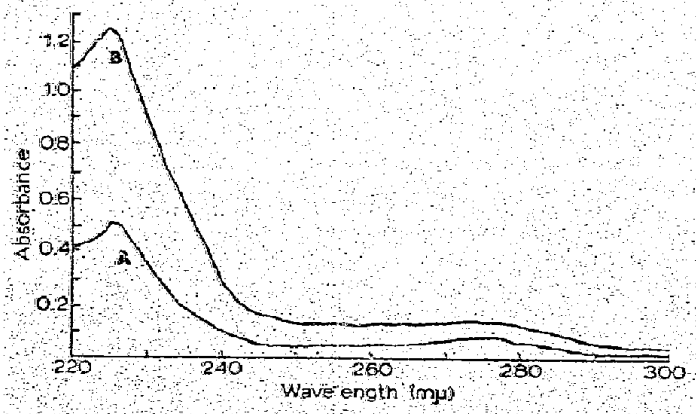

Fig. 1. UV absorption spectra of calf thymus histone and protein extracted from *V. carteri*. Cuvette width 1 cm.
A = calf thymus histone (Sigma) 0.11 mg/ml;
B = *V. carteri* histone 0.28 mg/ml.

Table 1
Amino acid analyses of histones from *V. carteri*, calf thymus [8], *N. crassa* [9], and acid soluble proteins of *R. palmata* [10]

Amino acid	*V. carteri*	Calf thymus	*N. crassa*	*R. palmata*
Lysine	9.7	14.5	11.2	3.8
Arginine	6.9	8.3	6.7	3.1
Histidine	2.8	2.0	1.8	3.1
Aspartic	8.3	4.9	8.4	7.6
Glutamic	9.7	8.2	10.7	12.2
Serine	4.9	5.9	5.9	5.9
Threonine	5.6	5.7	5.4	3.8
Alanine	11.1	13.4	11.5	10.6
Valine	8.3	6.3	6.8	10.4
Leucine	7.6	7.7	7.8	2.0
Isoleucine	4.2	4.3	4.5	7.3
Proline	5.6	4.9	5.7	0.0
Glycine	9.0	8.3	8.4	12.6
Phenylalanine	3.5	2.3	3.0	8.4
Tyrosine	2.1	2.5	2.2	3.5
Methionine	0.0	1.0	2.3	0.0
Cystine/2	0.0	0.0	0.0	0.0
Basic/acidic	1.1	1.9	1.0	0.5
LYS/ARG	1.40	1.75	1.67	1.23

Amounts of amino acid are expressed as moles per 100 moles of amino acid recovered. No corrections were applied for hydrolytic losses of any of the amino acids in *V. carteri*. Basic amino acids are lysine, arginine and histidine; acid ones are aspartic and glutamic acids.

range and shows a low, flattened curve that peaks at 276–278 mμ [7].

No tryptophan color reaction was observed in any of the samples of *V. carteri* or in the calf thymus histone samples (Sigma). It can be concluded that less than 0.002 mg tryptophan/mg histone were present from the sensitivity of the color reaction, using L-tryptophan and bovine serum albumin standards. This lack of tryptophan is characteristic of the histones previously studied [8].

Amino acid analyses were performed on the *V. carteri* protein samples and compared with amino acid analyses of histones from calf thymus [7], the fungus *Neurospora crassa* [9], and acid soluble proteins of the red alga *Rhodymenia palmata* [10] (table 1). The amino acid profile of the *V. carteri* samples was similar to that of *N. crassa*. The composition and ratios of *V. carteri* and *N. crassa* were closely similar, and differed from those of calf thymus in lysine, arginine, aspartic acid and glutamic

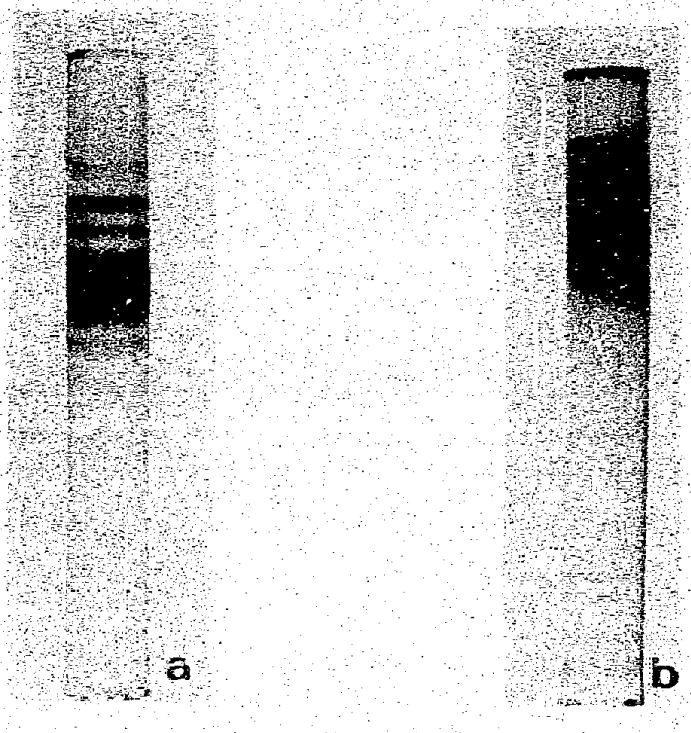

Fig. 2. Electrophoretic patterns of histones from calf thymus (gel a) and *V. carteri* (gel b). Migration is from top (+) to bottom (−).

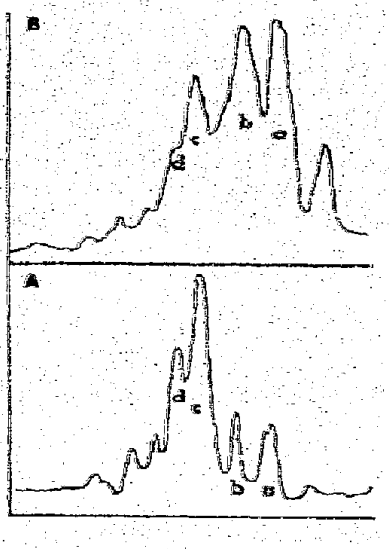

Fig. 3. Densitometer tracings of the polyacrylamide gels shown in fig. 1. Migration is from right to left. A = standard calf thymus histone; B = *V. carteri* histone. Explanation in text. The bands preceding *a* are variable in intensity and are believed to represent non-histone contaminants.

acid. The composition of *R. palmata* protein differed from all three of the above in many respects.

To determine the similarity in banding patterns between calf thymus and *V. carteri*, we electrophoresed the two sets of proteins and observed the resulting gel patterns (fig. 2) and densitometer tracings (fig. 3). These tracings (fig. 3) demonstrated some quantitative differences in various bands. For example, band *c* in the calf thymus gel was much heavier than bands *a* and *b*, but in the *V. carteri* gel, band *c* was slightly lower in intensity than *a* and *b*. A few less marked quantitative differences were also observed.

No apparent qualitative differences in the banding pattern were demonstrated in the three developmental stages of this organism after running at least six trials in each stage. However, we observed some quantitative differences, particularly in the bands in areas *a* and *b* (fig. 3, gel B). Band *a* appeared heavier than band *b* after spheroid release, whereas these bands seemed approximately equal in intensity both before cleavage and before release.

Histones have been studied extensively in higher organisms, particularly calf thymus and pea buds, and appear to have a role in the expression of genetic information. These proteins are similar across plant and animal kingdoms, and with the exceptions of erythrocytes in birds [11] and the F1 fraction [12], differ only quantitatively, that is in relative proportions [13]. Our results support the concept that the appearance of histones in the evolutionary scheme coincides with organisms having true chromosomes and exhibiting cellular differentiation, and thus may have roles in both chromosome structure and differential genetic expression. We have clearly shown that the overall electrophoretic banding pattern of histones is relatively consistent between organisms at the top and bottom of the evolutionary scheme. Our results also support the idea that individual histones can differ in quantity either from one organism to another or within the life cycle of a single organism. This quantitative variation is also consistent with the variation in amino acid profiles of total histones for organisms far down the evolutionary scale [9, 10] as compared to those of higher organisms.

Various *Volvox* mutants, blocked at specific steps

in cellular differentiation, are readily produced and characterized [14]. Thus lower eukaryotic organisms such as *Volvox* may permit the exploration of general roles of histones by relatively simple approaches, and may also reveal novel aspects of histone functions in chromosome structure and cellular differentiation.

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